UNIVERSIDADE D
COIMBRA

Joana Filipa Simões Lopes

**Relatórios de Estágio e Monografia intitulada "Macrophage Membrane-Coated Nanosystems for Biomedical Applications" referente à Unidade Curricular "Estágio", sob a orientação, da Dra. Carolina Marques, da Doutora Marília João Rocha e da Professora Doutora Ana Cláudia Santos, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas**

Setembro de 2021

UNIVERSIDADE D
COIMBRA

Joana Filipa Simões Lopes

Relatórios de Estágio e Monografia intitulada "Macrophage Membrane-Coated Nanosystems for Biomedical Applications" referente à Unidade Curricular "Estágio", sob a orientação, da Dra. Carolina Marques, da Doutora Marília João Rocha e da Professora Doutora Ana Cláudia Santos, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

Setembro de 2021

Eu, Joana Filipa Simões Lopes, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2016228661, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Macrophage Membrane-Coated Nanosystems for Biomedical Applications" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

Mais declaro que este Documento é um trabalho original e que toda e qualquer afirmação ou expressão, por mim utilizada, está referenciada na Bibliografia, segundo os critérios bibliográficos legalmente estabelecidos, salvaguardando sempre os Direitos de Autor, à exceção das minhas opiniões pessoais.

Coimbra, 2 de setembro de 2021.

Joana Filipa Simões Lopes

(Joana Filipa Simões Lopes)

## Agradecimentos

À Professora Doutora Ana Cláudia Santos, pela disponibilidade, orientação e auxílio prestado ao longo da elaboração da presente monografia.

À Dra. Carolina Marques e a toda a equipa da Farmácia Medeiros pela oportunidade de estágio, excelente integração, vivências partilhadas e por todos os ensinamentos transmitidos ao longo do estágio curricular.

À Doutora Marília João Rocha e a toda a equipa dos Serviços Farmacêuticos do CHUC, pelo acolhimento e integração, pela disponibilidade e ensinamentos transmitidos.

À minha família, especialmente aos meus pais, irmãos, irmã e avós por acreditarem sempre em mim e nas minhas capacidades, pelo apoio incondicional, por estarem sempre do meu lado e me ajudarem nos momentos mais difíceis.

Aos amigos que Coimbra me deu, por me acompanharem ao longo desta caminhada, por todos os momentos partilhados e por tornarem estes 5 anos a melhor experiência académica que podia ter vivido.

À Faculdade de Farmácia da Universidade de Coimbra e a todo o corpo docente e não docente.

A ti, Coimbra, pelas experiências de vida, pelos momentos vividos e pelas amizades que, certamente, levarei para a vida!

**A todos, muito obrigada!**

# Índice

## Parte I - Relatório de Estágio em Farmácia Comunitária



## Parte II - Relatório de Estágio em Farmácia Hospitalar

# Parte I

Relatório de Estágio em Farmácia Comunitária

**Farmácia Medeiros**

**Lista de Abreviaturas**

**ANF:** Associação Nacional das Farmácias

**DCI:** Denominação Comum Internacional

**FFUC:** Faculdade de Farmácia da Universidade de Coimbra

**MICF:** Mestrado Integrado em Ciências Farmacêuticas

**PVF:** Preço de Venda à Farmácia

**PVP:** Preço de Venda ao Público

**SWOT** (*Strenghts*, *Weaknesses*, *Opportunities*, *Threats*)**:** Pontos Fortes, Pontos Fracos, Oportunidades e Ameaças

## 1. Introdução

A Farmácia Comunitária ou de Oficina assume um papel preponderante na prestação de cuidados de saúde à população através da oferta de uma panóplia de serviços que visam garantir o uso racional do medicamento, a gestão correta da medicação e a identificação precoce de situações de risco. O Farmacêutico Comunitário, enquanto agente de saúde pública e especialista do medicamento, é muitas vezes o primeiro ponto de contacto com os utentes, que diariamente depositam um enorme voto de confiança no farmacêutico para ver resolvidos os seus problemas de saúde de forma célebre e exímia [1]. Deste modo, o farmacêutico comunitário ocupa uma posição privilegiada na sociedade para a promoção e educação para a saúde dada a sua maior proximidade com a população e à criação de vínculos de confiança com os utentes que, em última instância, permitem um acompanhamento farmacêutico mais adequado.

A realização de um estágio em Farmácia Comunitária corresponde à última e obrigatória fase de formação do ciclo de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC), proporcionando aos estudantes a oportunidade de aplicar na prática os conhecimentos teóricos adquiridos ao longo do percurso académico e de vivenciar a realidade do mercado de trabalho, contactando com uma realidade prática mais próxima com a do futuro profissional. O estágio em Farmácia Comunitária constitui uma etapa crucial do percurso académico para a aquisição de competências práticas e experiência profissional, ambas essenciais ao futuro exercício da profissão farmacêutica.

Neste sentido, culmino o meu percurso académico com um estágio curricular na Farmácia Medeiros, localizada na vila de Avelar, decorrido entre janeiro de 2021 e abril de 2021, com uma duração total de 648h, sob a orientação da Dra. Carolina Medeiros Vieira Marques que lidera a equipa técnica composta por farmacêuticos e adjuvantes técnicos na ausência da Diretora Técnica. A seleção criteriosa da Farmácia Medeiros para a realização do estágio curricular teve em conta o conhecimento prévio da equipa técnica, da organização e do modo de funcionamento interno da farmácia, dado ter realizado anteriormente um estágio extracurricular nesta farmácia.

No relatório de estágio que apresento no formato de uma análise SWOT irei descrever não só os pontos fortes (*Strengths*) e os pontos fracos (*Weakness*) da minha experiência, que resultam da minha reflexão pessoal a nível interno deste período correspondente ao estágio curricular em Farmácia Comunitária, mas também a nível externo as oportunidades (*Opportunities*) e as ameaças (*Threats*) serão mencionadas.

## 2. Farmácia Medeiros

A Farmácia Medeiros situada na Praça Costa Rego n.º 130, pertencente ao concelho de Ansião, destaca-se pelo seu património valioso e história de longa data. Fundada em 1905, esta farmácia centenária mantém atualmente uma localização estratégica junto ao hospital Fundação Nossa Senhora da Guia, e de inúmeros locais comerciais, estabelecimentos de ensino e de saúde, o que garante uma maior afluência de utentes à farmácia. A inexistência de concorrência nas redondezas, dado ser a única farmácia na vila de Avelar, aliado à sua localização privilegiada, assegura que, para além dos utentes fidelizados e habituais, uma grande diversidade de utentes frequente a farmácia.

A Direção Técnica da Farmácia Medeiros é assumida pela Dra. Maria Alice David Abreu Figueiredo Medeiros, que é simultaneamente a proprietária. Com uma bagagem histórica notável, ao longo dos seus 116 anos de existência, a Farmácia Medeiros foi conquistando a confiança da população e acumulando um número considerável de utentes fidelizados, que constituem a maior parte dos utentes da farmácia. A simpatia e dedicação da equipa técnica que integra a Farmácia Medeiros, assim como o ambiente acolhedor e de partilha que sobressaem neste espaço, possibilitam o estabelecimento de relações de maior confiança com a população, o que é uma base fundamental para garantir um acompanhamento mais próximo dos utentes e a prestação de um aconselhamento farmacológico e não farmacológico personalizado e direcionado.

Para responder de forma adequada e eficaz às necessidades dos utentes que a frequentam, a Farmácia Medeiros apresenta um horário de funcionamento alargado, mais concretamente das 9h às 20h nos dias úteis, e das 9h às 19h nos fins de semana e feriados, encontrando-se em serviço de disponibilidade fora deste horário.

## 3. Análise SWOT

### 3.1. PONTOS FORTES

#### 3.1.1. Equipa técnica da Farmácia Medeiros

O espírito de equipa, de entreajuda e cooperação são peças fundamentais para garantir o sucesso e bom funcionamento de qualquer instituição. A equipa técnica que integra a Farmácia Medeiros é dotada de uma enorme dedicação, experiência, competência e profissionalismo, sendo que a constante partilha de conhecimento entre os diferentes colaboradores da farmácia e a capacidade de trabalho em equipa foram, indiscutivelmente, um dos pontos fortes do meu estágio, pois contribuíram para uma melhor aquisição e consolidação das ferramentas essenciais ao exercício da profissão. Paralelamente, desde o

início do estágio que senti todo o apoio e abertura necessários para aplicar na prática os conhecimentos teóricos adquiridos ao longo do percurso académico, o que reconheço como um ponto forte do estágio.

A oportunidade de integrar uma equipa experiente e competente foi, sem dúvida, uma mais-valia na minha formação, pois permitiu-me adquirir competências de trabalho e de relação interpessoal, que contribuíram para o meu crescimento tanto a nível profissional como pessoal. Destaco, assim, a importância de todos os elementos que compõem a equipa técnica neste processo de aprendizagem e na minha integração, dada a total disponibilidade para qualquer questão que surgisse e a partilha do maior número de conhecimentos possível.

### 3.1.2. Planeamento do estágio

No que concerne ao plano de estágio, este foi projetado de forma a permitir uma aquisição gradual dos conhecimentos e o desempenho de tarefas, por ordem faseada de responsabilidade, em praticamente todas as valências da farmácia comunitária. De facto, a possibilidade de participar em todas as atividades da rotina diária da farmácia comunitária, desde a gestão dos medicamentos e de outros produtos de saúde até ao atendimento e indicação farmacêutica, o ato mais nobre desempenhado pelo farmacêutico comunitário, é claramente um dos pontos fortes que retiro desta experiência profissional.

Inicialmente, comecei por desempenhar funções relacionadas com o *back office* da farmácia, as quais, embora não envolvam contacto direto com os utentes, são da máxima importância para o correto funcionamento interno da farmácia e para o exercício adequado das funções no *front office*. Estas incluem a conferência e receção das encomendas através da verificação da quantidade recebida, do prazo de validade, do estado da embalagem, do preço de venda ao público (PVP) e do preço de venda à farmácia (PVF), gestão de devoluções, monitorização semanal da humidade e temperatura recorrendo a termohigrómetros devidamente calibrados, gestão dos *stocks* e dos prazos de validade, tratamento das reservas, transferência de produtos entre farmácias, fecho e encaminhamento dos contentores da Valormed aos fornecedores, e ainda, gestão mensal da saída de estupefacientes e psicotrópicos, sendo todas estas realizadas segundo as Boas Práticas para a Farmácia Comunitária [2]. A meu ver, a realização destas tarefas foi bastante positiva e profícua, uma vez que o contacto mais estreito com os produtos numa fase inicial do estágio contribuiu claramente para uma maior familiarização com os mesmos e facilitou a associação do(s) princípio (s) ativo (s) à respetiva embalagem e designação comercial.

Numa fase posterior do estágio comecei progressivamente a integrar e a participar ativamente nas tarefas do *front office*. De salientar que, desde o primeiro momento tive total

permissão para observar os atendimentos realizados pelos membros da equipa técnica, o que considero uma grande vantagem, pois permitiu-me adquirir ferramentas e competências que agilizaram a minha comunicação posterior com os utentes e a minha abordagem no atendimento através da colocação de perguntas-chave mais pertinentes. Por outro lado, a observação inicial dos atendimentos, antes de os realizar de forma mais autónoma, contribuiu para a minha familiarização com o *software* Sifarma 2000® nas diferentes modalidades na vertente do atendimento, incluindo, entre outros, o acesso ao histórico de consumo do utente, a pesquisa de informação científica do medicamento, e o rebate de pontos do cartão Saúda. Posteriormente, de forma gradual e sempre devidamente acompanhada, comecei a executar os atendimentos de forma mais independente.

### 3.1.3. Dinamização da farmácia e posicionamento *online*

A comunicação e divulgação dos produtos e das campanhas publicitárias em vigor na farmácia através de estratégias de *merchandising*, que se inserem no campo do *marketing* e visam estimular o ato de compra, foi uma prática com a qual tive um contacto muito próximo durante o período de estágio. Na Farmácia Medeiros, esta vertente publicitária junto da população é bastante notória, tanto no desenvolvimento de estratégias de venda na farmácia alusivas a dias temáticos, implicando, para tal, a reorganização dos lineares com disposição dos produtos com menor rotatividade nas zonas quentes e mais visíveis da farmácia, bem como através da divulgação de campanhas promocionais nas redes sociais.

Para o efeito, a Farmácia Medeiros dispõe de uma página de *Facebook,* onde publica mensalmente uma *Newsletter* alusiva a variadas temáticas acompanhada com os respetivos sintomas, aconselhamento farmacêutico, medidas não farmacológicas e ainda sugestões de produtos comercializados na farmácia indicados para cada situação. Esta inclui ainda as campanhas publicitárias em vigor e os serviços providenciados pela farmácia em cada mês, com o intuito de aumentar a afluência dos utentes à farmácia. Na qualidade de estagiária, o meu contributo na redação de algumas *Newsletters* serviu para aprofundar o meu conhecimento e a minha capacidade de divulgação de alguns produtos, o que posteriormente proporcionou uma melhor abordagem no atendimento.

### 3.1.4. Oferta de serviços farmacêuticos diferenciados

A diferenciação e o carácter inovador dos produtos e dos serviços prestados à população são um pilar essencial para o sucesso. Na Farmácia Medeiros a diversidade de serviços é claramente evidente, incluindo administração de injetáveis e de vacinas, consultas de nutrição, rastreiros de audição, avaliação dos parâmetros antropométricos, como o peso

e a altura, medição da pressão arterial, determinação dos parâmetros bioquímicos, incluindo a glicémia, concentração de colesterol total e de triglicerídeos, e ainda, consultas de dermocosmética com conselheiras da marca *Lierac*. Outros serviços oferecidos na Farmácia Medeiros incluem a cedência de medicamentos e muitos outros produtos de saúde a diferentes instituições, designadamente a lares de idosos, e a preparação individualizada da medicação com recurso a caixas organizadoras da medicação (*pill-boxes*) para idosos polimedicados e para outros utentes que, dada a complexidade do perfil farmacoterapêutico, justificam a organização semanal da medicação.

#### 3.1.4.1. Parceria com instituições

A Farmácia Medeiros colabora com diferentes instituições sociais, neste caso a residência geriátrica de Chão de Couce e do Avelar, através do fornecimento de diversos produtos de saúde conforme os pedidos e necessidades dos utentes que residem nestes locais. Este serviço revela-se da máxima importância para o funcionamento destas instituições, e contempla a dispensa, organização e entrega da medicação e dos demais produtos de saúde para cada indivíduo específico. Para tal, cada utente apresenta uma ficha aberta no Sifarma 2000® de forma a poder consultar o histórico de consumo e organizar a medicação, sendo habitual a realização de uma venda a crédito. A realização frequente desta tarefa durante o estágio proporcionou um maior contacto com as diferentes embalagens, nomes comerciais e formas farmacêuticas, o que se revelou bastante útil no atendimento.

#### 3.1.4.2. Organização semanal da medicação

A preparação individualizada da medicação é um serviço farmacêutico que procura ajudar os utentes a gerir a sua medicação, e assim, assegurar a adesão e efetividade do tratamento, sendo os indivíduos com regimes farmacoterapêuticos complexos e polimedicados os que mais beneficiam desta intervenção farmacêutica. Tal serviço implica a organização da medicação ao longo dos principais momentos do dia, por norma em regime semanal, tendo por base o regime posológico instituído [3]. Ao longo do estágio tive a oportunidade de participar na organização da medicação em *pill-boxes*, sobretudo para idosos residentes em lares e utentes polimedicados, o que me proporcionou uma visão geral dos diferentes regimes farmacoterapêuticos instituídos bem como da sua complexidade.

#### 3.1.4.3. Determinação da pressão arterial e parâmetros bioquímicos

A determinação dos parâmetros bioquímicos e da pressão arterial, em ambiente devidamente reservado no gabinete ao utente, representa um momento de eleição para intervir junto da população e advertir para a necessidade de corrigir eventuais

comportamentos de risco. Durante o estágio pude constatar a importância deste serviço na comunidade, dado ser amplamente solicitado pelos utentes, e a sua inegável contribuição na monitorização da efetividade terapêutica, identificação de situações de risco e de descompensação de doenças crónicas. A realização desta tarefa permitiu-me aperfeiçoar as técnicas de manipulação dos aparelhos de medição, bem como aplicar os meus conhecimentos farmacoterapêuticos na interpretação dos valores obtidos e sugestão de medidas não farmacológicas mais personalizadas.

### 3.1.5. Contacto com o novo módulo atendimento do Sifarma®

Para otimizar a gestão dos *stocks*, a receção de encomendas e agilizar o processo de atendimento, a Farmácia Medeiros tem implementado o Sifarma 2000®, um programa informático muito intuito que simplifica as atividades diárias e ajuda a minimizar os erros de origem humana. Considero que esta ferramenta informática contribui significativamente para o correto funcionamento da farmácia, sendo extremamente benéfica para melhorar e potencializar o desempenho dos colaboradores da farmácia.

Durante o estágio, para além do Sifarma 2000®, pude contactar com o novo módulo de atendimento do Sifarma® nas vertentes do atendimento e receção das encomendas, uma ferramenta informática mais apelativa que surge no sentido de tornar o atendimento mais interativo, rápido e célebre. A utilização, em paralelo, dos dois programas permitiu-me constatar as diferenças entre ambos, que se refletem essencialmente no mecanismo de venda suspensa, que não existe no novo módulo de atendimento, e na possibilidade de introduzir ou retirar produtos na etapa final do atendimento e processar diferentes atendimentos simultaneamente, o que não é possível no Sifarma 2000®. Deste modo, reconheço como um aspeto bastante positivo do meu estágio a possibilidade de trabalhar com os dois programas, pois acredito que será vantajoso no meu futuro profissional.

## 3.2. PONTOS FRACOS

### 3.2.1. Períodos de menor afluência de utentes

Um dos aspetos menos positivos do estágio foi a existência de períodos de menor afluência e movimento de utentes o que, necessariamente, condicionou a observação e execução dos atendimentos. No entanto, estes períodos de ausência de utentes foram aproveitados para explorar as diferentes funcionalidades do programa Sifarma 2000®, analisar os fluxogramas de indicação farmacêutica disponibilizados pela Associação Nacional das Farmácias (ANF), elaborar as *Newsletters*, assistir a formações *online* disponibilizadas em plataformas digitais, organizar os diferentes produtos nos lineares da farmácia segundo o

princípio "*first expired, first out"* e para a partilha e consolidação de conhecimentos relativos aos diferentes produtos comercializados na farmácia.

### 3.2.2. Preparação de medicamentos manipulados

A manipulação de medicamentos constitui uma prática farmacêutica fundamental para assegurar a personalização e adequação da terapêutica ao perfil fisiopatológico específico de cada doente, bem como às suas características intrínsecas e preferências, procurando preencher as lacunas dos medicamentos industriais.

Na Farmácia Medeiros, a preparação de medicamentos manipulados não é uma prática muito frequente, dado que a reduzida procura pela população abrangida pela farmácia não justifica o investimento neste serviço. Neste sentido, a minha intervenção na preparação de medicamentos manipulados durante o estágio foi pouco expressiva, o que considero um ponto fraco. Ainda assim, tive a oportunidade de participar na manipulação de uma fórmula magistral (pomada a 10 % de ácido salicílico e 20 % de ureia), na posterior rotulagem e na definição do PVP tendo por base os fundamentos preconizados no Decreto-Lei n.º 95/2004, de 22 de abril.

## 3.3. OPORTUNIDADES

### 3.3.1. Diversidade de utentes

Como supramencionado, a Farmácia Medeiros dispõe de um número considerável de clientes regulares, no entanto, a sua localização estratégica e privilegiada permite que um variado leque de utentes com diferentes recursos económicos, de diferentes estatutos sociais, de diferentes nacionalidades e com graus de literacia em saúde muito díspares frequentem a farmácia. Perante esta realidade, durante o estágio tive a oportunidade de contactar com diferentes perfis de clientes, situação que se relevou extremamente benéfica para o meu crescimento e aprendizagem, na medida em que me permitiu adquirir a sensibilidade requerida para perceber o estado de espírito do utente e as suas necessidades, bem como a capacidade de adaptação necessária para lidar com situações muito diversas que extrapolam a minha zona de conforto. A capacidade de adaptação e flexibilidade aos diferentes contextos e realidades são pontos-chave para garantir o sucesso e a satisfação dos utentes, pelo que, enquanto estagiária na Farmácia Medeiros considero ter tido a oportunidade de desenvolver estas competências essenciais ao futuro exercício da profissão.

### 3.3.2. Formação contínua

A atualização permanente dos conhecimentos técnicos e científicos, através de formações externas ou internas à própria farmácia, é de primordial importância para assegurar

a excelência da atividade farmacêutica, à luz dos conhecimentos científicos mais recentes [1]. Durante o estágio tive oportunidade de participar em formações internas orientadas por colaboradores da farmácia e também em formações conduzidas externamente à farmácia, quer em contexto *online* quer pela visita de Delegados de informação médica.

Relativamente às formações externas que tive oportunidade de assistir durante o período de estágio saliento algumas formações da área da dermocosmética, mais concretamente da *Bioderma*, *Papillon* e do *Laboratoire Native* Portugal, que engloba as marcas *Phyto, Lierac, Jowaé* e *Roger&Gallet*, do Espaço Animal direcionadas a variadas temáticas relacionadas com a saúde animal, do laboratório *Boiron* relativo aos produtos homeopáticos e também formações sobre higiene íntima feminina, diarreia, obstipação, entre outras. Reconheço estes momentos formativos como uma mais-valia para aprofundar e reunir conhecimentos sobre diversas condições patológicas, bem como para conhecer as particularidades dos produtos por forma a aconselhá-los de forma mais apropriada.

### 3.3.3. Produtos de Veterinária e de Dermocosmética

Por estar localizada numa zona rural, onde a preocupação com a saúde animal é mais pronunciada, a procura de produtos de uso veterinário e o aconselhamento farmacêutico nesta matéria constituem práticas rotineiras da Farmácia Medeiros, traduzindo-se numa maior proximidade com os produtos de uso veterinário benéfica para enriquecer e expandir o conhecimento nesta área diferenciadora e com claros benefícios para a farmácia. Assim, durante o estágio, deparei-me com uma grande e diversificada gama de produtos veterinários, obrigando-me a renovar e manter atualizados os meus conhecimentos por forma a solucionar as necessidades dos utentes de forma célebre e eficaz.

A dermocosmética destaca-se igualmente como uma área de extremo relevo e distinção para a Farmácia Medeiros, com a qual tive um contacto muito próximo durante o estágio tendo, inclusive, presenciado a introdução de uma nova marca na farmácia, a *Bioderma*, e as subsequentes visitas dos Delegados e momentos formativos, que constituíram uma excelente oportunidade para a aquisição de novos conhecimentos que, seguramente, serão muito úteis no meu futuro profissional.

## 3.4. AMEAÇAS

### 3.4.1. Medicamentos esgotados

A existência de medicamentos esgotados exige por parte do farmacêutico uma capacidade de adaptação e de aplicação dos conhecimentos por forma a contornar esta situação que, muitas vezes, se traduz em descontentamento por parte dos utentes, através da

sugestão de medicamentos alternativos igualmente seguros e efetivos. No entanto, a ausência de alternativas terapêuticas ao medicamento esgotado constitui uma ameaça tanto para o farmacêutico como para os utentes, uma vez que a incapacidade de solucionar estas situações é encarada pelos utentes com uma certa indignação, podendo fragilizar a relação de confiança com os profissionais de saúde, bem como a sua ideia preconcebida da farmácia enquanto espaço prestador de cuidados de saúde de qualidade. Por outro lado, a escassez de certos medicamentos repercute-se também negativamente no utente, dado que, privá-lo da sua medicação habitual pode comprometer o seu bem-estar e o estado geral de saúde.

### 3.4.2. Alteração do preço e da embalagem dos medicamentos

A alteração do PVP do medicamento, apesar de depender de fatores externos à farmácia, suscita nos utentes um sentimento de desconfiança e descrença no farmacêutico, apesar das iniciativas em explicar a origem destas alterações, comprometendo a relação com os utentes e a credibilidade da atividade do farmacêutico. Ao longo do estágio deparei-me com algumas situações em que a discrepância do PVP do medicamento, em relação ao indicado na guia de tratamento das receitas desmaterializadas, que corresponde ao preço do medicamento genérico mais barato no mercado, se traduziu numa confusão por parte dos utentes difícil de esclarecer, ocasionando uma certa insegurança nos serviços prestados.

Da mesma forma, outro aspeto que pude constatar durante o estágio que cria alguma confusão junto dos utentes é a alteração das embalagens dos medicamentos, nomeadamente a cor e a sua dimensão, uma vez que a maioria dos utentes reconhecem a sua medicação habitual pela aparência exterior das embalagens.

### 3.4.3. Ilegibilidade das receitas manuais

As receitas manuais, embora cada vez menos frequentes no quotidiano da farmácia comunitária, podem ser usadas em casos excecionais e devidamente identificados, no entanto, esta modalidade de prescrição requer uma atenção especial por parte do farmacêutico de forma a garantir o cumprimento dos requisitos necessárias à validação da mesma [4]. Para além disso, a ilegibilidade da caligrafia do médico prescritor, algo com que me deparei durante o estágio, facilita a ocorrência de erros na identificação e dispensa dos medicamentos devido à dificuldade em interpretar o conteúdo das prescrições, nomeadamente a denominação comum internacional (DCI) ou nome comercial do medicamento, a dosagem e a forma farmacêutica. Assim, a dupla verificação das prescrições manuais revela-se uma prática imprescindível para minimizar a ocorrência de erros na identificação dos medicamentos e aumentar a segurança do atendimento. Inevitavelmente, esta situação condiciona a qualidade

dos serviços prestados dada a dificuldade em gerir o tempo de atendimento, o que se revela inconveniente para os utentes.

## 4. Considerações Finais

Com este estágio curricular na Farmácia Comunitária, uma das valências da profissão farmacêutica com maior destaque e visibilidade na comunidade, pude verificar a premissa de que o papel do farmacêutico na sociedade ultrapassa largamente a simples troca de medicamentos e outros produtos de saúde, sendo um dos principais intervenientes na divulgação de informação na área da saúde, visando estimular a adoção de estilos de vida mais saudáveis, bem como na promoção do uso seguro, eficaz e racional do medicamento.

A natureza prática do estágio proporciona aos estudantes a oportunidade de aplicar e transpor para a realidade do mercado de trabalho as competências e conhecimentos multidisciplinares adquiridos ao longo do percurso académico, permitindo, ao mesmo tempo, adquirir uma visão mais alargada da componente prática da profissão farmacêutica e da sua importância na sociedade, cujo foco principal incide no utente e nas suas necessidades, e não no medicamento propriamente dito.

Assim, considero que o estágio em Farmácia Comunitária foi uma etapa imprescindível e bastante enriquecedora do meu percurso académico, na medida em que me permitiu reunir um conjunto de ferramentas e desenvolver competências de relacionamento interpessoal, que certamente serão fundamentais para um exercício adequada da profissão e para uma comunicação assertiva e esclarecedora com os utentes.

## 5. Referências Bibliográficas


1. ORDEM DOS FARMACÊUTICOS - **Código Deontológico da Ordem dos Farmacêuticos**. [Consultado a 20 de abril de 2021]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/documentos/codigo_deontologico_da_of_4436676175988472c14020.pdf

2. ORDEM DOS FARMACÊUTICOS - **Boas Práticas Farmacêuticas para a farmácia comunitária**. [Consultado a 21 de abril de 2021]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/documentos/boas_praticas_farmaceuticas_para_a_farmacia_comunitaria_2009_20853220715ab14785a01e8.pdf

3. ORDEM DOS FARMACÊUTICOS - **Norma Geral da Preparação Individualizada da Medicação (PIM).** [Consultado a 25 de abril de 2021]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/documentos/norma_geral_de_preparacao_individualizada_de_medicacao_9446071805b0edc3c64d3f.pdf

4. INFARMED - **Normas relativas à dispensa de medicamentos e produtos de saúde**. [Consultado a 30 de abril de 2021]. Disponível em: https://www.infarmed.pt/documents/15786/17838/Normas_Dispensa/4c1aea02-a266-4176-b3ee-a2983bdfe 790

5. INFOMED - **Resumo das Caraterísticas do Medicamento - Pandermil 10mg/g Pomada**. [Consultado a 20 de abril de 2021]. Disponível em: https://extranet.infarmed.pt/INFOMED-fo/detalhes-medicamento.xhtml

6. ASSOCIAÇÃO NACIONAL DAS FARMÁCIAS - **Fluxograma de Indicação Farmacêutica para alívio dos sintomas associados à picada de inseto**. [Consultado a 20 de abril de 2021]. Disponível em: http://www.anfonline.pt

7. INFOMED - **Resumo das Caraterísticas do Medicamento - Vibrocil Actilong.** [Consultado a 30 de abril de 2021]. Disponível em: https://extranet.infarmed.pt/INFOMED-fo/detalhes-medicamento.xhtml

8. ASSOCIAÇÃO NACIONAL DAS FARMÁCIAS - **Fluxograma de Indicação Farmacêutica para alívio dos sintomas associados à Constipação.** [Consultado a 30 de abril de 2021]. Disponível em: http://www.anfonline.pt

## Anexos - Casos Práticos

**Caso Prático 1 -** Uma jovem de cerca de 20 anos dirigiu-se à farmácia com queixas de prurido e comichão intensa junto ao tornozelo. Quando questionada sobre a origem destes sintomas, a senhora referiu que tinha sido picada por um inseto nessa manhã.

Comecei por questionar se sabia qual o tipo de inseto que a tinha picado, se tinha antecedentes pessoais de alergia a picada de insetos, e se para além da comichão e prurido manifestava outros sintomas como dor, tonturas, dificuldade em respirar ou sensação de desmaio, ao que a utente respondeu negativamente.

Após observação atenta da zona da picada, verifiquei que a mesma se apresentava muito inflamada, com um inchaço e vermelhidão evidente. Como a pele à volta da picada se apresentava íntegra, sem sinais de infeção, lesão ou fissuras, recomendei o Pandermil®, uma pomada à base de hidrocortisona indicada para o alívio da inflamação associada à picada do inseto [5]. Aconselhei a aplicação tópica da pomada na zona da picada, 2 vezes ao dia, em pequenas quantidades e durante um período máximo de 7 dias, reforçando que, se após este período não se observarem melhorias deveria consultar o médico. Reforcei ainda a importância de fazer o desmame gradual da hidrocortisona após os 7 dias de tratamento, alternando a aplicação da pomada com o creme hidratante diário. Para o alívio do prurido e comichão associados à picada do inseto aconselhei a toma diária de 1 comprimido de cetrizina na dosagem de 10 mg, um anti-histamínico H1 de 2ª geração com menor incidência de efeitos anticolinérgicos como a sonolência.

Como medidas não farmacológicas, recomendei a aplicação de gelo ou água fria na zona da picada para aliviar a inflamação e o prurido, e alertei para a importância de evitar coçar a zona da picada para evitar lesões e manter a integridade da pele [6].

**Caso Prático 2 -** Um senhor de cerca de 60 anos dirigiu-se à farmácia com queixas de congestão nasal e dores de garganta que tiveram início no dia anterior.

Comecei por perguntar se para além dos sintomas reportados, o senhor tinha febre ou dores no corpo, ao qual respondeu que não. Perante o quadro sintomático manifestado pelo utente, e sabendo que não tinha diagnóstico de glaucoma, com o objetivo de melhorar a congestão nasal recomendei a aplicação tópica de Vibrocil Actilong®, que é constituído pelo cloridrato de xilometazolina, um vasoconstritor tópico. Aconselhei o utente a fazer 1 pulverização em cada narina, 3 vezes ao dia, durante um período máximo de 10 dias, reforçando que a utilização de descongestionantes nasais deve ser feita por curtos períodos de tempo devido ao elevado risco de dependência e efeito congestão *rebound* [7]. Para além

disto, recomendei ainda a lavagem nasal diária com Rhinomer® (água do mar isotónica) para hidratar e descongestionar as fossas nasais.

Relativamente à dor de garganta reportada pelo utente, comecei por questionar os sintomas que apresentava, isto é, se apenas sentia irritação na garganta, ou se tinha a garganta inflamada e dor ao deglutir. O senhor respondeu que tinha a garganta vermelha e inflamada acompanhado de dor ao deglutir. Desta forma, aconselhei a toma de uma pastilha de Mebocaína Anti-Inflam® a cada 2h a 3h, até um máximo de 6 pastilhas por dia, devido às suas ações antisséticas, analgésicas e anti-inflamatórias conferidas pelo álcool diclorobenzílico e cloridrato de benzidamina. Como medidas não farmacológicas, reforcei a importância do repouso, da ingestão abundante de líquidos, e evitar mudanças bruscas de temperatura [8].

**Caso Prático 3 -** Uma senhora deslocou-se à farmácia solicitando uma papa para o bebé de 6 meses, até à data exclusivamente alimentado de leite materno, pois estava a iniciar a diversificação alimentar por indicação do pediatria. Referiu ainda que o bebé sofre de muitas cólicas, e como já utilizou o Aero-OM® e não notou melhorias, solicitou algo que o aliviasse.

Comecei por referir que existem dois tipos de papas, as lácteas preparadas com água, dado que já têm leite na sua composição, e as não lácteas preparadas com leite, podendo, por isso, ser preparadas com o próprio leite materno.

De seguida, questionei se já tinha introduzido o glúten na dieta, ao qual a senhora respondeu que não. Dada a situação, reforcei a importância de introduzir o glúten após os 6 meses, mas antes dos 9 meses de idade, e de forma gradual para reduzir o risco de doença celíaca. Assim, na introdução do glúten, aconselhei misturar uma colher de papa sem glúten com uma de papa com glúten e, progressivamente, reduzir a quantidade da primeira e aumentar a segunda. Esclareci ainda que neste processo de diversificação alimentar, após substituir uma refeição de leite por uma de papa ou sopa, torna-se importante oferecer água entre as refeições, algo que até então não era necessário.

Relativamente às cólicas reportadas pela senhora, perguntei se eram recorrentes e se o bebé tinha patologias associadas, ao que a senhora respondeu negativamente. Assim, dada a inefetividade do Aero-OM®, aconselhei o Colimil® *baby* na posologia de 1 mL duas vezes ao dia, dado apresentar propriedades carminativas, digestivas e antiespasmódicas conferidas pela camomila e erva-cidreira, e ainda ajuda a reforçar a flora intestinal e melhorar o funcionamento intestinal devido à presença de probióticos (*Lactobacillus acidophillus*). Alertei ainda para a importância das massagens abdominais, de forma a diminuir o desconforto e a dor das cólicas, e de encurtar o tempo entre refeições para que o bebé não esteja tão ávido e não ingira tanto ar durante as refeições, o que pode estar na origem das cólicas reportadas.

# Parte II

Relatório de Estágio em Farmácia Hospitalar

**Centro Hospitalar e Universitário de Coimbra**

**Lista de Abreviaturas**

**CAUL:** Certificado de Autorização de Utilização de Lote

**CHUC:** Centro Hospitalar e Universitário de Coimbra

**DDD:** Dose Diária Definida

**FFUC:** Faculdade de Farmácia da Universidade de Coimbra

**HG:** Hospital Geral

**HP:** Hospital Pediátrico

**HSC:** Hospital Sobral Cid

**HUC:** Hospitais da Universidade de Coimbra

**MBB:** Maternidade Bissaya Barreto

**MDM:** Maternidade Daniel de Matos

**MICF:** Mestrado Integrado em Ciências Farmacêuticas

**OMS:** Organização Mundial de Saúde

**SFH:** Serviços de Farmácia Hospitalar

**SWOT** (*Strenghts*, *Weaknesses*, *Opportunities*, *Threats*)**:** Pontos Fortes, Pontos Fracos, Oportunidades e Ameaças

**UMIV:** Unidade de Misturas Intravenosas

**UPC:** Unidade de Preparação de Citotóxicos

## I. Introdução

Os Farmacêuticos Hospitalares, enquanto especialistas do medicamento integrados em equipas multidisciplinares, participam ativamente em todo o circuito do medicamento no hospital, desde a seleção e aquisição, aprovisionamento e armazenamento, validação da prescrição médica, distribuição e preparação, de forma a garantir a eficácia, segurança, qualidade e gestão correta dos medicamentos [1, 2]. Todas estas atividades desempenhadas por farmacêuticos em ambiente hospitalar são abrangidas pelos Serviços de Farmácia Hospitalar (SFH), cujo objetivo primordial é assegurar a acessibilidade e racionalidade no uso dos medicamentos e produtos farmacêuticos, e assim, garantir a segurança do doente.

Para além do estágio em Farmácia Comunitária, o plano curricular do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) oferece a oportunidade de realizar um estágio em Farmácia Hospitalar, de forma a providenciar aos estudantes as ferramentas necessárias ao exercício nas diferentes valências da atividade farmacêutica. Neste sentido, dado o meu interesse e a ausência de experiência prévia nesta área profissional, optei pelo estágio no Centro Hospitalar e Universitário de Coimbra, E.P.E. (CHUC), tendo este decorrido entre maio de 2021 e junho de 2021 sob a orientação da Doutora Marília João Rocha.

No relatório de estágio que apresento no formato de uma análise SWOT irei descrever não só os pontos fortes (*Strengths*) e os pontos fracos (*Weakness*) da minha experiência, que resultam da minha reflexão pessoal a nível interno deste período correspondente ao estágio curricular em Farmácia Hospitalar, mas também a nível externo as oportunidades (*Opportunities*) e as ameaças (*Threats*) serão mencionadas.

## 2. Centro Hospitalar e Universitário de Coimbra, E.P.E.

O Decreto-Lei n.º 30/2011, de 2 de março, determinou a criação do CHUC, E.P.E. através da fusão de três unidades hospitalares: Hospitais da Universidade de Coimbra (HUC), Centro Hospitalar de Coimbra e Centro Hospitalar Psiquiátrico de Coimbra, com o objetivo de assegurar a qualidade e segurança dos cuidados de saúde prestados à população [3].

O CHUC, E.P.E. é um estabelecimento de saúde pública, reconhecido a nível nacional e internacional, que atualmente integra os Hospitais da Universidade de Coimbra (HUC), a Maternidade Daniel de Matos (MDM), a Maternidade Bissaya Barreto (MBB), o Hospital Sobral Cid (HSC), o Hospital Pediátrico (HP) e o Hospital Geral (HG), apresentando, por isso, uma elevada complexidade organizacional [3]. A formação, o ensino, a investigação e a prestação de cuidados de saúde diferenciadores constituem as suas principais missões [3, 4].

# 3. Análise SWOT

## 3.1. PONTOS FORTES

### 3.1.1. Receção e integração na equipa

Enquanto estagiária no CHUC, considero que a receção calorosa pelos profissionais que compõem a equipa dos SFH, aspeto transversal a todos os setores que integrei durante o estágio, assim como o enquadramento geral e apresentação dos principais setores dos SFH numa reunião inicial com os coordenadores farmacêuticos de cada setor, foram um alicerce fundamental para a minha inclusão nas rotinas de trabalho e consciencialização das tarefas inerentes a cada setor. De uma forma geral, todos os profissionais com que colaborei durante o estágio contribuíram neste processo de aprendizagem e formação, mostrando-se sempre disponíveis para esclarecer qualquer questão que surgisse e transmitir os conhecimentos necessários à compreensão das funções executadas em cada setor.

### 3.1.2. Plano de estágio e caderno do estagiário

A organização e estruturação prévia do plano de estágio foram claramente um pilar orientador para uma melhor integração e adaptação à realidade prática da profissão farmacêutica no ambiente hospitalar, proporcionando um melhor aproveitamento e desempenho no estágio. No primeiro dia de estágio decorreu uma reunião com a Doutora Marília João Rocha, na qual foi estabelecido o plano de estágio e entregue um caderno de estagiário onde vinham descritos os objetivos e conhecimentos a adquirir em cada setor, o que considero ter sido fundamental para uma melhor compreensão das suas missões e das atividades a desenvolver numa fase posterior do estágio.

### 3.1.3. Rotatividade por diferentes setores

A possibilidade de experienciar e executar diferentes funções é, sem dúvida, uma condição essencial do estágio, permitindo uma melhor elucidação das tarefas desempenhadas na prática profissional. Neste sentido, o meu estágio curricular no CHUC foi organizado segundo um esquema rotativo, permitindo-me contactar com quatro setores dos SFH em cada semana do estágio, sendo estes a Distribuição, Gestão e Aprovisionamento, Ensaios Clínicos e Farmacotecnia, tendo, neste último, integrado as diferentes áreas funcionais que o compõem, nomeadamente, a unidade de preparação de medicamentos não estéreis, Unidade de Misturas Intravenosas (UMIV), Unidade de Preparação de Citotóxicos (UPC) e a Radiofarmácia. Reconheço esta rotatividade pelos diferentes setores dos SFH como uma mais-valia na minha formação, tendo-me proporcionado uma visão mais abrangente do papel do Farmacêutico Hospitalar em todas as etapas que integram o circuito do medicamento no hospital.

### 3.1.4. Medicamentos sujeitos a legislação especial

Os medicamentos sujeitos a legislação especial, nos quais são incluídos os hemoderivados, estupefacientes e psicotrópicos, estão associados a um controlo especial, apresentando, por isso, circuitos de distribuição específicos no hospital. Durante o período de estágio no setor da Distribuição pude contactar com estes circuitos e conhecer as suas particularidades, que se revelam essenciais para garantir a rastreabilidade dos medicamentos.

No que concerne aos hemoderivados, por serem medicamentos derivados do plasma humano, a sua utilização exige o preenchimento de um modelo de requisição, distribuição e administração (modelo n.º 1804) que é composto por duas vias: a Via Farmácia que é arquivada nos Serviços Farmacêuticos e a Via Serviço que, após devidamente preenchida pelo enfermeiro, é anexada ao processo clínico do doente [5]. Na Via Farmácia, os quadros A e B são preenchidos pelo médico prescritor e o quadro C pelo farmacêutico, sendo necessário o registo do nome do hemoderivado, da quantidade, do lote, do laboratório fornecedor, do número do Certificado de Autorização de Utilização de Lote (CAUL) emitido pelo INFARMED e do número de registo de distribuição. A Via Serviço possui ainda um quadro adicional (quadro D) que é preenchido pelo enfermeiro no momento da administração [5].

Os estupefacientes e psicotrópicos são também medicamentos sujeitos a legislação especial, sendo a dispensa da exclusiva responsabilidade do farmacêutico. Durante o período de estágio pude colaborar com o farmacêutico responsável na cedência de psicotrópicos e na revisão dos *stocks* nas enfermarias, permitindo-me familiarizar com este circuito e com as medidas de segurança implementadas para evitar a sua utilização ilícita, tal como o armazenamento devidamente segregado dos restantes medicamentos.

### 3.1.5. Dispensa de medicamentos em ambulatório

A cedência de medicamentos em ambulatório, quer no Edifício de São Jerónimo destinada essencialmente a doentes oncológicos quer no edifício central dos HUC, foi uma experiência bastante enriquecedora no culminar do meu percurso académico, na medida em que me permitiu contactar com diferentes realidades e vivenciar situações muito díspares.

No Edifício de São Jerónimo, a dispensa da medicação em ambulatório é dirigida aos doentes que frequentam o hospital de dia de oncologia, pelo que, a área de atuação do farmacêutico centra-se essencialmente na validação de prescrições oncológicas e na dispensa de citotóxicos e fármacos adjuvantes, tal como os antieméticos. No ambulatório dos HUC, a diversidade de utentes e de condições patológicas é consideravelmente superior, permitindo-me contactar com um variado leque de medicamentos de uso restrito hospitalar com indicações muito diversas. Outro aspeto diferenciador foi a interação farmacêutico-utente,

tendo verificado um atendimento mais individualizado e personalizado em gabinetes próprios no Edifício São Jerónimo, ao passo que nos HUC, a elevada afluência de utentes acaba por comprometer este contacto mais próximo com os utentes.

Concluo, assim, que experienciar diferentes realidades no âmbito da dispensa de medicação em ambulatório foi uma clara vantagem nesta etapa de formação, em que o *background* existente sobre as especificidades dos medicamentos de uso restrito hospitalar e as respetivas patologias é ainda superficial.

### 3.1.6. Radiofarmácia - área funcional da farmacotecnia

A Radiofarmácia, uma das áreas funcionais da farmacotecnia situada no serviço da Medicina Nuclear nos HUC, é responsável pela preparação, controlo de qualidade e dispensa de preparações radiofarmacêuticas, cujas aplicações na clínica se estendem ao diagnóstico e terapia de várias doenças [6].

Dada a superior duração de estágio na Radiofarmácia (perfazendo um total de 2 semanas), considero que, colaborar com os farmacêuticos na preparação e no controlo de qualidade de produtos radiofarmacêuticos foi fundamental para o desenvolvimento de novas competências, contribuindo para expandir e enriquecer o meu conhecimento nesta área. Durante o estágio pude observar a preparação de alguns radiofármacos de tecnécio, cujo princípio básico reside na marcação de um composto sem radioatividade, também conhecido de "*kit* frio", com um radionuclídeo de pertecnetato de sódio ($^{99m}$Tc). Entre eles, destaco o Myoview® usado no diagnóstico de isquémia miocárdica e/ou enfarte do miocárdio, o Osteocis® usado na cintigrafia do esqueleto para deteção de áreas de osteogénese alteradas e o Nanotop® usado na pesquisa do gânglio sentinela em melanoma maligno e cancro da mama.

## 3.2. PONTOS FRACOS

### 3.2.1. Reduzido período de estágio

Considerando a elevada complexidade e multiplicidade das tarefas desempenhadas por farmacêuticos nos SFH, a duração do estágio de dois meses em Farmácia Hospitalar torna-se um aspeto menos positivo, dado não ser uma dimensão temporal suficiente para assimilar e interiorizar todas as especificidades que delas decorrem.

Embora reconheça este estágio curricular como uma primeira experiência muito completa e diversificada, no sentido em que pude contactar com a realidade da Farmácia Hospitalar em diferentes áreas de intervenção do farmacêutico, o tempo de permanência em cada setor não foi suficiente para explorar todas as atividades a eles associadas. Por outro lado, o reduzido período de estágio não permitiu experienciar todas as atividades executadas

em âmbito hospitalar, como a reconciliação da terapêutica e a monitorização farmacocinética de fármacos, o que considero ser fulcral para uma melhor consolidação dos conhecimentos teóricos adquiridos durante o percurso académico. Assim, considero ser necessário mais tempo para explorar todas as tarefas realizadas nos setores dos SFH.

## 3.3. OPORTUNIDADES

### 3.3.1. Aquisição de novos conhecimentos

A natureza prática do estágio proporciona aos estudantes não só a oportunidade de aplicar na prática os conhecimentos teóricos adquiridos ao longo dos anos de formação, mas também adquirir novos conhecimentos, até então, pouco explorados em contexto académico.

Durante o período de estágio em Farmácia Hospitalar pude cooperar com os farmacêuticos na cedência de citotóxicos e de medicamentos de uso restrito hospitalar em ambulatório, tal como são exemplo os medicamentos biológicos (anticorpos monoclonais), fármacos antivirais, imunossupressores e fármacos para a esclerose múltipla e artrite reumatóide, bem como no circuito de preparação de citotóxicos no UPC, que contempla uma etapa inicial de validação farmacêutica da prescrição médica, seguida da individualização, preparação e libertação do lote. No setor dos Ensaios Clínicos tive oportunidade de participar na cedência da medicação experimental e assistir a uma reunião de início do ensaio, permitindo-me conhecer, mais detalhadamente, os procedimentos internos deste setor.

Como já mencionado em 3.1.6., a minha passagem pela área da Radiofarmácia constituiu também uma importante fonte de novos conhecimentos, proporcionando uma boa oportunidade de complementar a formação académica.

### 3.3.2. Realização de um trabalho

Durante o estágio foi-me proposto analisar o padrão de consumo dos antibióticos no CHUC durante o período de 2018 a 2020, com o objetivo de identificar as classes de antibióticos mais consumidas. Para o efeito, tive necessidade de recorrer ao Microsoft Excel® e a uma plataforma informática desenvolvida pela Organização Mundial de Saúde (OMS), designada de *Antimicrobial Consumption Tool* (AMC *Tool*), para a gestão dos dados de consumo e para o cálculo da Dose Diária Definida (DDD), respetivamente, tendo sido os resultados apresentados tanto em DDD/100 camas/dia como em DDD/1000 habitantes/dia.

A realização desta tarefa durante o estágio traduziu-se numa excelente oportunidade para aprofundar e desenvolver novas competências a nível informático, permitindo a minha familiarização com o programa AMC *Tool*, com o qual ainda não tinha tido contacto prévio,

mas também para compreender melhor as tendências de consumo de antibióticos a nível hospitalar e as flutuações ocorridas ao longo dos últimos anos.

## 3.4. AMEAÇAS

### 3.4.1. Plano de estudos do MICF

Durante o estágio curricular em Farmácia Hospitalar pude constatar a importância e a aplicabilidade prática dos conteúdos abordados em algumas unidades curriculares do plano de estudos do MICF, sendo as unidades de Farmácia Hospitalar, Farmácia Clínica, Farmacologia e Virologia as mais direcionadas para esta saída profissional. De facto, a abordagem teórica dos diferentes setores dos SFH apresentada na unidade de Farmácia Hospitalar foi uma base fundamental para uma melhor integração no estágio. Por outro lado, os conteúdos abordados nas unidades de Farmácia Clínica e Farmacologia no âmbito da terapia oncológica e na unidade de Virologia relativos aos fármacos antivirais revelaram-se imprescindíveis durante o estágio.

Não obstante, considero que o plano de estudos do MICF, apesar de ser bastante abrangente e diversificado, ainda é pouco direcionado para esta possível área de intervenção do farmacêutico, e atendendo à sua especificidade e complexidade, considero que seria muito benéfico receber formação mais aprofundada neste âmbito durante o percurso académico.

## 4. Considerações Finais

O estágio curricular nos serviços farmacêuticos do CHUC foi, sem dúvida, uma etapa extremamente benéfica e enriquecedora do meu percurso académico, que possibilitou a concretização de um objetivo pessoal: conhecer, de uma forma geral, o quotidiano do farmacêutico hospitalar nos diferentes setores dos SFH.

Concluída esta primeira experiência de estágio neste ramo da profissão farmacêutica, considero ter adquirido uma visão geral e mais prática da dinâmica de funcionamento e das diferentes atividades executadas em cada setor dos SFH, bem como da importância do farmacêutico hospitalar enquanto elemento da equipa clínica. De facto, a rotatividade por diferentes setores e a possibilidade de participar na execução de tarefas muito diversificadas permitiu-me alargar e expandir o meu conhecimento, contribuindo para a aquisição de novas competências e para o meu crescimento profissional.

Concluo, portanto, que a possibilidade de estágio na vertente da Farmácia Hospitalar oferecida pelo MICF constitui uma oportunidade única de aquisição de novos conhecimentos, permitindo conhecer melhor uma das realidades da profissão farmacêutica cujo foco durante o percurso académico é menos substancial.

## 5. Referências Bibliográficas


1. BROU, M. H. L., FEIO, J. A. L., MESQUITA, E., RIBEIRO, R. M. P. F., BRITO, M. C. M., CRAVO, C., PINHEIRO, E. - **Manual da Farmácia Hospitalar.** Conselho Executivo da Farmácia Hospitalar. [Consultado a 19 de junho de 2021]. Disponível em: https://www.infarmed.pt/documents/15786/17838/manual.pdf/

2. ORDEM DOS FARMACÊUTICOS - **Manual de Boas Práticas de Farmácia Hospitalar.** [Consultado a 19 de junho de 2021]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/publicacoes/mbpfh_capitulo_i_vfinal_17815111995a8eee5ad0c17.pdf

3. CONSELHO DE ADMINISTRAÇÃO DO CHUC, E.P.E. - **Regulamento Interno do Centro Hospitalar e Universitário de Coimbra, E.P.E.** (2020). [Consultado a 21 de junho de 2021]. Disponível em: https://www.chuc.min-saude.pt/media/Regulamento_Interno/Regulamento_Interno_CHUC_-_Homologado_SES_2020.pdf

4. CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA, E.P.E. - Missão, Visão e Valores. [Consultado a 21 de junho de 2021]. Disponível em: https://www.chuc.min-saude.pt/paginas/centro-hospitalar/missao-visao-e-valores.php

5. INFARMED - Despacho conjunto n.º 1051/2000, de 14 de setembro. **Registo de medicamentos derivados de plasma.** [Consultado a 25 de junho de 2021]. Disponível em: https://www.infarmed.pt/documents/15786/1068535/despacho_1051-2000.pdf

6. OLIVEIRA, R., SANTOS, D., FERREIRA, D., COELHO, P., VEIGA, F. - **Preparações radiofarmacêuticas e suas aplicações**. Revista Brasileira de Ciências Farmacêuticas. Vol. 42, n.º 2 (2006), p. 151-165. [Consultado a 30 de junho de 2021].

# Parte III

Monografia

**“Macrophage Membrane-Coated Nanosystems for Biomedical Applications”**

**Resumo**

A nanotecnologia apresenta-se como uma abordagem promissora para aprimorar a libertação vetorizada de fármacos e de agentes de diagnóstico aos locais desejados, aumentando assim a sua acumulação em tecidos específicos, e reduzindo os efeitos adversos em tecidos não-alvo. Todavia, apesar das inúmeras vantagens das nanopartículas (NPs) em relação às abordagens de diagnóstico e às terapias convencionais, ainda subsistem alguns desafios que dificultam a sua aplicação clínica, nomeadamente a reduzida biocompatibilidade e a rápida eliminação da circulação sanguínea.

Para contornar estas limitações e melhorar as propriedades de "*biointerface"* dos nanomateriais, uma tecnologia biomimética, que utiliza membranas celulares naturais para revestir NPs através de abordagens *"top-down",* tem sido proposta. Esta tecnologia, baseada no revestimento de NPs com membranas celulares, é inspirada nas interações intercelulares que ocorrem de forma natural para orientar eficazmente as NPs aos locais desejados, melhorando, assim, os objetivos de eficácia terapêutica e de segurança. Além disso, a biocompatibilidade intrínseca da célula também tem despertado muito interesse, nomeadamente no que diz respeito ao aumento da capacidade de atravessar as barreiras biológicas, e ao facto de evitar a eliminação precoce pelo sistema imunológico, resultando num período de tempo de circulação sanguínea superior, e numa menor toxicidade *in vivo*. Até à data, um vasto leque de membranas celulares tem sido investigado com o objetivo de reproduzir as funções biológicas e a composição superficial complexa das células a partir das quais a membrana é isolada.

Considerando as propriedades singulares das células do sistema imunitário, que derivam dos componentes proteicos presentes na membrana, o revestimento das NPs com uma camada de uma membrana celular derivada de células imunitárias visa dotá-las de propriedades "furtivas" (do inglês, "*stealth*"), aumento da biocompatibilidade, e capacidades de vetorização ativa. Em particular, os macrófagos, como células fagocitárias cruciais do sistema imunitário inato e defensores naturais contra agentes invasores, respondem a sinais inflamatórios que induzem a sua migração para locais patológicos, exibindo assim um tropismo natural para tecidos inflamatórios e tumorais. Desta forma, os nanossistemas revestidos com uma membrana de macrófagos são concebidos de forma a combinar os benefícios dos macrófagos e das NPs, bem como a reforçar a capacidade de alcançar os tecidos alvo. Estudos recentes têm demonstrado o potencial destes nanossistemas biomiméticos a nível da libertação vetorizada de fármacos e de agentes de imagem em tumores, locais infeciosos e

inflamatórios, revelando resultados encorajadores para aplicações de diagnóstico, terapia, e teranóstico, num amplo espetro de doenças.

Neste trabalho de revisão, os procedimentos detalhados de preparação, a caracterização, e a aplicabilidade biomédica das nanopartículas revestidas com membrana de macrófagos são discutidas. Adicionalmente, são também mencionados os desafios para a implementação clínica, e, por fim, as perspetivas futuras desta nanotecnologia emergente.

**Palavras-chave:** "*biointerface*", célula do sistema imunitário, macrófago, nanossistema revestido por membrana de macrófago, nanotecnologia biomimética, revestimento com membrana celular.

**Abstract**

Nanotechnology has emerged as a promising approach to improve the targeted delivery of drugs and diagnostic agents to desired tissues, thereby increasing site-specific accumulation and reducing adverse effects at non-target sites. However, despite the many advantages of nanoparticles (NPs) over the conventional therapeutic and diagnostic approaches, some challenges hindering their clinical application, such as poor biocompatibility and rapid elimination from blood circulation, still need to be addressed.

To circumvent these limitations and increase the biointerfacing properties of nanomaterials, a biomimetic technology using natural cell membranes to camouflage NPs *via* top-down approaches has been proposed. This cell membrane-cloaking technology takes inspiration from the naturally occurring intercellular interactions to efficiently guide NPs to desired locations, thereby increasing both therapeutic efficacy and safety goals. In addition, the cell's intrinsic biocompatibility has also aroused great interest to increase the ability to cross biological barriers and avoid elimination by the immune system, resulting in enhanced blood circulation time and lower toxicity *in vivo*. To date, a large variety of cell-derived membranes have been investigated, with the aim of mimicking the biological functions and the complex surface composition of the cells from which the membrane is isolated.

In line with the distinctive features of immune cells, which come from their protein components in the outer membrane, wrapping NPs with a layer of an immune cell-derived membrane aims to endow NPs with stealth properties, enhanced biocompatibility and active targeting abilities. In particular, macrophages, as key phagocytic cells of the innate immune system and natural defenders against invaders, respond to inflammatory signals that elicit their migration to pathological sites, thereby exhibiting a natural tropism for inflammatory/tumor tissues. Hence, macrophage membrane-coated nanosystems are designed to combine the advantages of both macrophages and NPs and reinforce the ability to reach target sites. Recent studies have demonstrated the potential of these biomimetic nanosystems for targeted delivery of drugs and imaging agents to tumors, infectious and inflammatory sites, revealing encouraging results for diagnosis, therapy, and theranostics, in a broad spectrum of diseases.

In this review, the detailed preparation procedures, the characterization, and the biomedical applicability of these bioinspired macrophage membrane-coated nanosystems are discussed. In addition, challenges for clinical implementation and future perspectives of this emergent nanotechnology are also mentioned.

**Keywords:** biointerfacing, immune cell, macrophage, macrophage membrane-coated nanosystem, biomimetic nanotechnology, cell membrane coating.

**Abbreviations**

**AD:** Alzheimer's disease

**AgNC:** Silver nanocluster

**APC:** Antigen-presenting cell

**Arg1:** Arginase 1

**AuNS**: Gold nanoshell

**Aβ:** Beta-amyloid

**BBB:** Blood-brain barrier

**CCL2:** CC-chemokine ligand 2

**CCR2:** CC-chemokine receptor 2

**Ce6:** Chlorin e6

**COVID-2019:** Coronavirus disease 2019

**CTC:** Circulating tumor cell

**CuS:** Copper sulfide

**DLA:** Dalton Lymphoma Ascites

**DLS:** Dynamic light scattering

**DOX:** Doxorubicin

**DXM:** Dexamethasone

**EPR:** Enhanced permeability and retention

**EV:** Extracellular vesicle

**$Fe_3O_4$ NP:** Iron oxide nanoparticle

**GS:** Genistein

**GSNC:** Gold-silver nanocage

**ICAM-1:** Intercellular adhesion molecule 1

**ICD:** Immunogenic cell death

**IDO1:** Indoleamine 2,3-dioxygenase 1

**IND:** Indoximod

**iNOS:** Inducible nitric oxide synthase

**JMSNM:** Janus mesoporous silica nanomotor

**LFA-1:** Lymphocyte function-associated antigen 1

**LPS:** Bacterial lipopolysaccharide

**Mac-1:** Macrophage-1 antigen

**MCM:** Macrophage cell membrane

**MHC:** Major histocompatibility complex

**MNC:** Magnetic nanocluster

**MPS:** Mononuclear phagocytic system

**MRI:** Magnetic resonance imaging

**NIR:** Near-infrared

**NP:** Nanoparticle

**PDT:** Photodynamic therapy

**PEG:** Polyethylene glycol

**PLGA:** Poly (lactic-*co*-glycolic acid)

**PLNP:** Persistent luminescence nanoparticle

**PSGL-1:** Glycoprotein P-selectin ligand 1

**PTT:** Photothermal therapy

**PTX:** Paclitaxel

**QD:** Quaternary quantum dot

**RBC:** Red blood cell

**ROS:** Reactive oxygen species

**SDS-PAGE:** Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

**SEM:** Scanning electron microscopy

**SIRPα:** Signal-regulatory protein alpha

**SLN:** Solid lipid nanoparticle

**TAM:** Tumor-associated macrophage

**TEM:** Transmission electron microscopy

**TLR:** Toll-like receptor

**TME:** Tumor microenvironment

**UCNP:** Upconverting nanoparticle

**UV:** Ultraviolet

**VCAM-1:** Vascular cell adhesion protein 1

**VEGF:** Vascular endothelial growth factor

**WBC:** White blood cell

## I. Introduction

The emergence of nanoparticle (NP)-based delivery systems marked an important turning point in the diagnosis, prevention and treatment of many diseases, as they can protect the payload from degradation and premature leakage, provide a controlled drug release, alleviate the systemic toxicity of conventional drugs, encapsulate multiple compounds, enable large-scale production and can be rationally modified in terms of size, surface charge or surface ligands to enhance drug delivery to target sites [1-3].

Although nanotechnology has provided significant improvements in both efficacy and safety compared to conventional modalities, NPs are likely to be recognized and eliminated by the mononuclear phagocytic system (MPS) before they can fulfil their delivery task due to their exogenous nature and poor biocompatibility, resulting in lower circulation time [1, 4, 5]. In addition, their inability to overcome biological barriers may contribute for decreasing the delivery efficacy of therapeutic and diagnostic compounds to sites of interest, thus compromising the successful clinical application of NP-based nanomedicines [6, 7].

Being able to remain unnoticed by the MPS and interact with the complex biological environments and surrounding cells has been pointed out as a crucial prerequisite towards effective clinical translation *in vivo* [8]. Therefore, designing NPs with active targeting capabilities and high affinity to target cells has gained increasing attention, which is achieved by functionalizing the NPs surface with specific targeting ligands, such as antibodies, peptides, aptamers and other small molecules, capable of interacting with receptors overexpressed in pathological sites [5, 8, 9]. To enhance blood circulation time, the modification of the NPs surface with the polymer polyethylene glycol (PEG) remains a standard practice, as PEGylation can confer stealth properties and reduce the immune-mediated clearance [10, 11]. However, the immunogenicity of the artificial polymer and the complexity of bottom-up ligand incorporation approaches remain a concern, which highlights the need for novel surface modification approaches to enhance the performance of delivery nanoplatforms both by increasing their ability to actively target the desired sites and reducing NPs uptake by the immune system [8, 11, 12].

In this regard, there has been a paradigm change in the design of NPs, employing bioinspired principles to produce biocompatible and long-circulation cell-based delivery nanosystems capable of mimicking the biological features of source cells while reproducing the advantageous physicochemical properties of NPs [7, 13]. Although the use of whole cells as carriers has also been studied, current research in this field is primarily focused on cell membrane coatings for the surface functionalization of NP cores [4, 6, 14]. This top-down

approach consists of wrapping a NP inner core with a thin layer of a natural cell membrane, which preserves the intact proteolipid composition and the complex set of surface proteins essential for effective biointerfacing, thus endowing NPs with the desirable biofunctions of the parent cells and the ability to reproduce the cell behaviours [6, 8, 15].

So far, a diversified range of cell membranes have been employed in biomimetic coatings. For instance, red blood cell (RBC) membrane coatings have pioneered this biomimetic technology, and since then, a wide diversity of cell membranes have been studied, including those derived from platelets, white blood cells (WBCs), stem cells, bacteria, cancer cells and others, with each particular cell type conferring unique and specialized biofunctionalities that are related to their intrinsic properties [8, 16].

WBCs, also referred to as leukocytes, are important components of the immune system and are categorized in two major subsets: granulocytes and agranulocytes. Granulocytes, which are distinguished by the presence of cytoplasmatic granules, comprise neutrophils, eosinophils and basophiles, whereas agranulocytes include lymphocytes and monocytes [6, 17]. The growing interest on WBCs for delivery purposes comes from their unique ability to be recruited and guided by chemoattractant gradients, bind and cross the vascular wall and enter sites of inflammation, which has been associated with cancer development and progression, suggesting their promising use as carriers of drugs and imaging agents in inflammation-associated disorders [6, 18].

Amongst WBCs, monocyte-derived macrophages have been widely studied due to their important role in regulating both the innate and adaptive immunity through clearance of tumor cells and other foreign particles (e.g. microorganisms) by phagocytosis and antigen presentation [19, 20]. Furthermore, macrophages have an inherent ability to escape immune clearance and target inflamed/tumor and infectious tissues *via* chemotactic signals, which can be harnessed for delivery purposes. Besides, the well-known ability of macrophages to target and bind tumor cells *via* cell-cell interaction, makes them very attractive for improving cancer therapeutic and diagnostic outcomes, while reducing unwanted systemic toxicity [4, 21, 22]. Therefore, on account of these multifunctional roles, the biomimetic approach of cloaking macrophage cell membranes (MCMs) onto the NPs surface has been applied in several biomedical applications, from cancer to treatment of inflammatory and infectious diseases, indicating its widespread use for a wide range of diseases.

This review summarizes the latest advances and original research conducted in recent years in the field of immune cell membrane-coating nanotechnology, especially regarding to MCM-coated nanosystems for drug delivery and other targeted applications, such as imaging,

phototherapy, immunotherapy, detoxification and vaccination. First, the genesis, phenotypic diversity, heterogeneous functions, and surface markers of macrophages will be discussed in detail. Then, particularly attention will be paid to the preparation methods, characterization, and biomedical applications of these next-generation systems. Lastly, the futures prospects and main challenges for the successful implementation in the biomedical field will be addressed.

## 2. Macrophages as key immune mediators

Macrophages are mononuclear phagocytes, a type of WBC derived from blood circulating monocytes, that are found in tissues throughout the body, playing a key role in immune surveillance [6, 23]. As major immunomodulatory cells, they perform a central role to maintain homeostasis and protect the human body by regulating the innate and adaptive immunity. Nevertheless, beyond their crucial protective role, certain macrophage phenotypes are believed to be involved in the pathogenesis of several diseases by suppressing inflammatory responses and inducing tissue repair, meaning that macrophages are a unique cell type with decisive effects in both disease and health [24, 25].

### 2.1. Surface and physiology

#### 2.1.1. Genesis of macrophages

Myeloid cells are derived from progenitor myeloid cells residing in bone marrow and encompass, among others, monocytes, macrophages, granulocytes and dendritic cells [18]. Tissue macrophages are phagocytic immune cells belonging to the MPS, that can be either established before birth and self-sustained over time independently of monocytes or derived from circulating monocytes [2, 18]. After birth, continuous replenishment of tissue macrophages is imperative to maintain homeostasis and depends on monocytes´ production from hematopoietic stem cells (HSCs) present in the bone marrow and their subsequent migration towards injured sites, where they undergo various modifications to become a dendritic cell or tissue macrophage [18, 26, 27]. The migration of monocytes and macrophages towards inflamed/ tumor tissues is mediated by several chemoattractant gradients released by tumor cells within the tumor microenvironment (TME), such as CC-chemokine ligand 2 (CCL2), CC-chemokine ligand 5 (CCL5) and colony stimulating factor-1 (CSF-1) [5, 7] Therefore, since macrophages are naturally recruited to sites of inflammation, which is a major hallmark of neoplastic diseases, it can be assumed that these immune cells can also be attracted to tumor tissues *via* tumor-derived inflammatory mediators [4, 6, 19].

### 2.1.2. Phenotypic diversity of macrophages

The immune cells derived from the myeloid lineage are highly flexible and plastic and can switch and adopt different phenotypes according to the environmental stimuli [18]. Indeed, heterogeneous populations of macrophages are found in the TME with distinct effects on tumor development and progression.

Tumor-associated macrophages (TAMs), which are macrophages that have migrated to tumor tissues, can be polarized into two contrary phenotypes through classical or alternative pathway according to specific signals and stimuli in the TME. These phenotypes differ from each other in their surface receptors, cytokines and chemokines production and inflammatory functions, which allows their classification into classically activated or M1 macrophages and alternatively activated or M2 macrophages [19, 20, 23]. TAMs play a central role in modulating cancer immunity and tumor development, since M1 macrophages phagocytize and kill tumor cells leading to tumor suppression, whereas M2 macrophages support tumor growth, progression and metastasis [23, 28].

In the initial stages of tumor development, bacterial lipopolysaccharide (LPS, also known as endotoxin) and pro-inflammatory cytokines, such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF-α), instruct non-polarized macrophages (M0) to adopt the M1 phenotype, which is characterized for pro-inflammatory properties, enhanced phagocytic activities and antigen presentation abilities to antitumor T cells, playing a key role in activating adaptive immunity for stronger anticancer immune responses [20, 23, 29]. M1 macrophages secrete several pro-inflammatory cytokines, including TNF-α, IL-6, IL-12 and IL-1β, chemokines like CXCL-10, reactive oxygen species (ROS) and oxide nitric (NO) *via* inducible nitric oxide synthase (iNOS) pathway, which promote tissue damage and tumor suppression. The main markers of M1 macrophages involve Toll-like receptors (TLR), such as TLR 4 capable of binding to LPS, the co-stimulatory molecules CD 80 and CD 86, iNOS and major histocompatibility complex II (MHC-II), as depicted in Figure 1 [24, 30].

On the contrary, in advanced stages of the tumor, anti-inflammatory factors released in the TME, such as interleukin 10 (IL-10), interleukin 13 (IL-13) and interleukin 14 (IL-14), instruct macrophages to embrace the M2 phenotype, which has pro-tumor activities, anti-inflammatory properties and is directly involved in fostering an immunosuppressive TME, enabling tumor cells to avoid elimination by the immune system, growth and spread to establish a metastatic focus [6, 23, 29]. 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. As

shown in Figure 1, the main markers of M2 macrophages involve mannose receptor (CD 206), scavenger receptor (CD 163), arginase I (Arg 1) and CD 200 R [24, 30].

There is strong evidence that TAMs are usually reprogramed to the pro-tumorigenic M2 phenotype, indicating that macrophage recruitment to tumor sites is related to a worse prognostic in most cancers [27]. Given the pivotal role performed by TAMs in cancer immunity, exploiting the dichotomy in M1/M2 macrophages functions to modulate the immunosuppressive TME has received increasing attention. In this regard, the depletion of TAMS or the inhibition of macrophage recruitment to tumor sites may attenuate the tumor immunosuppressive response by decreasing the number of TAMs, and thus, preventing tumor progression [20, 23, 28]. Another promising strategy in cancer immunotherapy is to reprogram TAMs in the M1 phenotype, exploiting the antitumor properties of M1 macrophages to inhibit tumor progression and metastasis. This current therapy has the potential to reinforce the antitumor activity of macrophages within the TME by converting the immunosuppressive TME in the pro-inflammatory TME and, by doing so, contribute to robust the immune responses against cancer [15, 23].

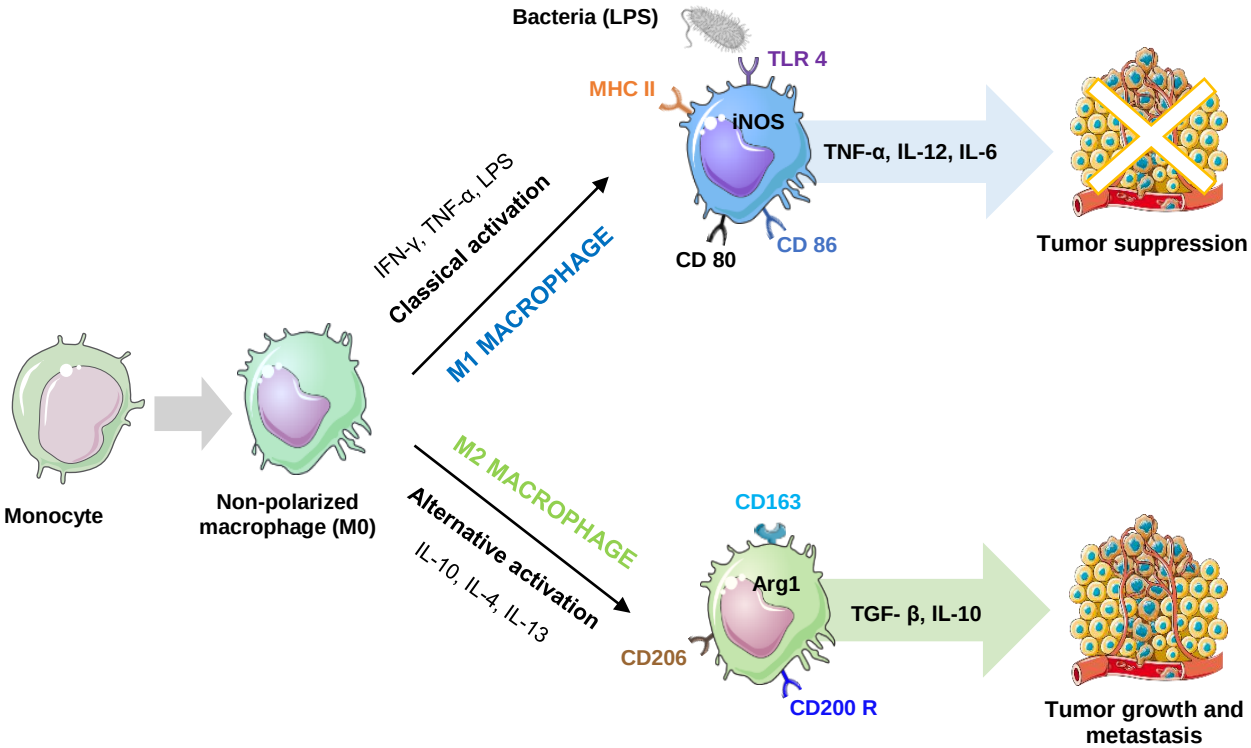


**Figure 1 -** Schematic representation of M0 macrophage polarization pathways in response to specific mediators and respective markers for each phenotype. M1 macrophages are involved in secreting inflammatory factors that kill tumor cells and are activated through a classical pathway. M2 macrophages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

## 2.2. Cell-cell communication and physiological / pathophysiological processes

Macrophages are key sentinel cells of the innate immune system by virtue of their unique ability to identify, engulf and phagocytize tumor cells and microorganisms, which comes from their crucial membrane markers capable of distinguishing foreign particles from "their own" [19, 31]. In addition, these surface markers also play a key role in modulating the communication of macrophages with their neighboring environments and other cells, such as tumor cells occupying the TME, by activating or suppressing specific signals [6, 13].

As previously mentioned, the ability to avoid MPS clearance is a major feature of WBCs, which is conferred by the "self-marker" CD47 and leucocyte common antigen (CD45) [32, 33]. Besides, macrophages have a natural tropism for inflammatory/tumor sites due to the presence of specific proteins on their membrane surface, like cell adhesion molecules (integrins and selectins) and chemokine receptors, capable of binding to specific adhesion molecules and inflammatory chemokines highly expressed in the inflamed endothelium to initiate transendothelial migration, in a phenomenon called diapedesis [6, 19, 33]. Growing evidence has revealed that macrophages, in addition to their inflammation-targeting capabilities, can also actively target metastatic cancer cells and penetrate the blood-brain barrier (BBB) *via* precise membrane receptors-ligands interactions, making them very attractive and versatile carriers for delivery purposes [8, 23, 34]. A summary of the main surface markers involved in these multiple roles of macrophages is presented in Table 1.

As crucial components of the TME, macrophages mediate complex interactions with tumor cells that can dictate tumor progression and cancer immunity [7]. Macrophages express on their membrane surface the signal-regulatory protein alpha (SIRPα) that bind specifically to "self-marker" CD47, a transmembrane protein expressed in all healthy cells and some cancer cells [20, 35]. The SIRPα /CD47 interaction produces a "don't eat me" signal that not only hinders the phagocytosis of healthy cells by macrophages, but also prevents the immune clearance of CD47-overexpressing cancer cells, promoting an immunosuppressive environment that contributes favorably to tumor growth and poor prognosis of several solid tumors [20]. Therefore, blocking this intercellular interaction can be a powerful strategy in cancer immunotherapy to increase the phagocytic activity of macrophages, and then, to enhance the antitumor activity of TAMs [23, 28].

**Table 1 -** Overview of the main macrophage membrane markers and their functions.

| Classification of the membrane marker | Macrophage membrane marker | Counter-receptor/ Ligand | Function | Ref. |
|---|---|---|---|---|
| Cell adhesion molecule - selectins | L - selectin, PSGL-1 | E-selectin, P-selectin (on endothelium) | Cell-cell adhesion (firm adhesion to endothelium) | [17-19] |
| 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 towards inflamed and tumor tissues | [6, 13, 17] |
| Chemokine receptor | CCR2 | CCL2 | Strong chemotaxis that elicits macrophage migration to tumor and inflammatory sites | [13, 23] |
| 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 | [23] |

**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; VCAM-1, vascular cell adhesion protein 1; VLA-4, very late antigen 4.

## 2.3. Macrophages as nanocarriers for diagnostics and therapeutics

In the last few years there has been a growing interest in using macrophages for diagnostic and therapeutic purposes. Specifically, in the context of cancer, leveraging TAMs as targets for cancer therapy and biomarkers for cancer diagnosis and prognosis has become very appealing [36]. In one example of macrophages used for early detection of cancer, Aalipour *et al.* synthesized a highly sensitive macrophage-based *in vivo* sensor, exploiting the repolarization of macrophages in the pro-tumorigenic M2 phenotype to detect the presence of 4T1 breast tumors smaller than 50 $mm^3$ [37]. Assuming that tumor-penetrating M2 macrophages (TAMs) overexpress specific markers involved in promoting an immunosuppressive TME, such as Arg 1, Ym 1, Mrc 1 and Fizz 1, macrophages were genetically modified to secrete an artificial bioluminescent reporter upon activation of the Arg 1 promoter, which could be detected by bioluminescent imaging and blood measurement of the secreted reporter. The main improvement of this biocompatible sensor is the enhanced tropism for tumor sites, since macrophages have an innate aptitude to be recruited to tumor

locations, helping to overcome the poor sensitivity and specificity of the conventional cancer diagnostic techniques [37].

For therapeutic applications, more and more attention has been given to M1 macrophages as drug carriers because of their biocompatibility, their antigen presentation activities, their ability to phagocytize malignant cells, and their specificity for neoplastic tissues and inflammation sites [6, 18]. Indeed, the potential of these phagocytic cells for targeted delivery is multifaceted, as they can target both primary tumors and metastatic cancers and can infiltrate deep inside hypoxic regions of tumors, providing an excellent opportunity to achieve these poorly perfused areas usually related with resistance to radiotherapy and chemotherapy [2, 7]. Supporting this, traditional NPs can only gain limited access to deep tumor tissue through the enhanced permeability and retention (EPR) effect, due to the elevated interstitial pressure and poor tumor vasculature, while macrophages can still infiltrate in these regions, even with raised interstitial pressure, highlighting their potential to successfully deliver anticancer drugs to deep neoplastic tissue [7, 18].

Similar to other drug delivery vehicles, macrophage-based delivery systems can protect their therapeutic payload from the recognition and clearance by the MPS and refine the pharmacokinetic properties of loaded drugs since both hepatic and renal excretion are limited, resulting in longer circulation time *in vivo* [7, 18]. As outlined in Figure 2, several promising macrophage-based systems have currently been used as carriers for targeted delivery of therapeutic agents to the TME and sites of inflammation, thereby increasing the therapeutic efficacy and preventing off-target toxicity. These include: (1) live macrophages as drug delivery vehicles; (2) macrophage-derived extracellular vesicles (EVs) as drug delivery vehicles; (3) macrophage-like proteolipid nanovesicles as drug delivery vehicles, and (4) macrophage-derived membranes as drug delivery vehicles [22, 23].

### 2.3.1. Macrophages as drug delivery vehicles

In the first strategy, the intrinsic phagocytic activity of M1 macrophages is exploited to build *ex vivo* delivery platforms by incubating live macrophages with drugs or drug-loaded NPs [7]. Since macrophages can naturally phagocytize foreign materials, they can engulf drugs or nanosized systems and deliver them precisely to sites where macrophages tend to gather. The most conventional approach is to design macrophages to carry NPs rather than directly transporting drugs, because NPs help to decrease the toxicity of therapeutic agents to macrophages, resulting in better therapeutic outcomes by allowing an increase in the drug content [2, 5, 23]. Perhaps the most serious disadvantage of internalizing NPs is the possible degradation of therapeutic cargo in macrophages phagosomes after being engulfed, leading to

a reduction in drug release from macrophages. To address this issue, NPs can be immobilized on macrophage surface instead of being phagocytosed, allowing to maintain the integrity of the cargo and the targeting abilities of macrophages. Therefore, NPs can be internalized or attached to macrophages in a "hitchhiking" approach for targeted drug delivery [2, 7, 34].

### 2.3.2. Macrophage-derived extracellular vesicles as drug delivery vehicles

M1 macrophage-derived EVs have also gained importance as potential candidates in drug delivery, as EVs secreted by M1 macrophages exhibit surface proteins identical to those expressed on the parent cells and, therefore, may inherit their tumor and inflammation targeting capabilities [23, 28]. EVs perform a crucial role in cell-cell communication and according to their size and origin they can be classified in exosomes, microvesicles and apoptotic bodies. The literature on macrophage-derived EVs shows a variety of approaches, including the use of macrophage-secreted exosomes loaded directly with therapeutic agents for targeted delivery to tumors and the application of macrophage-derived EVs membrane-coated NPs for several biomedical purposes, including treatment of rheumatoid arthritis and management of lung metastasis derived from orthotopic breast cancer [23, 38, 39]. In these studies, it was hypothesized that the enhanced ability of NPs to target inflammatory locations and tumors arose from their membrane coating, since the membrane derived from macrophage-secreted EVs expresses a protein profile similar to that of macrophages, bestowing NPs with the desirable macrophages´ biofunctions [38, 39].

Nevertheless, there are limitations to how far the concept of employing exosomes as drug carriers can be taken. Extraction and isolation of exosomes remains a real challenge due to the insufficient production of EVs from cells, the extremely low yield of EVs isolation procedures and the great possibility of disrupting the integrity and functional effects of the EVs during isolation processes [23, 40]. To circumvent these problems, Jang *et al.* proposed the development of exosome-like nanovesicles by extruding DOX-loaded macrophages as an alternative to drug delivery, since these nanovesicles containing DOX can mimic the protein profile, size and tumor-homing features of macrophage-secreted exosomes with the added benefit of higher manufacturing yield [41]. Another relevant delivery nanoplatform based on macrophage-derived EVs reported in the literature by Rayamajhi *et al.* consists of hybrid exosomes obtained by fusing exosomes secreted from macrophages with synthetic liposomes, conceiving a robust hybrid delivery system that combines the tumor-homing features of exosomes and the conventional properties of liposomes [40].

### 2.3.3. Macrophage-like proteolipid nanovesicles as drug delivery vehicles

Using macrophage-like proteolipid nanovesicles that closely mimic the complex protein composition and unique features of these immune cells as drug delivery platforms is another promising strategy for achieving targeted therapy [22]. In this context, Molinaro *et al.* developed hybrid liposomes, called leukosomes, composed of membrane proteins isolated from macrophages anchored to a synthetic phospholipidic bilayer for efficient delivery of dexamethasone (DXM) to inflamed sites and doxorubicin (DOX) to treat both melanoma and breast cancer [42, 43]. The resulting nanosized vesicles showed to preserve CD 45, CD47, LFA-1, Mac-1 and PSGL-1, thus displaying an enhanced ability to recognize and adhere to inflamed vasculature and reduce macrophage uptake. Indeed, DXM-loaded leukosomes revealed a fifth increase in accumulation at sites of inflammation compared to liposomes, whereas DOX-loaded leukosomes showed a superior targeting ability to 4T1 breast cancer cells and B16 melanoma cells with higher accumulation in tumor-related vessels than free DOX. In summary, the results obtained from these studies showed the promising role of leukosomes for effective management of localized inflammation and chemotherapeutic drug (DOX) delivery to 4T1 and B16 tumors [42, 43].

### 2.3.4. Macrophage-derived membranes as drug delivery vehicles

Despite the exciting potential of all these macrophage-based drug vehicles, current research is focused on MCM-coated NPs as appealing drug delivery vehicles for biomedical applications. The site-specific targeting and immune evasion abilities exhibited by macrophage cells are essentially a consequence of their membrane surface proteins, which can be faithfully preserved and transferred to the NPs surface by wrapping them with macrophage-derived membranes using a "ghost cell" approach [7].

However, apart from using single cell-derived membranes, other cloaking materials have been investigated in this biomimetic approach, including hybrid cell membranes that incorporate multiple functionalities of different cell membranes [11, 44]. For instance, Gong *et al.* prepared a macrophage-4T1 breast cancer cell hybrid membrane by fusing both cell membranes and used it to transport poly (lactic-*co*-glycolic acid) (PLGA) NPs containing DOX, aiming the targeted drug delivery to pulmonary metastasis deriving from breast cancer [45]. The authors proved that the hybrid cell membrane retained the protein markers of macrophages and cancer cells as well as their particular biological properties, thus equipping the resulting NPs with the homotypic tumor-targeting capability of cancer cells and the metastasis-targeting ability of macrophages. Because of these multifunctional properties, the biomimetic nanoplatform produced a substantial reduction in lung metastatic nodules and

efficiently prolonged survival time *in vivo*, indicating that biomimetic NPs coated with a macrophage-4T1 cancer cell hybrid membrane are exciting therapeutic tools for pulmonary metastasis from breast cancer [45].

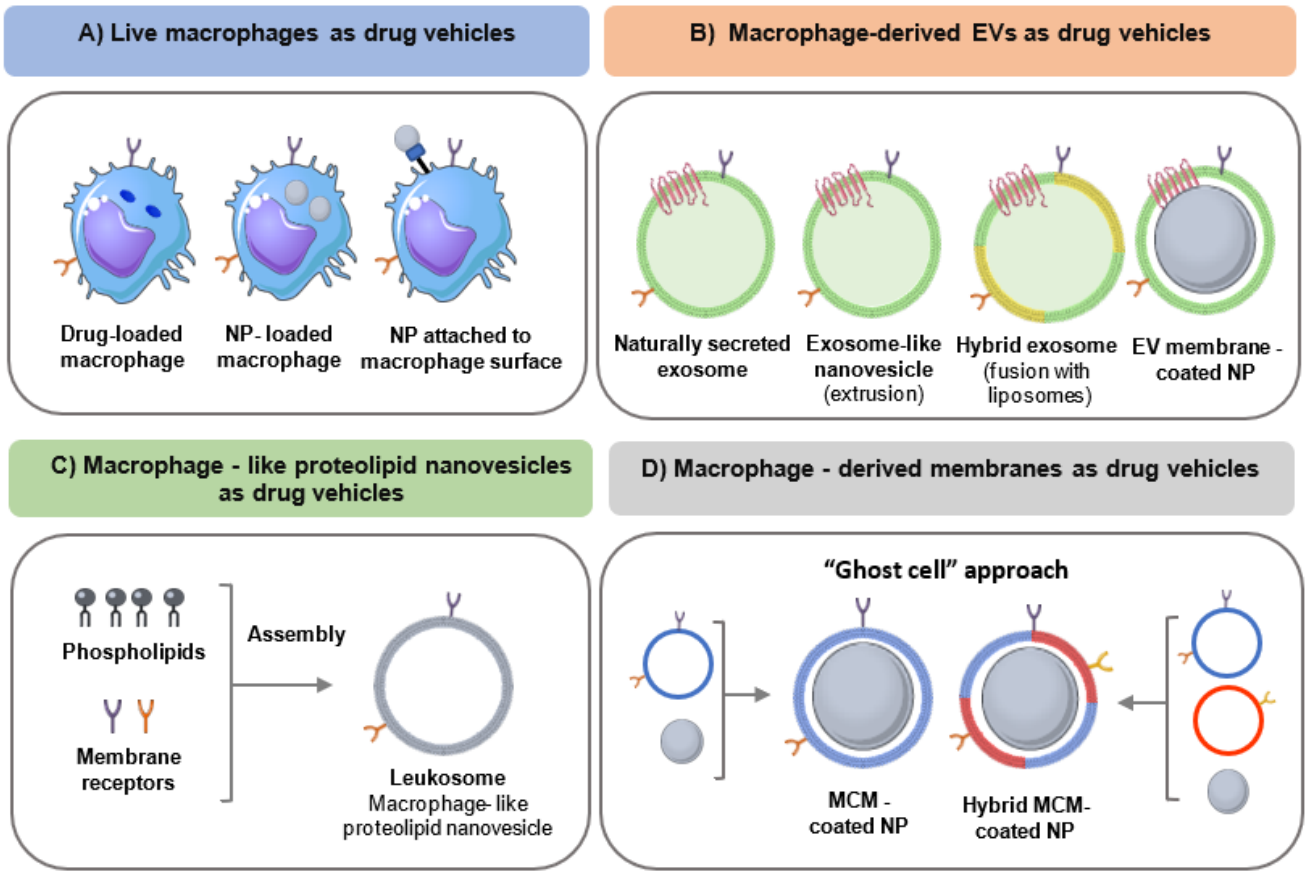


**Figure 2 -** Overview of the bioinspired macrophage-based therapeutics for active targeted drug delivery, consisting of A) alive macrophages (encapsulation of drugs and NPs or NPs surface conjugation), B) macrophage-derived EVs (natural macrophage-secreted exosomes, exosome-like nanovesicles obtained by extruding macrophage cells, hybrid exosomes and macrophage-secreted EV membranes), C) macrophage-like proteolipid nanovesicles, and D) macrophage-derived membranes (single MCMs or hybrid MCMs yielded by fusion with another cell membranes types).
**Abbreviations:** EV, extracellular vesicle; MCM, macrophage cell membrane; NP, nanoparticle.

## 3. Macrophage cell membrane coatings

Amongst coating materials, WBC membranes have attracted considerable attention because they collaborate in unique functions related to immune escape and site-specific targeting, especially to inflamed tissues and tumors, making their use very attractive for biomimetic coatings [8, 19]. In particular, MCM coatings have proven to endow NPs with the ability to evade clearance by the phagocytic immune system, circulate in bloodstream for a longer time, home to infectious and inflammatory tissues and actively bind tumor cells, allowing a more specific and targeted therapy and diagnosis [22, 46].

### 3.1. Preparation of macrophage membrane-coated nanosystems

The fabrication of MCM-coated NPs requires a few sequential number of steps and, briefly, is achieved by coating the previously prepared NP inner core with an MCM-derived vesicle. As shown in Figure 3, the preparation steps involve: (I) Extraction of the outer membrane and isolation of membrane vesicles, also 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 co-extrusion, sonication or electrostatic interaction, thus achieving MCM-coated NPs. In the following, an overview of these three fundamental steps will be presented.

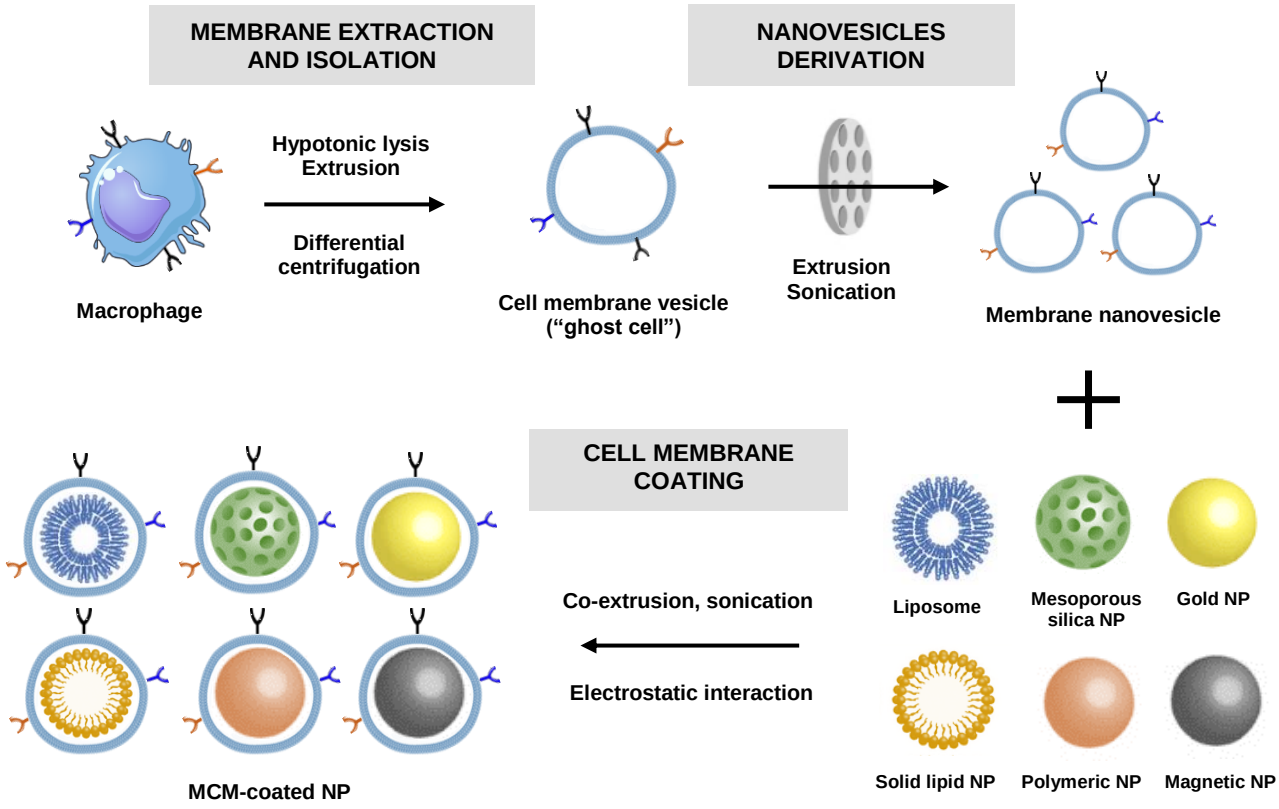


**Figure 3 -** Schematic illustration of the several steps required for coating different types of NPs with MCM nanovesicles, including extraction of the outer membrane from macrophages, derivation of nanovesicles, preparation of the NP inner cone and fusion of the NP with a cell membrane nanovesicle *via* top-down approaches. **Abbreviations:** MCM, macrophage cell membrane; NP, nanoparticle.

#### 3.1.1. Extraction of outer membranes and nanovesicles derivation

The conventional methods used to extract the biomembrane from natural cells are mainly based on cell disruption and lysis, however, the selection of the exact methodology is governed by the cell type [6, 11]. Several gently techniques that disrupt the cell structure and induce cell lysis have been applied to empty source cells and remove their intracellular contents, such as hypotonic lysis buffer, freeze and thaw, sonication, extrusion, and dounce

homogenizer, while preserving the surface membrane proteins intact to fully ensure the bioactivity and biofunctions of the cell membrane for successful biointeractivity [11, 47].

The first step to fabricate MCM-cloaked NPs involves extracting the outer membrane from previously isolated macrophage cells. Since WBCs have complex intracellular components and are nucleated, the membrane extraction procedures are more complicated and require a combination of cell disruption methods to remove the nucleus and cytoplasmic components, normally hypotonic lysis treatment and extrusion, followed by differential centrifugation and purification of the isolated membranes [6, 11, 46, 48]. Finally, the obtained membranes are mechanically extruded through variable-sized pores on a polycarbonate membrane and sonicated to form a nanosized vesicle that mimics the complete proteolipid composition of the original MCMs [10, 46, 48].

### 3.1.2. Preparation of the nanoparticle core

Then, the following step includes the preparation of the NP core and loading the payload that is intended to be delivered to the desired location on the NPs, which can be diagnostic or therapeutic agents [11]. Since the cell membrane-cloaking nanotechnology has emerged, a wide diversity of nanoconstrutions made with different materials has been applied as inner cores and then coated with MCM vesicles, including, among others, gold-based NPs, lipid-based NPs, inorganic NPs like mesoporous silica NPs, upconverting nanoparticles (UCNPs) or magnetic iron oxide nanoparticles ($Fe_3O_4$ NPs), and organic polymeric NPs like chitosan NPs, albumin NPs or PLGA NPs [46, 49, 50]. It is worth mentioning that the selection of the NP material must consider the specific characteristics and needs of the payload. Even so, regardless of the core material, it has been proven that the negative zeta potential of the nanoparticulate core facilitates the correct orientation of the cell membranes around the NP surface, due to the establishment of electrostatic repulsive forces between the negatively charged NP core and the negatively charged extracellular membrane constituents [31, 51].

### 3.1.3. Coating process

Finally, after obtaining the cell membrane nanovesicle and the NP inner core, 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, consisting of membrane extrusion through a porous membrane, sonication and electrostatic interaction [48, 51].

The first and most commonly used coating method is based on physical extrusion, in which the NP cores and cell membrane vesicles are co-extruded several times through a

porous membrane to achieve the final MCM-camouflaged NPs [10, 48]. Lately, a sonication coating approach has emerged, in which both components are mixed and co-incubated under ultrasound, being exposed to disruptive forces derived from ultrasonic energy to similarly form MCM-coated NPs, with the additional advantage of losing less material compared to physical extrusion [8, 10]. Another reported method to coat NPs with cell membranes relies on electrostatic interaction between the positively charged NP inner core and negatively charged membrane vesicles, resulting in spontaneous generation of membrane-cloaked NPs. In this approach, the strong electrostatic attraction induces the disruption of cell membranes, which is required for successful membrane coating [51]. An overview of the principle of these techniques and their main limitations is presented in Table 2.

Apart from these fusion methods, an electroporation technique using live macrophages instead of purified cell membranes has very recently been reported to prepare MCM-coated inorganic NPs, in order to solve problems related to loss of cell membrane integrity when using an extrusion or sonication approach [52]. In this approach, the NPs are first incubated and phagocytosed by macrophages and then, the resulting NPs-loaded macrophages are exposed to an external electric field, consequently opening pores on the cell membranes through which only the cellular content is released [52].

**Table 2 -** Coating techniques for preparing macrophage cell membrane (MCM)-coated nanosystems.

| Fusion method | Principle of the technique | Main limitations | Ref. |
|---|---|---|---|
| Co-extrusion (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<br>• Time-consuming<br>• Possible disruption of cell membrane integrity | [11, 49, 51] |
| Sonication | The ultrasonic energy induces the spontaneous reconstruction of the cell membrane around the NP core | • High variation in size<br>• Non-uniform membrane coating<br>• Possible disruption of cell membrane integrity | [11, 49, 51] |
| Electrostatic interaction | Spontaneous assembly through electrostatic attractions between the positively charged NP inner core and negatively charged membrane vesicles | • Non-complete membrane coating might be formed | [51] |

**Abbreviations:** NP, nanoparticle.

### 3.2. Characterization of macrophage membrane-coated nanosystems

After designing biomimetic nanosystems, their physicochemical and biological characterization is a mandatory step to confirm the successful cloaking of the natural cell membrane around the NP core. For this purpose, several strategies relying on the analysis of size, zeta potential and protein profile, before and after the coating process, have captured considerable attention [11, 48].

Cell membrane coatings modify the size and zeta potential (surface charge) of NPs, and to visualize the changes in surface morphology some methodologies are reported in the literature [11, 53]. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are amongst the techniques used to assess surface morphology, while the surface zeta potential and size of NPs are often measured by dynamic light scattering (DLS). The resulting NPs successfully cloaked with MCM vesicles reveal a clear core-shell nanostructure, with an outer membrane layer around the NP surface being detected in TEM images, and a slightly larger size compared to uncoated NPs, with an increase of about 10 nm-20 nm in particle size being indicative of successful membrane coating [6, 48]. Beyond techniques based on examining the size distribution of coated NPs, other approaches rely on measuring the surface zeta potential. Once covered with cell membrane vesicles, the NPs exhibit a negative surface charge similar to that of the original cells [23].

However, although the evaluation of physicochemical properties can confirm the correct assembly of the core-shell nanostructure, it is clearly not enough to ensure the bioactivity of membrane-coated NPs [11]. Hence, other techniques are also required to assess their biological characteristics and prove the expression of relevant macrophages´ surface markers. Western blotting and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) are common techniques used to investigate the protein composition of coated NPs and identify specific membrane markers [48, 54]. To ensure that MCM vesicles, together with their associated proteins, have been successfully assembled onto the NP surface, the final nanoformulation should exhibit a protein profile similar to that of the purified MCMs [48].

## 4. Applications of macrophage membrane-coated nanosystems

MCM-camouflaged NPs, recently designed to merge the advantages of both macrophage cells and NPs, have been successfully used for a wide variety of biomedical applications, from cancer imaging and therapy to management of inflammatory disorders and infectious diseases. Because of their immune evasion properties, their ability to target inflammatory/tumor sites and bind tumor cells, MCM-coated NPs have been widely used as

carriers of imaging agents, therapeutic drugs, immunomodulators, photosensitizers and photothermal agents for cancer therapy, imaging, and theranostics. However, using the MCM as a carrier of NPs, in such a way that gives them the desirable macrophage biofunctions, has been explored in several other directions beyond cancer, including atherosclerosis, Alzheimer´s disease (AD), management of infectious diseases (bacterial and viral infections) and sepsis (detoxification) (Figure 4). A summary of the main biomedical applications is presented in Table 3 (Appendix I) and Table 4 (Appendix II).

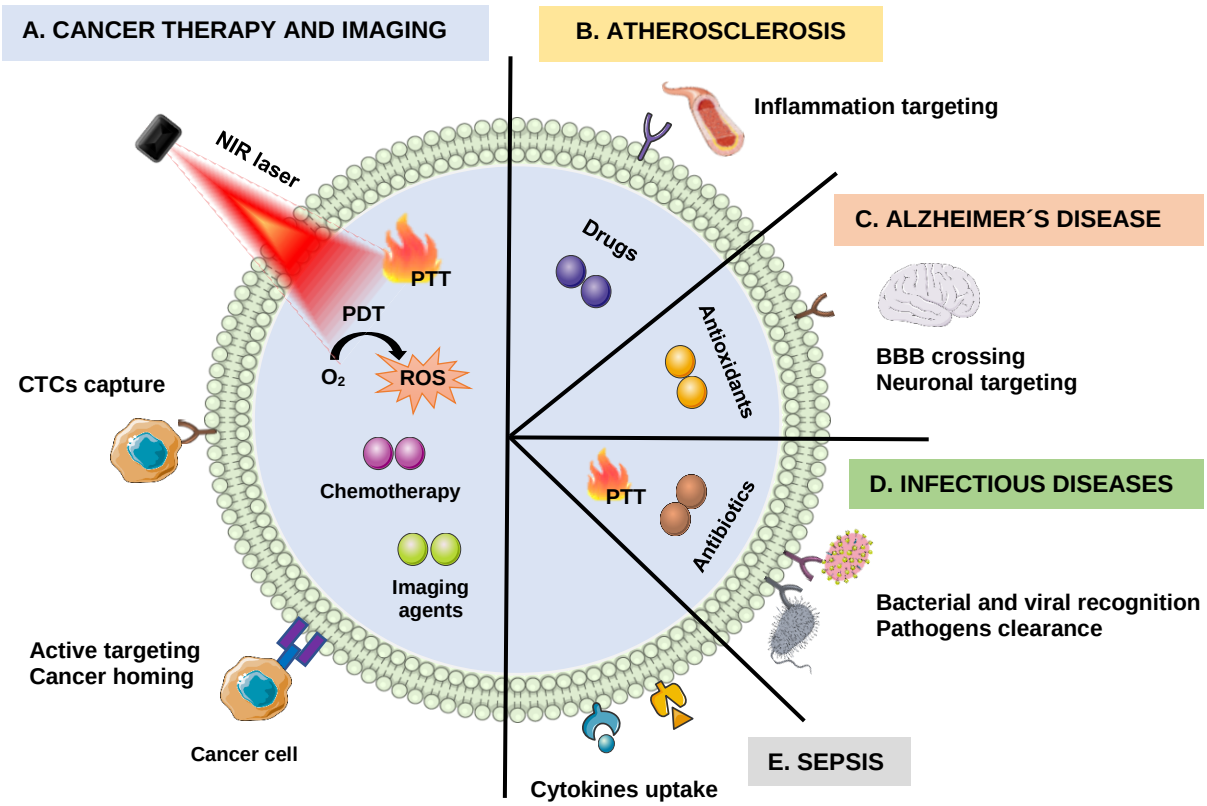


**Figure 4 -** Schematic of the biomedical applications of macrophage membrane-coated nanosystems for A) cancer therapy and imaging, B) atherosclerosis, C) Alzheimer´s disease, D) infectious diseases, and E) sepsis. **Abbreviations:** BBB, blood-brain barrier; CTC, circulating tumor cell; NIR, near-infrared;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

## 4.1. Cancer applications

Current research in the field of cancer therapy has been exploiting the unique ability of macrophages to be recruited to tumor sites to improve antitumor outcomes in both primary tumors and metastatic cancers. Recent research in this area has shown promising results of MCM-camouflaged nanosystems for chemotherapy delivery to primary tumors, antimetastatic therapy, antiangiogenic therapy, anti-proliferative cancer therapy, cancer immunotherapy, phototherapy, cancer imaging, cancer theranostics and capture of circulating tumor cells (CTCs).

### 4.1.1. Chemotherapy delivery to primary tumors

Chemotherapy is a conventional strategy in cancer therapy, however its application in clinical practice is limited due to the poor tumor specificity causing damage to surrounding healthy cells and severe side effects [55, 56]. Hence, the application of MCM-coated NPs as chemotherapeutics carriers has sought to improve tumor-targeted delivery and reduce off-target toxicity. As an example, Xuan *et al.* developed a biomimetic nanoplatform by wrapping DOX-loaded mesoporous silica nanocapsules (MSNCs) with macrophage-derived membrane vesicles for tumor-targeted chemotherapy [57]. *In vivo* studies showed prolonged blood circulation time compared to uncoated NPs, enhanced ability to target tumor cells and selective accumulation at tumor tissue, likely due to their enhanced ability to recognize the tumor vasculature and actively target tumor cells, which are imparted by the MCM cloaking. In general, coated MSNCs have shown to successfully guide the anticancer drug DOX to tumor sites, leading to highly effective chemotherapy and extraordinary ablation of breast cancer [57]. In another similar study, Cao *et al.* exploited MCM-coated albumin NPs as a means of delivering paclitaxel (PTX) to melanoma cells *via* active targeting mechanisms. *In vivo* studies showed superior uptake by tumor cells, leading to notorious antitumor effects and highly effective tumor eradication with chemotherapy [58].

Despite the very promising use of MCM-camouflaged nanosystems to carry chemotherapeutics to tumors, some challenges still remain in this biomimetic approach, especially in releasing the therapeutic payload, after being internalized by tumor cells, through the barrier formed by the membrane vesicle coating [59]. To tackle this issue, Zhang *et al.* synthetized a biomimetic platform, named cskc-PPiP/PTX@Ma, by cloaking a PTX-loaded polymer-based NP sensitive to pH fluctuations in the tumor inflammatory microenvironment with a macrophage-derived membrane [59]. Based on the proton sponge effect, polymeric cores were functionalized with a cationic 2-aminoethyldiisopropyl ligand (PPiP) so that the internal NP could behave as sponge for $H^+$ in the mildly acidic extracellular TME, which ultimately dictates expansion and rupture of the membrane coating. Therefore, the incorporation of this cationic chemical group into polymer materials enables the removal of the external membrane cloak, after being exposed to the acidic TME, thereby allowing the NPs to escape from the disrupted membrane coating and efficiently deliver drugs towards tumor sites (Appendix III A and III B). The authors also conjugated a targeting ligand with raised affinity for the insulin-like growth factor 1 receptor (IGF1R), which is abnormally expressed by tumor cells, on the surface of polymeric NPs to further enhance tumor accumulation efficacy. *In vivo* studies showed enhanced tumor uptake and retention, resulting

in superior tumor ablation compared to the pH-insensitive control group and non-coated group (Appendix III C and III D). Overall, biomimetic NPs showed to combine the buffering properties of polymeric NP cores and the active tumor-targeting abilities of MCMs, thus exhibiting enhanced biocompatibility, preferential accumulation in tumor tissues and gradual drug release in response to external and internal pH stimulation of the TME [59].

### 4.1.2. Antimetastatic therapy

So far, the mentioned studies mainly focused on addressing primary tumor sites, however, further applications of macrophages-mimetic nanosystems have been found in targeting metastatic cancers [8]. In a large study, Cao *et al.* took advantage of the higher affinity binding of macrophages to metastatic 4T1 breast cancer cells, which overexpress VCAM-1, *via* their α4β1 integrin to actively target and suppress lung metastasis of breast cancer [60]. To do so, pH-sensitive liposomes carrying the anticancer drug emtansine were cloaked with macrophage-derived membrane vesicles, creating a macrophage-like nanosystem named MEL. Owing to 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 non-coated liposomes. Summing up the results of experimental studies, MEL selectively targeted sites of pulmonary metastasis, thus providing more efficient drug delivery to the lungs, stronger antimetastatic efficacy and outstanding suppression of lung metastasis [60].

Likewise, in a similar study aimed at suppressing lung metastatic nodules from breast cancer, Li *et al.* projected a biomimetic nanoplatform, named DOX-MPK@MDL, by cloaking liposomes with membranes derived from macrophages for controlled and localized DOX release into lung metastatic cells [61]. Briefly, they first introduced pH-sensitive DOX prodrugs (DOX-MPK) into DNA tetrahedron dendrimers and then sequentially cloaked them with a lipid bilayer and a macrophage-derived membrane using a self-assembly technique (Appendix IV A). The DNA tetrahedron dendrimers helped to improve the stability of the nanosystem due to their biocompatibility, higher loading capacity and stable structure [61]. *In vitro* and *in vivo* studies showed enhanced biocompatibility, prolonged blood circulation time, superior uptake by lung metastatic cells and pH-induced DOX release from the DOX prodrugs under the acidic conditions of the TME, contributing to enhance the efficacy of antimetastatic therapy and mitigate the high cardiotoxicity of DOX by preventing non-specific release (Appendix IV B and IV C). In summary, DOX-MPK@MDL enabled a superior reduction of lung metastatic nodules and markedly reduced the DOX-triggered cardiotoxicity by preferentially accumulating in tumor tissue, therefore causing less damage to cardiac tissue (Appendix IV D - IV F) [61].

### 4.1.3. Antiangiogenic therapy

The concerns related to systemic toxicity of chemotherapy, the standard therapy in metastatic breast cancer, led to the development of alternative therapies through modulation of the angiogenic pathway [62]. Taking advantage of the anticancer effects of saikosaponin D, a triterpene saponin, Sun *et al.* camouflaged saikosaponin D-loaded PLGA NPs with T7 peptide-inserted MCM vesicles, developing a biomimetic nanoplatform with potential for targeted antiangiogenic therapy [62]. *In vitro* and *in vivo* studies showed that MCM-coated PLGA NPs efficiently reduced macrophage clearance and selectively targeted tumor cells, which was a result of their membrane coating and specific recognition of transferrin receptors overexpressed in tumor cells through the T7 peptide, contributing to inhibit both *in situ* tumor growth and breast cancer-derived metastasis. It was demonstrated that the remarkable suppression of tumor growth by saikosaponin D was mediated by downregulation of angiogenesis-associated pathways, in particular MAPK/ERK and PI3K/AKT. Therefore, this highlights the potential use of this biomimetic nanoplatform for the management of advanced stages of breast cancer with disseminated metastasis, likely due to its enhanced ability to target tumor sites and suppress cancer metastasis by modulating the expression of angiogenesis-related factors, including vascular endothelial growth factor (VEGF) [62].

### 4.1.4. Anti-proliferative cancer therapy

Macrophages are specialized immune cells characterized by secreting a wide range of cytokines, such as TNF-α, whose production from the transmembrane TNF-α, its precursor present on the cell membrane, is triggered upon exposure to LPS or other factors [13]. Based on the well-recognized anti-proliferative activity of the transmembrane TNF-α, and its potential to kill tumor cells, Bhattacharyya *et al.* wrapped biodegradable and biocompatible polymeric chitosan NPs with an engineered TNF-α-coupled MCM [63]. For this, THP-1 human monocytes derived from a leukemia monocytic cell line initially differentiated into macrophages after exposure to phorbol 12-myristate 13-acetate (PMA) and then, they were stimulated by LPS to produce TNF-α. That is, in this study, the ability of macrophages to produce TNF-α upon LPS induction was exploited to produce a TNF-α-embedded membrane [63]. *In vitro* studies demonstrated the enhanced cytotoxic effects of membrane-cloaked NPs against different cancer cell lines, and when used to treat tumor spheroids, the NPs were able to efficiently suppress tumor growth and reduce cell viability in a dose-dependent manner by inducing apoptosis of tumor cells, indicating their potential for cancer therapy [63].

### 4.1.5. Cancer immunotherapy

Immunotherapy, as a type of cancer therapy that attempts to trigger the patient's own immune system to recognize and eliminate tumor cells, finds applications ranging from cancer vaccines to adoptive T-cell transfer therapy and immune checkpoint blockade, with the aim of eliciting robust anticancer immune responses and eradicating cancer [64 - 66].

Adoptive T-cell transfer therapy consists of stimulating autologous T cells *ex vivo* by incubating them with antigen-presenting cells (APCs), and then they are transferred into the patient to accumulate in tumors and destroy tumor cells. However, using natural APCs is often time-consuming and laborious [67]. To overcome these hurdles, Zhang *et al.* constructed artificial APCs, named magnetosomes, by coating $Fe_3O_4$ magnetic nanoclusters (MNCs) with an engineered azide-attached membrane derived from macrophages through electrostatic interaction, to allow the incorporation of dibenzocyclooctyne (DBCO)-linked T cell stimulatory signals to the nanosystem surface *via* chemical reaction [67]. *Ex vivo* studies showed efficient CD8+ T-cell stimulation, and when injected in tumor-bearing mice, the nanoplatform effectively guided the activated T cells to tumor sites under a magnetic field, while monitoring the tumor accumulation *in vivo* by magnetic resonance imaging (MRI). In summary, the resultant magnetosomes showed to be endowed with stealth properties from the MCM cloaking and both magnetic and superparamagnetic features from MNCs, leading to superior tumor eradication *in vivo* without significant toxicity [67].

Ongoing research on cancer immunotherapy has pointed out the exciting prospects of TAMs repolarization approaches towards the anti-tumorigenic M1 phenotype to mitigate the immunosuppressive TME and stimulate stronger anticancer immune responses [68]. Encouraged by the macrophage polarization abilities of $Fe_3O_4$ NPs and imiquimod (R837), a Toll-like receptor 7 (TLR 7) agonist, Liu *et al.* wrapped polymeric PLGA NPs containing both R837 and magnetic $Fe_3O_4$ NPs with membranes derived from LPS-induced M1 macrophages [68]. In the *in vivo* studies, the resulting NPs were highly efficient in impairing breast cancer growth when injected into tumor-bearing mice, which was a consequence of the synergistic effects of R837 and magnetic $Fe_3O_4$ NPs that enabled the polarization of TAMs to M1 macrophages by activating the NF-kB and IRF5 pathways, correspondingly. The authors found that the biomimetic NPs could induce the polarization of macrophages towards the M1 phenotype in an extension of 2.88, producing remarkable antitumor effects by shaping the TME and inducing strong immunotherapy against cancer [68].

### 4.1.6. Phototherapy

Phototherapy is a promising light-triggered therapeutic approach comprising photothermal therapy (PTT) and photodynamic therapy (PDT), both of which require laser irradiation to generate heat or ROS, respectively, which are capable of destroying and killing tumor cells [64, 69]. Based on the macrophages´ ability to guide therapeutics to tumor sites, avoid clearance by the MPS and prolong blood circulation time in *vivo*, several emerging studies have turned their focus to macrophage-mimicking NPs containing photothermal agents or photosensitizers as a means to considerably enhance the cytotoxic effects of phototherapy and reduce the viability of cancer cells, without harming healthy tissues.

#### 4.1.6.1. Photothermal therapy

Photothermal cancer therapy is one application of MCM-coated NPs, in which the NP core is a photothermal agent capable of absorbing light in the near-infrared (NIR) range and converting it into heat, resulting in efficient damage to cancer cells and potentially cell death through hyperthermia. In recent years, must research has been devoted to PTT as a minimally invasive cancer phototherapy, due to its potential to selectively destroy cancer cells *via* thermal ablation, without causing significant damage to un-irradiated normal tissues [70].

Inorganic $Fe_3O_4$ NPs have been widely studied for PTT applications due to their remarkable photoabsorbing properties and their ability to generate hyperthermia when irradiated with an NIR laser [71]. As an example, Meng *et al.* wrapped macrophage-derived membrane vesicles onto $Fe_3O_4$ NPs to enhance breast cancer therapy [71]. As opposed to non-coated NPs, which displayed poor tumor accumulation, the biomimetic nanoplatform, named $Fe_3O_4$ NPs@MM, demonstrated superior uptake by tumor cells and selective accumulation in neoplastic tissue. *In vivo* administration of $Fe_3O_4$ NPs@MM followed by irradiation of tumor areas with an NIR laser successfully induced photothermal injuries to the neoplastic cells, leading to extraordinary reduction of tumor growth and nearly complete tumor elimination. Together, these findings provide important insights into cancer therapy, with $Fe_3O_4$ NPs@MM showing promise to enhance the effectiveness of PTT *in vivo* by combining the biocompatibility, high circulation time and tumor-targeting ability of MCMs and the photothermal properties of $Fe_3O_4$ NPs cores [71]. Good nanoshells (AuNSs), which are recognized for their ideal NIR absorption capacity, have also been harnessed for photothermal cancer therapy [72]. For instance, Xuan *et al.* coated NIR fluorescent dye cyanine 7 (Cy7)-loaded AuNSs with macrophage-derived membranes, yielding a biomimetic nanoplatform with potential for both fluorescent imaging and PTT [72]. *In vitro* and *in vivo* studies demonstrated enhanced uptake by tumor cells, longer blood circulation and efficient hyperthermia-induced

tumor cell death. This study concluded that, by capitalizing on the enhanced tumor tropism of MCMs and the photothermal properties of AuNSs, the efficacy of PTT *in vivo* can be greatly improved [72].

Taking advantage of the NIR-activated propulsion of Janus mesoporous silica nanomotor (JMSNM) and its potential for PTT, Xuan *et al.* camouflaged JMSNMs with a macrophage-derived membrane only in one of the sides and showed that the biomimetic MCM-cloaked JMSNMs could actively target cancer cells and circulate in biological media undetected by the immune system, resulting in superior accumulation on the surface of tumor cells [73]. *In vitro* studies showed that the resulting nanosystem could open and perforate the tumor cell membranes, once irradiated with NIR light, leading to highly efficient killing of cancer cells by inducing irreparable damage to cell membranes. Overall, this NIR-driven biomimetic nanosystem showed to be a very promising therapeutic approach for photothermal cancer therapy *in vitro* [73].

The main obstacle in the treatment of neurological disorders is the BBB that hinders the passage of therapeutic agents into the central nervous system, and so, the development of novel strategies capable of bypassing the BBB and targeting tumor sites is a current need in orthotopic glioblastoma therapy [74]. To tackle this challenge, Lai *et al.* designed a biomimetic nanoplatform that could be used for both diagnostic and therapeutic purposes by wrapping NIR-Ib fluorescence IR-792 dyes-loaded liposomes with macrophage-derived membranes [74]. The resulting nanosystem showed to efficiently overcome the hurdle of BBB, due to the 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 target tumor cells, thereby enabling the efficient delivery of IR-792 dyes to glioblastoma sites, which performed a dual function, acting as an imaging agent and heat generator for PTT. *In vivo* studies showed enhanced cytotoxic effects against tumor cells under NIR radiation with NIR-Ib fluorescence imaging guidance, resulting in remarkable antitumor efficacy and prolonged survival time of glioblastoma-bearing mice [74].

Hollow bismuth selenide ($Bi_2 Se_3$) NPs were also investigated because of their ideal properties for PTT, X-ray computed tomography (CT) and infrared thermal (IRT) imaging, making them interesting nanomaterials for the development of multimodal systems [75]. In this regard, Zhao *et al.* developed MCM-coated $Bi_2 Se_3$ NPs loaded with quercetin and showed that the resulting NPs were endowed not only with immune escape abilities, higher biocompatibility and active tumor-targeting capabilities, but also with the ability to be recruited to tumor sites in response to chemotactic signals produced by CCL2 [75]. In the experimental

studies, this heat-generating platform showed the most striking effect in reducing tumor growth and inducing cell death under NIR radiation, which is partly due to the role of quercetin in sensitizing cancer cells to PTT by depleting thermoresistance-linked chaperones, such as heat shock protein 70 (Hsp70), that protect cancer cells from hyperthermia-induced apoptosis. Moreover, the biomimetic nanosystem showed a higher efficacy in suppressing lung metastasis from breast cancer, which resulted from its ability to negatively regulate metalloproteinase 9 (MMP 9) and protein kinase B (Akt), both of which are implicated in tumor growth, invasion, and metastasis. Overall, the researchers presented a novel strategy to improve PTT efficacy by combining therapeutic agents with synergistic anticancer effects [75].

Several combinatorial cancer therapies have been reported in recent years, aiming to achieve better therapeutic outcomes compared to individual treatments. For instance, the combination of PTT and chemotherapy was studied by Ji *et al.* and has proven to be a highly effective therapeutic approach for hepatocellular carcinoma [76]. In this study, sorafenib-loaded copper sulfide (CuS) NPs were coated with a macrophage-hepatic cancer cell (H22) hybrid membrane, to which was attached anti-VEGF receptor (VEGFR) antibodies, producing a hybrid nanosystem, named CuS-SF@MCV, that reunites the surface markers of both macrophages and cancer cells, thereby exhibiting immune escape abilities and enhanced tropism for homotypic tumor cells (Appendix V A and V B) [76]. The combination of CuS-SF@MCV and NIR irradiation enabled a substantial suppression of tumor growth, which is attributed to the photothermal effects of CuS NPs and synergistic antimetastatic effects of anti-VEFGR antibodies and sorafenib, a well-recognized chemotherapeutic drug that inhibits protein kinases in the MEK/ERK and PI3K/AKT pathways (Appendix V C and V D). Additionally, this synergistic chemo-PTT enabled a substantial increase in the survival rate of tumor-bearing mice, with no variations in body weigh being observed, indicating the biosafety of the nanosystem and its therapeutic potential (Appendix V E and V F) [76].

#### 4.1.6.2. Photodynamic therapy

Beyond PTT, very recent studies have extended the application of MCM-camouflaged NPs to PDT, which requires the delivery of photosensitizers to cancer sites and their subsequent activation by laser irradiation, leading to the generation of ROS, essentially singlet oxygen ($^1O_2$), from the surrounding oxygen molecules ($O_2$) within the tumor tissue [69]. In turn, ROS play a crucial role in amplifying anticancer immunity by inducing photooxidative damage to tumor cells and thereby increasing the uptake, processing, and presentation of tumor-associated antigens by APCs to T cells [77].

In an attempt to develop a synergistic approach against melanoma and breast cancer that combines PDT, immunotherapy and chemotherapy, Hu *et al.* designed a biomimetic nanoplatform composed of DOX-attached PEGylated bilirubin NPs containing both the photosensitizer chlorin e6 (Ce6) and the IDO1 inhibitor, indoximod (IND), and then covered with membranes derived from M1-polarized macrophages [77]. *In vitro* and *in vivo* studies showed enhanced tumor accumulation, longer 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 not only markedly inhibited the primary tumor growth in murine models of B16F10 and 4T1 cancers, but also exerted excellent suppressive effects on tumor recurrence and metastasis [77]. Similarly, Liu *et al.* reported a macrophage-like nanosystem capable of releasing PTX, Ce6 and IND in a NIR laser-responsive manner, producing a combinatorial chemophotoimmunotherapy for breast cancer [78]. To this end, they used cell membranes derived from LPS-induced M1 macrophages to coat a hydrophobic bilirubin/ Ce6 core containing both PTX and IND. In combination with immunotherapy by IND, PDT by Ce6 and chemotherapy by PTX, the final nanoassembly strongly induced anticancer immunity and exhibited the most remarkable antitumor activity *in vivo* and also inhibited lung metastasis, suggesting its promising role in improving outcomes in cancer therapy [78]. In summary, both studies presented a synergistic therapy in a single nanosystem capable of inducing strong anticancer immune responses through a combinatorial mechanism.

Photoabsorbing nanomaterials serving as chemotherapeutics carriers and generators of both heat and ROS have also been studied. In a recent effort to treat metastatic breast cancer using a chemophototherapy approach, Poudel *et al.* coated macrophage-derived membranes onto NIR-absorbing CuS NPs containing PTX, yielding a biomimetic nanosystem that was able to destroy and eliminate 4T1 tumors through chemotherapy, PDT and PTT [79]. Given the tumor-targeting ability of the MCM, the final biomimetic nanoplatform could actively target and be internalized by 4T1 cancer cells *via* α4β1 integrin /VCAM-1 interaction, which was significantly reinforced by systemic co-administration of the tumor-targeting peptide iRGD. In the *in vivo* studies, treatment with coated NPs, iRGD and NIR led to a marked increase in tumor temperature and a notorious tumor eradication with minimal off-target toxicity, accomplishing a superior anticancer efficacy than non-coated NPs thanks to the multiple properties and functions carried out by the nanosystem [79].

### 4.1.7. Cancer imaging

Cancer imaging is a crucial non-invasive modality for early detection of cancer, monitorization of tumor progression and investigation of metastasis. Several types of imaging techniques have been explored for cancer diagnosis, including fluorescence imaging that employs fluorescent probes capable of converting NIR light into visible light [64]. Among them, β-Na $YF_4$: $Er^{3+}$,$Yb^{3+}$ UCNPs have captured attention because of their remarkable optical properties, lower toxicity, higher photostability, and excellent light penetration depth [80]. In a study which set out to improve cancer targeting and imaging, Rao *et al.* cloaked UCNPs with a macrophage-derived membrane vesicle and showed that the membrane coating could greatly enhance the performance of these imaging agents *in vivo* because of its native ability to target neoplastic tissues [80]. The resulting MCM-coated UCNPs displayed superior uptake by tumor cells *in vitro*, good biocompatibility *in vivo,* longer circulation time and superior fluorescence intensity in tumor tissue compared to non-coated UCNPs. These results are consistent with those of other studies and suggest that these macrophage-like imaging nanoprobes have great potential for improving *in vivo* fluorescence imaging of tumors [80].

### 4.1.8. Cancer theranostics

Recent developments in the field of cell-mimetic nanotechnology have led to a proliferation of studies exploring MCM-coated NPs for theranostic applications, which combine diagnostic imaging and therapy into a single nanosystem [81].

By drawing on the concept of biomimetic theranostic nanosystems, Liang *et al.* synthetized a multimodal macrophage-like superparticle aiming the targeted co-delivery of drugs and imaging agents to suppress lung metastasis of breast cancer, while monitoring the therapeutic efficacy and tumor distribution of NPs *in vivo* [82]. To this end, DOX and fluorescent quaternary quantum dots (QDs) were co-loaded in liposomes and then cloaked with a macrophage-derived membrane. Similar to the aforementioned studies, the MCM cloaking 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 α4β1 integrin. *In vivo* studies showed that the biomimetic platform efficiently targeted DOX and QDs towards cancer cells, leading to superior suppression of lung metastasis and stronger fluorescence intensity in tumor tissue. The present study makes 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 [82].

Similarly, Chen *et al.* presented a theranostic nanosystem, named PTX@MPLMC, opening a new avenue in the development of personalized strategies for cancer management by combining both diagnostic and therapeutic functions [83]. To achieve this, they first designed persistent luminescence NP (PLNP)@metal-organic framework (MOF)-derived mesoporous carbon nanocomposites (PLMC), in which $Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4$: $Cr^{3+}$ PLNP served as the imaging source due to its exceptional capacity to emit long-term NIR persistence luminescence upon exposure to light emitting diodes (LED), which can be harnessed for *in vivo* persistent fluorescence imaging. Then, PTX-loaded PLMC cores were cloaked with macrophage-derived membranes hoping to achieve targeted drug delivery, reduced uptake by the MPS and effective chemotherapy with luminescence imaging guidance. *In vivo* studies showed the enhanced tumor-targeting ability of PTX@MPLMC, increasing both the tumor accumulation and luminescence intensity in tumor locations. Collectively, these findings outline that NIR-releasing PTX@MPLMC can optimize the delivery of PTX for superior inhibition of tumor growth and tumor eradication, while enabling the tracking of PTX@MPLMC *in vivo* by luminescence signals released from PLMC inner cores [83].

In the context of cancer theranostic applications, silver nanoclusters (AgNCs) have also attracted strong attention due to their intrinsic cytotoxic effects and ideal fluorescent properties. In this regard, Girigoswami *et al.* camouflaged AgNCs with macrophage-derived membranes to design a biomimetic system for Dalton Lymphoma Ascites (DLA) theranostics [84]. When incubated with DLA tumor cells *in vitro*, the MCM-cloaked AgNCs showed superior efficacy in inducing tumor cell death compared to non-coated AgNCs, even at lower doses, which highlights the great potential of this biomimetic nanosystem as an anticancer agent against DLA tumors. *In vivo* studies revealed that membrane-coated AgNCs efficiently homed to DLA tumor tissues, enabling the visualization of tumors through fluorescent signals released by AgNC cores. Together these findings reveal that the anticancer effects and fluorescent imaging properties of AgNCs coupled with the tumor-targeting abilities of MCMs hold great promise for DLA-targeted theranostics [84].

### 4.1.9. Capture of circulating tumor cells

Macrophages can bind tumor cells both at tumor sites and in circulation through the α4β1integrin/VCAM-1 interaction, enabling them to survive and spread through the circulation to form cancer metastasis [11, 34]. The migration of CTCs through the bloodstream has been recognized as a decisive step in the development of metastasis, and therefore, modalities based on detecting and counting CTCs in the bloodstream have potential applications in cancer diagnosis, prognosis, and therapy. However, the low occurrence rate of CTCs in bloodstream

and the nonspecific binding of WBCs may hamper the successful clinic application of this approach [85]. To address these issues, Xiong *et al.* coated positively charged MNCs with a negatively charged azide-coupled membrane derived from macrophages *via* electrostatic interaction, to which was attached the antibody of epithelial cell adhesion molecule (EpCAM), an antigen selectively expressed in adenocarcinomas [85]. The resulting biomimetic platform, named immune-magnetosome (IMS), was highly competent not only in recognizing EpCAM-expressing tumor cells due to its surface modification, but the MCM coating also helped to reduce nonspecific adsorption and interaction with surrounding WBCs, due to the repelling effect of WBCs. The combination of these multiple advantages in IMS enabled the capture of around 90 % tumor cells from whole blood in 15 min without any interference from WBCs interaction, which highlights its great potential for highly efficient CTCs capture [85].

### 4.2. Atherosclerosis

Inspired by the macrophages´ ability to recognize and target the inflamed endothelium, Wang *et al.* wrapped PLGA NPs containing rapamycin, an mTOR pathway inhibitor compound, with a macrophage-derived membrane for the treatment of atherosclerosis [86]. The resulting biomimetic PLGA NPs demonstrated to be able not only to suppress macrophages-mediated phagocytosis *in vitro*, but also showed to actively target atherosclerotic plaques *in vivo* by binding to the VCAM-1 expressed on inflamed vasculature, thereby enabling for more efficient drug delivery to atherosclerotic lesions compared to non-coated NPs and efficient reduction of atherosclerosis progression. Furthermore, no pathological damage to major organs or significant side effects were observed compared to the control group, thus confirming the biosafety and biocompatibility of the nanosystem [86].

Early detection of atherosclerosis and targeted therapy largely contribute to improve anti-atheroscloric outcomes, however the current diagnostic and therapeutic modalities are still uncapable of fulfilling these requirements [87]. To circumvent these limitations, Wu *et al.* projected a multifunctional nanosystem composed of an MNC inner core cloaked with a simvastatin-embedeed MCM, to which was attached an apolipoprotein A-I mimetic 4F peptide (AP), for early diagnostics of atherosclerosis through MRI and combinatorial therapy by simvastatin and AP (Appendix VI A) [87]. *In vitro* and *in vivo* studies showed that the biomimetic nanoplatform efficiently targeted early atherosclerotic lesions because of the inflammation targeting capabilities of MCMs and specific interaction of AP with foam cells present in the atherosclerotic plaques, which are macrophages that have internalized low density lipoproteins (LDL), thus being directly implicated in establishing atherosclerotic plaques. The synergistic effects of AP and simvastatin enabled a remarkable suppression of atherosclerotic

lesions *in vivo* by inducing the LDL efflux from foam cells and reducing inflammatory cytokines, respectively (Appendix VI B - VI D). Thus, this biomimetic nanoplatform showed enormous potential for achieving a more efficient and targeted atherosclerosis diagnosis and therapy [87].

### 4.3. Alzheimer's disease

AD is a well-studied neurodegenerative disorder characterized by progressive accumulation of beta-amyloid (Aβ) in the brain, which culminates in the loss of neuronal synapses and neuronal cell apoptosis. Mitochondrial dysfunction, resulting from excessive ROS generation, has been considered a key precipitating event in AD by eliciting the production and aggregation of Aβ, and therefore, therapeutic modalities based on targeted delivery of antioxidants to neuronal mitochondria have attracted strong attention [88].

Recently, Han *et al.* built a dual-functionalized nanoplatform consisting of solid lipid NPs (SLNs) loaded with genistein (GS), an antioxidant, anti-inflammatory and neuroprotector flavonoid, which were cloaked with macrophages-derived membranes to solve problems related to GS's low ability to cross the BBB and target neuronal mitochondria (Appendix VII A and VII B) [88]. By posteriorly co-incorporating the triphenylphosphine (TPP), a positively charged ligand, and rabies virus glycoprotein (RVG29), a specific neuronal-targeting and BBB-crossing ligand, on the surface of MCMs, the biomimetic nanoplatform could more efficiently penetrate the BBB and selectively reach the neuronal cells, particularly the mitochondria (Appendix VII C and VII D). *In vitro* and *in vivo* studies showed enhanced uptake by neuronal cells, superior BBB-penetration ability, enhanced ability to target the negatively charged neuronal mitochondria, and efficient mitochondrial ROS elimination due to the antioxidant effect of GS (Appendix VII E and VII F). Hence, the combination of TPP, RVG 29 and MCMs on a single nanoplatform enabled a significant inhibition of AD progression by using a neuronal mitochondrial-targeted strategy to accomplish effective GS delivery [88].

### 4.4. Sepsis

Macrophage-mimetic nanosystems have also been used for detoxification purposes because of their exceptional capacity to absorb and remove bacterial endotoxins and pro-inflammatory cytokines, which is essentially attributed to specific receptors located on the cell membrane. Accordingly, Thamphiwatana *et al.* conceived polymeric PLGA NP inner cores coated with MCMs and found that the resulting biomimetic NPs could be used to treat sepsis, a life-threatening pathological condition caused by an extensive inflammatory reaction that leads to an overreaction of the immune system, as they were able to efficiently neutralize and sequester LPS and several pro-inflammatory cytokines *in vitro* from standardized solutions

[89]. Western blotting confirmed the presence of CD126, CD120 a/ b, CD119 in the prepared membrane-coated NPs, which engage with IL-6, TNF and IFN-γ (inflammatory cytokines) respectively, as well as the LPS-binding receptors TLR 4 and CD 14. *In vivo* studies showed that membrane-coated NPs markedly reduced both pro-inflammatory cytokines and bacterial count in several organs and prolonged survival time of the mice. Overall, these encouraging findings paved the way for the development of novel strategies for sepsis treatment [89].

## 4.5. Infectious diseases

### 4.5.1. Bacterial infections

MCM-coated NPs containing a wide repertoire of pathogen recognition receptors, such as TLR 4 and TLR 2, can mimic the bacterial targeting capabilities of macrophage source cells. In a novel study, Wang *et al.* cloaked membranes of macrophages pre-treated with *Staphylococcus aureus (S. aureus)* onto gold-silver nanocages (GSNCs) with good NIR absorption capacity to enhance bacterial targeting and antibacterial treatment with PTT [90]. It was demonstrated that bacterial pre-treatment could significantly increase the amount of bacterial recognition receptors on the membrane surface, thus amplifying the ability of membrane-coated GSNCs to recognize and bind bacteria. *In vivo* studies showed longer retention at infection sites in comparison to non-coated GSNCs and efficient hyperthermia-induced bacterial destruction under an NIR laser, thus confirming the enhanced antibacterial efficacy of this biomimetic platform against local bacterial infections [90].

The absence of effective strategies in current clinical practice to manage intracellular bacterial infections, which are particularly more complicated to treat and far more severe than extracellular infections, motivated Li *et al.* to create a biomimetic nanosystem by wrapping antimicrobial-conjugated NPs (ANPs), composed of triclosan and ciprofloxacin, with macrophage-derived membranes for the treatment of intracellular infections caused by S. *aureus* [91]. The biomimetic NPs have shown to be specifically internalized *in vitro* by macrophages infected with intracellular *S. aureus*, thus releasing the conjugated antimicrobials for efficient intracellular bacterial killing without compromising healthy macrophages, which can be attributed to the negative zeta potential and TLRs expression in the projected NPs that increase their uptake by positively charged S. *aureus*-infected macrophages. Overall, membrane-coated ANPs showed to provide a superior elimination of intracellular *S. aureus* infection compared to uncoated ANPs and free ciprofloxacin, therefore confirming their upgraded ability to mitigate the severity of intracellular bacterial infections [91].

The increasing worldwide prevalence of multidrug-resistant bacterial infections is rapidly becoming a serious and life-threatening public health problem, largely due to the slowdown in the development of novel antibiotics [92]. As Wang *et al.* and Li *et al.,* based on the natural interaction of macrophages with pathogenic agents, Wei *et al.* designed a biomimetic platform capable of capturing and neutralizing bacterial toxins, as well as boosting immune responses against *Pseudomonas aeruginosa (P. aeruginosa)*, a Gram-negative bacterium mainly responsible for nosocomial pneumonia, by loading highly toxic *P. aeruginosa* secretions, named PaS-1/-2, in the preassembled MCM-coated PLGA NPs [92]. 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 antibacterial immunity 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 providing superior outcomes in the management of bacterial infections [92].

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. Very recently, Shi *et al.* cloaked magnetic composite NPs composed of $Fe_3O_4$ NPs, titanium dioxide ($TiO_2$), an antibacterial agent with ultraviolet (UV) light-induced ROS generation property, and calcium phosphate ($Ca_3(PO_4)_2$), which served as a bone substitute to promote bone regeneration, with macrophage-derived membranes using a state-of-the-art electroporation-based coating technique [52]. *In vitro* and *in vivo* studies revealed that, by combining the excellent ability of MCMs to neutralize cytokines and toxins, 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 responses caused by bacterial infection, stimulate bone tissue formation and destroy bacteria when irradiated with UV light, thus achieving enhanced anti-inflammatory and antibacterial efficacy against bone infection [52].

### 4.5.2. Viral infections

The ongoing pandemic crisis caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a very 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-2019) [93]. As crucial members of the primary line of defense against invaders, alveolar macrophages express specific membrane receptors involved in cytokines uptake and binding of spike protein on the coronavirus surface, thus suppressing

virus infection by diverting them from target cells and blocking virus cellular entry, while mitigating the strong inflammatory and immune responses. In a recent study aiming to develop a multifunctional biomimetic nanosystem against coronavirus infection, Li *et al.* camouflaged polymeric PLGA NP cores containing 2PTE-2NDTA, an efficient photothermal agent, with alveolar macrophages-derived membranes (Appendix VIII A) [94]. Under NIR radiation, the biomimetic nanoplatform, named TN@AM NP was highly efficient in converting NIR light into heat, even for very low concentrations, producing a substantial temperature increase beneficial for photothermal viral ablation, due to the heat sensitivity of SARS-CoV-2. In the experimental studies, the combinatorial therapy with TN@AM NPs and NIR irradiation produced a notorious decrease in both cytokine expression and viral count in the lungs, extended the survival time of the infected mice and reduced the lung tissue damage, indicating its potential to be used in clinical practice for the treatment of COVID-19 (Appendix VIII B - VIII E) [94].

In another effort to reduce the violent inflammatory responses associated with coronavirus infection, Tan *et al.* cloaked macrophage-derived membranes onto PLGA NPs containing lopinavir, an antiviral drug, constructing a nanosystem with potential for both antiviral and anti-inflammatory therapy (Appendix IX A) [95]. By inheriting crucial cytokine binding receptors, including IL-6R and IL-1βR, the biomimetic nanoplatform could effectively absorb inflammatory cytokines and suppress macrophage and neutrophil activation, thereby alleviating the strong inflammatory responses (Appendix IX B and IX C). In addition, the presence of angiotensin-converting enzyme 2 (ACE II) enabled the nanosystem to specifically bind to spike protein on the coronavirus surface, thereby allowing the targeted drug delivery to sites of infection. *In vivo* studies showed superior reduction of inflammatory cytokines levels and efficient viral destruction, which resulted in prolonged survival time of infected mice and reduced inflammation-induced lung tissue damage (Appendix IX D - IX F). Hence, this biomimetic platform showed great potential for alleviating the strong inflammatory responses for the treatment of COVID-19 [95].

## 5. Challenges and future prospects

Despite the bright future prospects of immune cell-mimetic nanosystems and the tremendous progress made in recent years by camouflaging NPs with macrophage-derived membranes in the field of targeted drug delivery and immune modulation, there are some remaining issues that need to be surpassed before they can be accepted as standard treatments in clinical practice [7, 23]. The main challenges to the successful clinical translation of MCM-coated NPs, which mainly derive from the novelty and infancy of this biomimetic technology, include the high complexity of the preparation methods, great heterogeneity of WBCs

functions depending on the sources, possible epigenetic modifications of WBCs during isolation and purification procedures, immunogenicity, low reproducibility, large-scale production and safety issues related to the coating technique that may damage the integrity and structure of membrane proteins and compromise the biofunctions of cell membranes [4]. Furthermore, the lack of understanding about the triggering mechanisms of macrophages migration and polarization, and the high complexity of immune responses within the TME 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 [22, 23].

Using the immune cell membrane to functionalize 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 accurate and efficient accumulation of these nanosystems in inflamed locations and neoplastic tissues [6]. However, despite these obvious advantages, producing macrophages on a clinical scale suitable for universal use represents a very demanding task due to immunological and safety concerns arising from the presence of proteins involved in triggering immune responses on cell membranes. Therefore, due to the high risk of immune rejection when using allogeneic cells, human macrophages must be genetically modified after being extracted to reduce undesirable side effects [4, 22]. Another choice consists of using patient's own derived cells, termed autologous cells, as membranes sources for NPs coating, which can significantly improve the safety profile of these bioinspired nanosystems. Since the immune system recognize these modified autologous cells as "self", developing personalized therapies based on autologous cell membranes hold great promise in the future for drug delivery purposes [6, 7].

Another challenge concerns the absence of standardized protocols for macrophages extraction and purification, which may be responsible for reducing batch-to-batch consistency. Ensuring reproducibility between batches is very challenging, not only because WBCs may undergo changes in gene expression during *in vitro* manipulation, but also their functions may fluctuate according to the sources [7]. Therefore, there is an emerging need to develop novel large-scale production techniques that can reduce batch-to-batch variability and assess the properties and quality of macrophages [22, 23].

To date, several fusion techniques have been proposed for coating immune cell membranes onto NPs, however, the diversified efficiency of these methods and the current lack of standardized protocols may compromise the results obtained with these approaches [6]. Moreover, another concerning issue related to the coating method is the possible disruption of cell membranes and biofunctionality loss of the membrane proteins, which may compromise their natural functions and trigger strong immune responses against the damaged

proteins markers, thus raising both efficacy and safety issues. Hence, the development of optimized cell membrane coating protocols is a key step towards the clinical translation of these biomimetic nanosystems. In addition, the development of standardized criteria and quality control parameters for cell membrane-coated NPs is also urgently needed to assess the presence of microorganisms, toxins, and other contaminants [4, 6, 22].

In addition to directly transporting therapeutic compounds to target sites, cell-based platforms were also 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 the promotor, triggering gene expression and systemic release of the protein of interest [6, 7, 18]. These "cell-factories" are a very promising strategy for achieving a more effective therapeutic dose, better therapeutic results, and greater patient acceptance and satisfaction by reducing the number of parenteral administrations required [6, 7].

Due to the remarkable features of immune cell membrane-coated NPs, in the future, more and more research will focus on optimizing and improving their targeting capabilities and, therefore, further advances in cancer-targeted therapies can be expected owing to the development of biomimetic systems with even greater efficiency and tumor specificity [6].

## 6. Conclusion

Immune cell membrane-coating technology is an emergent and nature-inspired approach that harnesses the higher biocompatibility, longer blood circulation time and enhanced specificity of immune cells for inflamed tissues and tumors. This strategy overcomes the shortcomings of nanomaterials, improves the delivery of therapeutic agents and diagnostic compounds to sites of interest and, consequently, enhances the clinical outcomes achieved with NP-based systems. By using a functional and intact membrane vesicle derived from immune cells to cloak NPs *via* top-down approaches, the resulting core-shell NPs can inherit the complex protein profile of cell membranes, as well as the unique biological features of the parent cells, enabling them to replicate the cellular biofunctions *in vivo*.

Among immune cells, macrophages have been extensively investigated for therapeutic and diagnostic purposes in various diseases, especially for cancer and inflammatory disorders, due to their immune evasion properties and natural tropism for inflamed tissues, primary tumors and metastatic cancers. Indeed, these versatile phagocytic cells are equipped with a battery of surface markers that allow for specific binding to tumor cells *via* cell-cell adhesion, transendothelial migration to inflamed tissues, and recognition and destruction of microorganisms. Moreover, macrophages are crucial players of the TME that can determine

cancer immunity and tumor progression according to the signals received. Therefore, because of these unique features, using macrophages for drug delivery and cancer immunotherapy is receiving increasing attention.

Recently, macrophage-derived membranes that mimic the exact composition of the parent cells have been widely used for decorating the NPs surface, providing these nanomaterials with longer blood circulation time, higher biocompatibility, gradual release of the payload, improved cell-cell interactions, and enhanced ability to recognize, bind, and phagocytose tumor cells and microorganisms, such as bacteria and virus. A considerable number of experimental studies have shown the huge potential of MCM-coated NPs for diagnostic, therapeutic and theranostic purposes in a wide range of diseases, from cancer to inflammatory disorders, like sepsis, infections disorders, AD and atherosclerosis, by enabling a preferential and targeted delivery of imaging agents and therapeutic drugs to sites of interest.

However, despite emerging evidence of better therapeutic efficacy and reduced toxicity *in vivo*, these next-generation macrophage-based nanosystems still struggle with some critical barriers that may compromise their clinical application. Thus, given the enormous potential of these nanosystems to revolutionize the therapy and diagnostics of various diseases in the future, these limitations must be urgently addressed for these strategies to be successfully implemented in clinical practice.

## 7. References


1. Li, Y., *et al.*, *Cell membrane-engineered hybrid soft nanocomposites for biomedical applications.* Journal of Materials Chemistry B, 2020. **8**(26): p. 5578-96.

2. Banskota, S., P. Yousefpour, and A. Chilkoti, *Cell-Based Biohybrid Drug Delivery Systems: The Best of the Synthetic and Natural Worlds.* Macromolecular bioscience, 2017. **17**(1).

3. Anselmo, A.C. and S. Mitragotri, *Cell-mediated delivery of nanoparticles: taking advantage of circulatory cells to target nanoparticles.* Journal of controlled release : official journal of the Controlled Release Society, 2014. **190**: p. 531-41.

4. Li, R., *et al.*, *Cell membrane-based nanoparticles: a new biomimetic platform for tumor diagnosis and treatment.* Acta pharmaceutica Sinica B, 2018. **8**(1): p. 14-22.

5. Thanuja, M.Y., C. Anupama, and S.H. Ranganath, *Bioengineered cellular and cell membrane-derived vehicles for actively targeted drug delivery: So near and yet so far.* Adv Drug Deliv Rev, 2018. **132**: p. 57-80.

6. Oroojalian, F., *et al.*, *Immune Cell Membrane-Coated Biomimetic Nanoparticles for Targeted Cancer Therapy.* Small, 2021. **17**(12): p. e2006484.

7. Huang, Y., X. Gao, and J. Chen, *Leukocyte-derived biomimetic nanoparticulate drug delivery systems for cancer therapy.* Acta pharmaceutica Sinica B, 2018. **8**(1): p. 4-13.

8. Fang, R.H., *et al.*, *Cell Membrane Coating Nanotechnology.* Advanced materials, 2018. **30**(23): p. e1706759.

9. Valcourt, D.M., *et al.*, *Advances in targeted nanotherapeutics: From bioconjugation to biomimicry.* Nano Res, 2018. **11**(10): p. 4999-5016.

10. Dash, P., A.M. Piras, and M. Dash, *Cell membrane coated nanocarriers - an efficient biomimetic platform for targeted therapy.* Journal of controlled release: official journal of the Controlled Release Society, 2020. **327**: p. 546-70.

11. Liu, Y., *et al.*, *Cell Membrane Coating Technology: A Promising Strategy for Biomedical Applications.* Nano-Micro Letters, 2019. **11**(1).

12. Kroll, A.V., R.H. Fang, and L. Zhang, *Biointerfacing and Applications of Cell Membrane-Coated Nanoparticles.* Bioconjugate chemistry, 2017. **28**(1): p. 23-32.

13. Gong, P., *et al.*, *Immunocyte Membrane-Coated Nanoparticles for Cancer Immunotherapy.* Cancers, 2020. **13**(1).

14. Wang, Y., *et al.*, *Cell-Membrane-Display Nanotechnology.* Advanced healthcare materials, 2021. **10**(1): p. e2001014.

15. Liu, W.L., *et al.*, *Recent Advances of Cell Membrane-Coated Nanomaterials for Biomedical Applications.* Advanced Functional Materials, 2020. **30**(39): p. 2003559.

16. Yang, J., *et al.*, *Biologically modified nanoparticles as theranostic bionanomaterials.* Progress in Materials Science, 2021. **118**: p. 100768.

17. Hussain, Z., *et al.*, *Cell membrane cloaked nanomedicines for bio-imaging and immunotherapy of cancer: Improved pharmacokinetics, cell internalization and anticancer efficacy.* Journal of controlled release : official journal of the Controlled Release Society, 2021. **335**: p. 130-57.

18. Combes, F., E. Meyer, and N.N. Sanders, *Immune cells as tumor drug delivery vehicles.* Journal of controlled release : official journal of the Controlled Release Society, 2020. **327**: p. 70-87.

19. Yan, H., *et al.*, *Engineering Cell Membrane-Based Nanotherapeutics to Target Inflammation.* Advanced Science, 2019. **6**(15): p. 1900605.

20. Zhou, X., X. Liu, and L. Huang, *Macrophage-Mediated Tumor Cell Phagocytosis: Opportunity for Nanomedicine Intervention.* Adv Funct Mater, 2021. **31**(5).

21. Zargar, S.M., *et al.*, *A Review of Controlled Drug Delivery Systems Based on Cells and Cell Membranes.* Journal of medical signals and sensors, 2019. **9**(3): p. 181-9.

22. Jahromi, L.P., *et al.*, *Chemically Engineered Immune Cell-Derived Microrobots and Biomimetic Nanoparticles: Emerging Biodiagnostic and Therapeutic Tools.* Adv Sci (Weinh), 2021. **8**(8): p. 2002499.

23. Xia, Y., *et al.*, *Engineering Macrophages for Cancer Immunotherapy and Drug Delivery.* Advanced materials, 2020. **32**(40): p. e2002054.

24. Shapouri-Moghaddam, A., *et al.*, *Macrophage plasticity, polarization, and function in health and disease.* Journal of cellular physiology, 2018. **233**(9): p. 6425-40.

25. Sreejit, G., *et al.*, *Origins and diversity of macrophages in health and disease.* Clinical & Translational Immunology, 2020. **9**: p. e1222.

26. Li, C., *et al.*, *Artificial Engineering of Immune Cells for Improved Immunotherapy.* Advanced NanoBiomed Research, 2021. **1**(6): p. 2000081.

27. DeNardo, D.G. and B. Ruffell, *Macrophages as regulators of tumour immunity and immunotherapy.* Nature reviews Immunology, 2019. **19**(6): p. 369-82.

28. Li, Z., *et al.*, *Cell-Based Delivery Systems: Emerging Carriers for Immunotherapy.* Advanced Functional Materials, 2021. **31**(23): p. 2100088.

29. Messex, J.K., C.J. Byrd, and G.Y. Liou, *Signaling of Macrophages that Contours the Tumor Microenvironment for Promoting Cancer Development.* Cells, 2020. **9**(4).

30. Najafi, M., *et al.*, *Macrophage polarity in cancer: A review.* J Cell Biochem, 2019. **120**(3): p. 2756-65.

31. Jimenez-Jimenez, C., M. Manzano, and M. Vallet-Regi, *Nanoparticles Coated with Cell Membranes for Biomedical Applications.* Biology, 2020. **9**(11).

32. Ai, X., *et al.*, *Recent Advances of Membrane-Cloaked Nanoplatforms for Biomedical Applications.* Bioconjugate chemistry, 2018. **29**(4): p. 838-51.

33. Parodi, A., *et al.*, *Bio-inspired engineering of cell- and virus-like nanoparticles for drug delivery.* Biomaterials, 2017. **147**: p. 155-68.

34. Gong, X., *et al.*, *Emerging Approaches of Cell-Based Nanosystems to Target Cancer Metastasis.* Advanced Functional Materials, 2019. **29**(48): p. 1903441.

35. Matlung, H.L., *et al.*, *The CD47-SIRPalpha signaling axis as an innate immune checkpoint in cancer.* Immunological reviews, 2017. **276**(1): p. 145-64.

36. Yang, L. and Y. Zhang, *Tumor-associated macrophages: from basic research to clinical application.* Journal of hematology & oncology, 2017. **10**(1): p. 58.

37. Aalipour, A., *et al.*, *Engineered immune cells as highly sensitive cancer diagnostics.* Nature biotechnology, 2019. **37**(5): p. 531-9.

38. Li, R., *et al.*, *Route to Rheumatoid Arthritis by Macrophage-Derived Microvesicle-Coated Nanoparticles*. Nano letters, 2019. **19**(1): p. 124-34.

39. Xiong, F., *et al.*, *Pursuing Specific Chemotherapy of Orthotopic Breast Cancer with Lung Metastasis from Docking Nanoparticles Driven by Bioinspired Exosomes.* Nano letters, 2019. **19**(5): p. 3256-66.

40. Rayamajhi, S., *et al.*, *Macrophage-derived exosome-mimetic hybrid vesicles for tumor targeted drug delivery.* Acta biomaterialia, 2019. **94**: p. 482-94.

41. Jang, S.C., *et al.*, *Bioinspired Exosome-Mimetic Nanovesicles for Targeted Delivery of Chemotherapeutics to MalignantTumors.* ACS nano, 2013. **7**(9): p. 7698- 710.

42. Molinaro, R., *et al.*, *Biomimetic proteolipid vesicles for targeting inflamed tissues.* Nature materials, 2016. **15**(9): p. 1037-46.

43. Molinaro, R., *et al.*, *Macrophage-derived nanovesicles exert intrinsic anti-inflammatory properties and prolong survival in sepsis through a direct interaction with macrophages.* Nanoscale, 2019. **11**(28): p. 13576-86.

44. Chen, H.Y., *et al.*, *Hybrid cell membrane-coated nanoparticles: A multifunctional biomimetic platform for cancer diagnosis and therapy.* Acta biomaterialia, 2020. **112**: p. 1-13.

45. Gong, C., *et al.*, *Macrophage-cancer hybrid membrane-coated nanoparticles for targeting lung metastasis in breast cancer therapy.* Journal of nanobiotechnology, 2020. **18**(1): p. 92.

46. Vijayan, V., S. Uthaman, and I.K. Park, *Cell Membrane Coated Nanoparticles: An Emerging Biomimetic Nanoplatform for Targeted Bioimaging and Therapy.* Advances in experimental medicine and biology, 2018. **1064**: p. 45-59.

47. He, Z., Y. Zhang, and N. Feng, *Cell membrane-coated nanosized active targeted drug delivery systems homing to tumor cells: A review.* Materials Science and Engineering: C, 2020. **106**: p. 110298.

48. Choi, B., *et al.*, *Recent trends in cell membrane-cloaked nanoparticles for therapeutic applications.* Methods, 2020. **177**: p. 2-14.

49. Vijayan, V., S. Uthaman, and I-K. Park, *Cell Membrane-Camouflaged Nanoparticles: A Promising Biomimetic Strategy for Cancer Theragnostics.* Polymers, 2018. **10**(9): p. 983.

50. Zhai, Y., *et al.*, *Preparation and Application of Cell Membrane-Camouflaged Nanoparticles for Cancer Therapy.* Theranostics, 2017. **7**(10): p. 2575-92.

51. Xu, C.H., *et al.*, *Cell membrane-camouflaged nanoparticles as drug carriers for cancer therapy.* Acta biomaterialia, 2020. **105**: p. 1-14.

52. Shi, M., *et al.*, *An electroporation strategy to synthesize the membrane-coated nanoparticles for enhanced anti-inflammation therapy in bone infection.* Theranostics, 2021. **11**(5): p. 2349-63.

53. Jha, A., *et al.*, *Biomimetic nanoarchitecturing: A disguised attack on cancer cells.* Journal of controlled release : official journal of the Controlled Release Society, 2021. **329**: p. 413-33.

54. Guido, C., *et al.*, *Biomimetic Nanocarriers for Cancer Target Therapy.* Bioengineering, 2020. **7**(3).

55. Chen, Y. and K. Cheng, *Advances of biological-camouflaged nanoparticles delivery system.* Nano Research, 2020. **13**(10): p. 2617-24.

56. Pereira-Silva, M., *et al.*, *Biomimetic cancer cell membrane-coated nanosystems as next-generation cancer therapies.* Expert opinion on drug delivery, 2020. **17**(11): p. 1515-8.

57. Xuan, M., *et al.*, *Macrophage Cell Membrane Camouflaged Mesoporous Silica Nanocapsules for In Vivo Cancer Therapy.* Advanced healthcare materials, 2015. **4**(11): p. 1645-52.

58. Cao, X., *et al.*, *Paclitaxel-Loaded Macrophage Membrane Camouflaged Albumin Nanoparticles for Targeted Cancer Therapy.* International journal of nanomedicine, 2020. **15**: p. 1915-28.

59. Zhang, Y., *et al.*, *Macrophage-Membrane-Coated Nanoparticles for Tumor-Targeted Chemotherapy.* Nano letters, 2018. **18**(3): p. 1908-15.

60. Cao, H., *et al.*, *Liposomes Coated with Isolated Macrophage Membrane Can Target Lung Metastasis of Breast Cancer.* ACS nano, 2016. **10**(8): p. 7738-48.

61. Li, Y., *et al.*, *Fabricating an intelligent cell-like nano-prodrug via hierarchical self-assembly based on the DNA skeleton for suppressing lung metastasis of breast cancer.* Biomaterials science, 2019. **7**(9): p. 3652-61.

62. Sun, K., *et al.*, *Saikosaponin D loaded macrophage membrane-biomimetic nanoparticles target angiogenic signaling for breast cancer therapy.* Applied Materials Today, 2020. **18**: p. 100-505.

63. Bhattacharyya, S. and S.S. Ghosh, *Transmembrane TNFalpha-Expressed Macrophage Membrane-Coated Chitosan Nanoparticles as Cancer Therapeutics.* ACS omega, 2020. **5**(3): p. 1572-80.

64. Chen, X., *et al.*, *Orchestration of biomimetic membrane coating and nanotherapeutics in personalized anticancer therapy.* Biomaterials science, 2021. **9**(3): p. 590-625.

65. Raza, F., *et al.*, *Recent Advances in Cell Membrane-Derived Biomimetic Nanotechnology for Cancer Immunotherapy.* Advanced healthcare materials, 2021. **10**(6): p. e2002081.

66. Chen, Z., Q. Hu, and Z. Gu, *Leveraging Engineering of Cells for Drug Delivery.* Accounts of chemical research, 2018. **51**(3): p. 668-77.

67. Zhang, Q., *et al.*, *Biomimetic Magnetosomes as Versatile Artificial Antigen-Presenting Cells to Potentiate T-Cell-Based Anticancer Therapy.* ACS nano, 2017. **11**(11): p. 10724-32.

68. Liu, L., *et al.*, *A Biomimetic Polymer Magnetic Nanocarrier Polarizing Tumor-Associated Macrophages for Potentiating Immunotherapy.* Small, 2020. **16**(38): p. e2003543.

69. Zhen, X., P. Cheng, and K. Pu, *Recent Advances in Cell Membrane-Camouflaged Nanoparticles for Cancer Phototherapy.* Small, 2019. **15**(1): p. e1804105.

70. Wu, M., *et al.*, *Cell membrane camouflaged nanoparticles: a new biomimetic platform for cancer photothermal therapy.* International journal of nanomedicine, 2019. **14**: p. 4431-48.

71. Meng, Q.F., *et al.*, *Macrophage membrane-coated iron oxide nanoparticles for enhanced photothermal tumor therapy.* Nanotechnology, 2018. **29**(13): p. 134004.

72. Xuan, M., *et al.*, *Macrophage Cell Membrane Camouflaged Au Nanoshells for in Vivo Prolonged Circulation Life and Enhanced Cancer Photothermal Therapy.* ACS applied materials & interfaces, 2016. **8**(15): p. 9610-8.

73. Xuan, M., *et al.*, *Self-Propelled Nanomotors for Thermomechanically Percolating Cell Membranes.* Angewandte Chemie, 2018. **57**(38): p. 12463-7.

74. Lai, J., *et al.*, *Scaffolds biomimicking macrophages for a glioblastoma NIR-Ib imaging guided photothermal therapeutic strategy by crossing Blood-Brain Barrier.* Biomaterials, 2019. **211**: p. 48-56.

75. Zhao, H., *et al.*, *C-C Chemokine Ligand 2 (CCL2) Recruits Macrophage-Membrane-Camouflaged Hollow Bismuth Selenide Nanoparticles To Facilitate Photothermal Sensitivity and Inhibit Lung Metastasis of Breast Cancer.* ACS applied materials & interfaces, 2018. **10**(37): p. 31124-35.

76. Ji, B., *et al.*, *Hybrid membrane camouflaged copper sulfide nanoparticles for photothermal-chemotherapy of hepatocellular carcinoma.* Acta biomaterialia, 2020. **111**: p. 363-72.

77. Hu, C., *et al.*, *Phagocyte-membrane-coated and laser-responsive nanoparticles control primary and metastatic cancer by inducing anti-tumor immunity.* Biomaterials, 2020. **255**: p. 120159.

78. Liu, R., *et al.*, *Macrophage-mimic shape changeable nanomedicine retained in tumor for multimodal therapy of breast cancer.* Journal of controlled release : official journal of the Controlled Release Society, 2020. **321**: p. 589-601.

79. Poudel, K., *et al.*, *Macrophage-Membrane-Camouflaged Disintegrable and Excretable Nanoconstruct for Deep Tumor Penetration.* ACS applied materials & interfaces, 2020. **12**(51): p. 56767-81.

80. Rao, L., *et al.*, *Effective cancer targeting and imaging using macrophage membrane-camouflaged upconversion nanoparticles.* Journal of biomedical materials research Part A, 2017. **105**(2): p. 521-30.

81. Li, T., *et al.*, *Cell Membrane Coated-Biomimetic Nanoplatforms Toward Cancer Theranostics.* Frontiers in bioengineering and biotechnology, 2020. **8**: p. 371.

82. Liang, B., *et al.*, *Biomimetic theranostic strategy for anti-metastasis therapy of breast cancer via the macrophage membrane camouflaged superparticles.* Materials science & engineering C, Materials for biological applications, 2020. **115**: p. 111097.

83. Chen, L-J., *et al.*, *Macrophage membrane coated persistent luminescence nanoparticle@MOF-derived mesoporous carbon core-shell nanocomposites for autofluorescence-free imaging-guided chemotherapy.* Journal of the American Chemical Society, 2020. **8**: p. 8071-83.

84. Girigoswami, A., *et al.*, *Camouflaged Nanosilver with Excitation Wavelength Dependent High Quantum Yield for Targeted Theranostic.* Scientific reports, 2018. **8**(1): p. 16459.

85. Xiong, K., *et al.*, *Biomimetic Immuno-Magnetosomes for High-Performance Enrichment of Circulating Tumor Cells.* Advanced materials, 2016. **28**(36): p. 7929-35.

86. Wang, Y., *et al.*, *Macrophage membrane functionalized biomimetic nanoparticles for targeted anti-atherosclerosis applications.* Theranostics, 2021. **11**(1): p. 164-80.

87. Wu, G., *et al.*, *A self-driven bioinspired nanovehicle by leukocyte membrane-hitchhiking for early detection and treatment of atherosclerosis.* Biomaterials, 2020. **250**: p. 119963.

88. Han, Y., *et al.*, *Macrophage membrane-coated nanocarriers Co-Modified by RVG29 and TPP improve brain neuronal mitochondria-targeting and therapeutic efficacy in Alzheimer's disease mice.* Bioactive materials, 2021. **6**(2): p. 529-42.

89. Thamphiwatana, S., *et al.*, *Macrophage-like nanoparticles concurrently absorbing endotoxins and proinflammatory cytokines for sepsis management.* Proceedings of the National Academy of Sciences of the United States of America, 2017. **114**(43): p. 11488-93.

90. Wang, C., *et al.*, *Pretreated Macrophage-Membrane-Coated Gold Nanocages for Precise Drug Delivery for Treatment of Bacterial Infections.* Advanced materials, 2018. **30**(46): p. e1804023.

91. Li, Y., *et al.*, *Coating of a Novel Antimicrobial Nanoparticle with a Macrophage Membrane for the Selective Entry into Infected Macrophages and Killing of Intracellular Staphylococci.* Advanced Functional Materials, 2020. **30**(48): p. 2004942.

92. Wei, X., *et al.*, *Multiantigenic Nanotoxoids for Antivirulence Vaccination against Antibiotic-Resistant Gram-Negative Bacteria*. Nano letters, 2019. **19**(7): p. 4760-9.

93. Pereira-Silva, M., *et al.*, *Unleashing the potential of cell membrane-based nanoparticles for COVID-19 treatment and vaccination.* Expert opinion on drug delivery, 2021. p. 1-20.

94. Li, B., *et al.*, *Antiviral and Anti-Inflammatory Treatment with Multifunctional Alveolar Macrophage-Like Nanoparticles in a Surrogate Mouse Model of COVID-19.* Advanced Science, 2021. **8**

95. Tan, Q., *et al.*, *Macrophage biomimetic nanocarriers for anti-inflammation and targeted antiviral treatment in COVID-19.* Journal of nanobiotechnology, 2021.**19**(1): p. 173.

# 8. Appendixes

## Appendix I

**Table 3 -** Some biomedical applications of the nanosystems coated with a single macrophage cell membrane (MCM) or hybrid MCM in the field of cancer therapy and diagnosis.

| Application | Cell membrane cloaking | Inner core | Cargo(es) | Coating method | Tumor model | Principal outcomes | Ref. |
|---|---|---|---|---|---|---|---|
| **Chemotherapy delivery to primary tumors** | RAW 264.7 macrophage membrane | Mesoporous silica nanocapsule (MSNC) | Doxorubicin (DOX) | Co-extrusion through 100 nm porous membrane (20 times) | 4T1 breast cancer mouse model | • Encapsulation efficiency > 35,7 %<br>• Prolonged blood circulation time<br>• Specific accumulation at tumor tissues<br>• Effective cancer ablation with chemotherapy | [57] |
| | RAW 264.7 macrophage membrane | Albumin nanoparticle (NP) | Paclitaxel (PTX) | Co-extrusion through 200 nm porous membrane | B16F10 melanoma mouse model | • Immune evasion<br>• Superior antitumor efficacy<br>• Targeted chemotherapy against malignant melanoma *in vivo* | [58] |
| | Macrophage membrane | pH responsive polymer-based NP funcionalized with a targeting ligand | PTX | Co-extrusion | Orthotopic MDA-MB-231 breast cancer mouse model | • Controlled and stepwise release of PTX in the acidic pH within the tumor microenvironment (TME)<br>• Active tumor targeting ability | [59] |
| **Antimetastatic therapy** | RAW 264.7 macrophage membrane | Liposome | Emtansine | Co-extrusion through 400 nm and 200 nm porous membrane | 4T1 breast cancer mice model with lung metastasis | • Efficient reduction of macrophage uptake and good biocompatibility<br>• Efficient drug uptake by metastatic 4T1 breast cancer cells in the lung tissue<br>• Efficient suppression of lung metastasis from breast cancer | [60] |
| | RAW 264.7 macrophage membrane | DNA tetrahedron dendrimer-Liposome | DOX prodrug (DOX-MPK) | Co-extrusion through 200 nm porous membrane | 4T1 breast cancer mice model with lung metastasis | • Selective accumulation at sites of lung metastasis<br>• 2.1- fold increase in lung accumulation compared to non-coated NPs after 4 h of administration<br>• Controlled DOX release in response to the acidic pH within the TME | [61] |
| **Antiangiogenic therapy** | T7 peptide- inserted macrophage membrane | Poly (lactic-*co*-glycolic acid) (PLGA) NP | Saikosaponin D | Co-extrusion | 4T1 breast cancer mouse model | • Targeted anti-angiogenic therapy with minimal side effects<br>• Suppression of primary breast cancer growth and lung metastasis | [62] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Antiproliferative cancer therapy** | TNF-α- attached macrophage membrane | Chitosan NP | - | Co-extrusion through 200 nm porous membrane | MDA-MB-231, MCF-7 and HeLa cancer cell lines | • Good biocompatibility<br>• Efficient cytotoxic effects against different cancer cells lines *in vitro* | [63] |
| **Cancer immunotherapy** | Azide-attached macrophage membrane | $Fe_3O_4$ magnetic nanocluster | - | Electrostatic interaction | EG-7 tumor-bearing mice model | • Promising as antigen-presenting cell to CD8+T cells *ex vivo*<br>• Efficient accumulation at tumor sites with magnetic guidance *in vivo*<br>• Good magnetic resonance imaging (MRI) properties *in vivo* | [67] |
| | Bacterial lipopolysaccharide (LPS)-induced M1 macrophage membrane | PLGA NP | $Fe_3O_4$ NP and imiquimod (R87) | Co-extrusion through 200 nm porous membrane | Orthotopic 4T1 breast cancer mice model | • Synergistic effects for stronger antitumor immunity<br>• Good M2-to -M1 macrophage conversion efficiency | [68] |
| **Photothermal therapy (PTT)** | RAW 264.7 macrophage membrane | $Fe_3O_4$ NP | - | Co-extrusion (11 times) | Xenograft mouse model of MCF-7 breast cancer | • Higher biocompatibility<br>• Longer circulation time<br>• Effective photothermal cancer ablation *in vivo* | [71] |
| | Macrophage membrane | Gold nanoshell (AuNS) | Near-infrared (NIR) fluorescent dye cyanine 7 (Cy7) | Sonication and extrusion through 200 nm porous membrane | 4T1 breast cancer-bearing mice model | • Potential for fluorescence imaging and tumor-targeted PTT *in vivo*<br>• Efficient suppression of 4T1 tumor growth (almost complete tumor eradication after 25 days of treatment) | [72] |
| | Macrophage membrane | Janus mesoporous silica nanomotor | - | Sonication (100 W) | - | • Superior uptake by 4T1 tumor cells *in vitro* and immune escape<br>• Photothermal effects *in vitro* | [73] |
| | Macrophage membrane | DSPE-PEG liposome | NIR-Ib fluorescence IR-792 dye | Co-extrusion (20 times) | Orthotopic U87L glioblastoma - bearing mice model | • Efficient blood-brain barrier (BBB) penetration ability and tumor homing<br>• Photothermal cancer ablation with NIR-Ib fluorescence imaging guidance<br>• Extended survival time of glioblastoma-bearing mice to 22 days (superior to other groups) | [74] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Macrophage membrane | Hollow bismuth selenide NP | Quercetin | Co-extrusion | 4T1 breast cancer mice model with lung metastasis | • Dual tumor targeting ability through CCR2/CCL2 recruitment and α4β1 integrin /VCAM-1 interaction<br>• Good *in vivo* X-ray computed tomography (CT) and infrared thermal (IRT) imaging performance<br>• Photothermal effect<br>• Synergistic effects for potentiating PTT *in vivo* and inhibiting lung metastasis from breast cancer | [75] |
| | RAW 264.7 macrophage-hepatic cancer cell (H22) hybrid membrane | Cooper sulfide (CuS) NP | Sorafenib | Sonication | Hepatocellular carcinoma-bearing mice model | • Higher homotypic tumor targeting ability and immune evasion<br>• Synergistic chemo-PTT<br>• Good NIR absorption capacity for photothermal tumor ablation | [76] |
| **Photodynamic therapy (PDT)** | LPS -induced M1 macrophage membrane | PEGylated bilirubin NP | DOX, indoximod (IND) and chlorin e6 (Ce6) | Sonication (100 W, 2 min) and extrusion | B16F10 and 4T1 cancer- bearing mice models | • Tumor-targeted co-delivery of DOX, IND and Ce6<br>• Efficient reactive oxygen species (ROS) generation ability under NIR light irradiation<br>• Synergistic effects for inducing strong anticancer immune responses | [77] |
| | LPS-induced M1 macrophage membrane | bilirubin/Ce6 core | PTX, IND and Ce6 | Sonication (65W, 5 s) | 4T1 breast cancer-bearing mice model | • Tumor-targeted co-delivery of PTX, IND and Ce6<br>• Promising for multimodal therapy through immunotherapy, chemotherapy and PDT, resulting in efficient suppression of primary tumor growth and lung metastasis | [78] |
| | RAW 264.7 macrophage membrane | CuS NP | PTX | Co-extrusion through 200 nm porous membrane (20 times) | 4T1 breast cancer-bearing mice model | • Superior uptake by 4T1 cancer cells through the synergistic targeting effects of α4β1 integrin and iRGD peptide<br>• Good NIR absorption capacity for ROS and heat generation<br>• Effective tumor eradication through PTT, PDT and chemotherapy | [79] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Cancer imaging** | RAW 264.7 macrophage membrane | Upconverting NP (UCNP) | - | Co-extrusion through 200 nm porous membrane | Xenograft mouse model of MCF-7 breast cancer | • Immune evasion<br>• Enhanced biocompatibility *in vivo*<br>• Superior active tumor targeting ability<br>• Good performance of the fluorescent UCNP, resulting in efficient cancer imaging *in vivo* | [80] |
| **Cancer theranostics** | RAW 264.7 macrophage membrane | Liposome | DOX and quaternary quantum dots (QDs) | Co-extrusion through 200 nm porous membrane | 4T1 breast cancer mice model with lung metastasis | • Capacity to load compounds with different hydrophobicity<br>• Tumor-targeted fluorescent imaging *in vivo* and efficient tumor ablation | [82] |
| | J774A.1 macrophage membrane | Persistence luminescence NP (PLNP)-based inner core | PTX | - | SCC-7 squamous epithelial cancer mice model | • Superior drug loading capacity<br>• Effective chemotherapy with luminescence imaging guidance | [83] |
| | Macrophage membrane | Silver nanocluster (AgNC) | - | Sonication (1h) | Dalton Lymphoma Ascites (DLA) tumor-bearing mice model | • Efficient cytotoxicity against DLA tumor cells *in vitro*<br>• Good fluorescent imaging properties *in vivo*<br>• Potential for DLA tumor-targeted theranostics | [84] |
| **Capture of circulating tumor cells (CTCs)** | Azide-attached macrophage membrane | $Fe_3O_4$ magnetic nanocluster | - | Electrostatic interaction | - | • Efficient capture of CTCs *in vitro* without interference from leucocytes interaction | [85] |

## Appendix II

**Table 4 -** Biomedical applications of macrophage cell membrane (MCM)- coated nanosystems in several diseases, including atherosclerosis, Alzheimer's disease (AD), sepsis, bacterial infections and viral infections.

| Application | Cell membrane cloaking | Inner core | Cargo(es) | Coating method | Mice model | Principal outcomes | Ref. |
|---|---|---|---|---|---|---|---|
| **Atherosclerosis** | RAW 264.7 macrophage membrane | Poly (lactic-*co*-glycolic acid) nanoparticle (PLGA NP) | Rapamycin | Sonication (100 W for 3 min) and extrusion (10 times) | ApoE deficient (Apo E-) mice with atherosclerosis | • Good biocompatibility<br>• Increased targeting ability to atherosclerotic plaques<br>• Inhibition of atherosclerosis progression without significant toxicity | [86] |
| | Simvastatin-embedeed apolipoprotein A-I mimetic 4F peptide (AP)-attached J774A.1 macrophage membrane | $Fe_3O_4$ magnetic nanocluster | - | Electrostatic interaction | ApoE- mice with early atherosclerotic lesions | • Early detection of atherosclerosis *via* magnetic resonance imaging (MRI)<br>• Synergistic effects for efficient atherosclerosis treatment | [87] |
| **Alzheimer´s disease (AD)** | Peritoneal macrophage membrane funcionalized with rabies virus glycoprotein (RVG29) and triphenylphosphine (TPP) | Solid lipid NP | Genistein | Co-extrusion through 100 nm porous membrane (≥ 5 times) | AD mice model (APP/PS1 transgenic mice model) | • Higher blood-brain barrier (BBB) penetration ability and neuronal mitochondria targeting<br>• Efficient mitochondrial reactive oxygen species (ROS) elimination for AD treatment | [88] |
| **Sepsis** | J774A.1 macrophage membrane | PLGA NP | - | Sonication (100 W for 2 min) | Mouse bacteremia model caused by *Escherichia coli* | • Efficient bacterial lipopolysaccharide (LPS) and cytokines absorption<br>• Superior outcomes in sepsis management<br>• Extended survival time of mice | [89] |
| **Bacterial infections** | Macrophage membrane pretreated with *Staphylococcus aureus* | Gold-silver nanocage (GSNC) | - | Co-extrusion | Local infection mouse model caused by *S. aureus* | • Higher bacterial recognition ability<br>• Good near-infrared (NIR) absorption capacity for enhanced photothermal therapy (PTT)<br>• Promising for treatment of local bacterial infection | [90] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | J774A.1 macrophage membrane | Antimicrobial-conjugated NP (ANP) | Triclosan and ciprofloxacin | Sonication (40 s) | Mouse acute peritoneal infection model | • Efficient treatment of intracellular *S. aureus* infection<br>• Superior antibacterial efficacy with a 4-fold reduction in peritoneal bacterial burden compared to control group | [91] |
| | J774A.1 macrophage 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<br>• 2-fold reduction in bacterial burden in the lungs after 35 days of subcutaneous vaccination | [92] |
| | RAW 264.7 macrophage 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 | • Superior bacterial recognition ability<br>• Cytokines and bacterial LPS uptake for superior antibacterial treatment<br>• Efficient ROS generation ability under ultraviolet (UV) light irradiation<br>• Bone tissue regeneration | [52] |
| **Viral infections** | Alveolar macrophage membrane | PLGA NP | 2PTE-2NDTA | Sonication | Surrogate model of COVID-19 by murine coronavirus | • Photothermal viral ablation<br>• Inflammatory cytokines uptake<br>• Efficient reduction of viral load in the lungs | [94] |
| | RAW 264.7 macrophage membrane | PLGA NP | Lopinavir | Sonication (100 W for 5 min) | Mouse model of coronavirus infection | • Efficient alleviation of inflammatory responses caused by coronavirus infection<br>• Reduction of viral load<br>• Extended survival time of infected mice to 60% | [95] |

## Appendix III

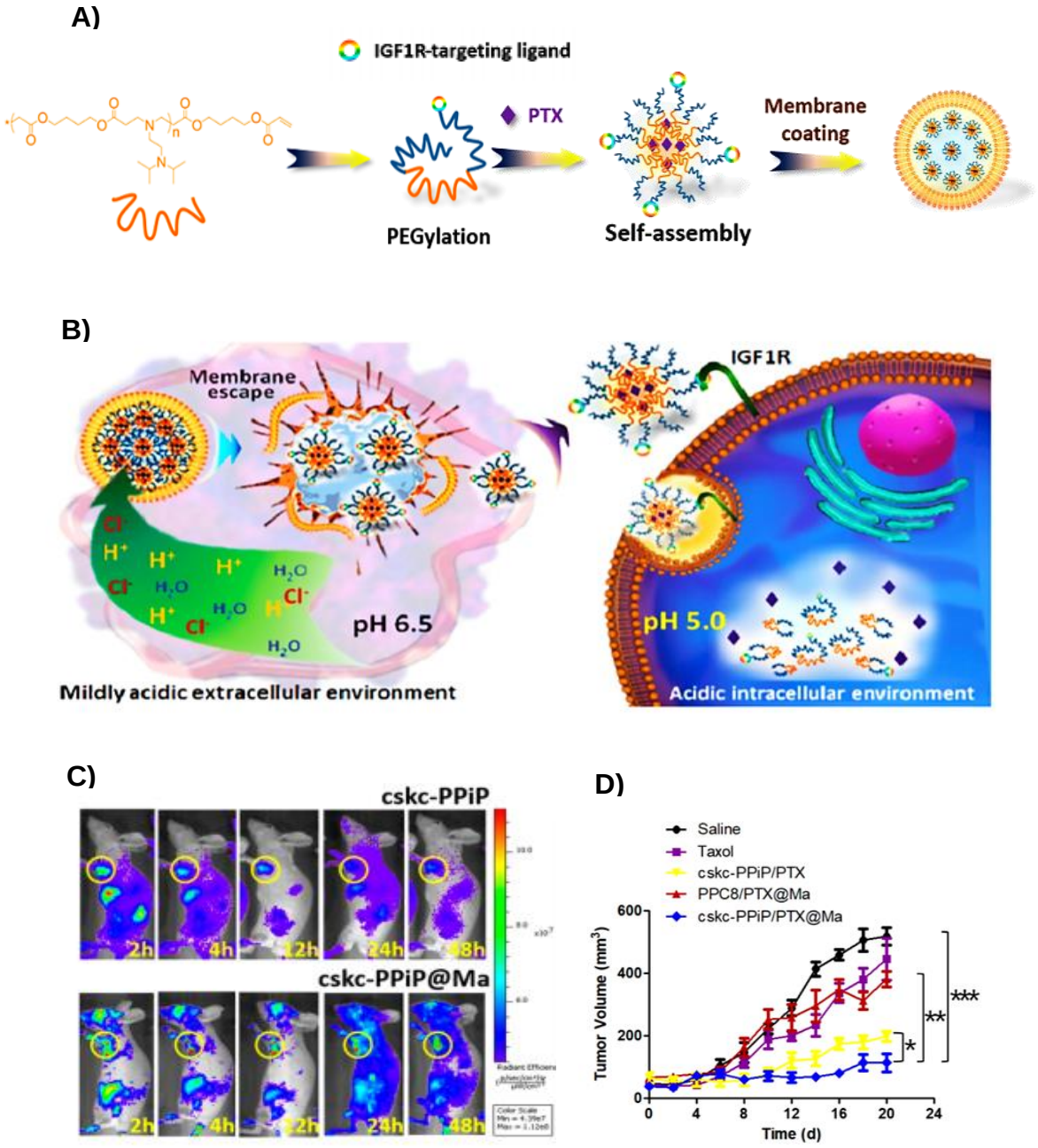


**Figure 5 -** A) Schematic illustration of cskc-PPiP/PTX@Ma preparation. B) Representation of cell membrane disruption under the acidic conditions of the TME, membrane escape, active tumor-targeting *via* IGF1R signaling and drug delivery to tumor cells. C) IVIS images obtained at different times after injection of NIR 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 from reference[59]. Copyright American Chemical Society (2018).

**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; TME, tumor microenvironment.

## Appendix IV

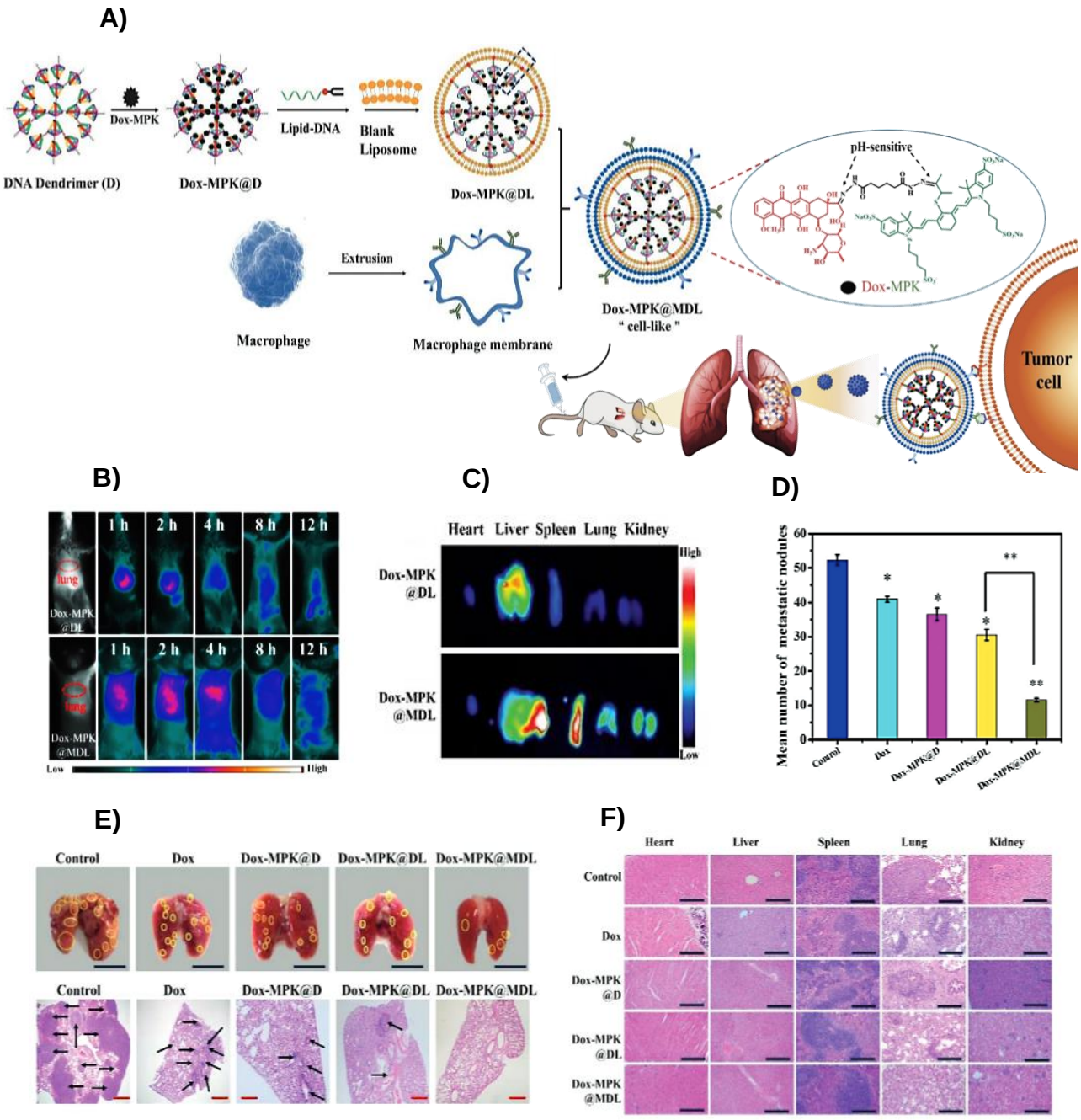


**Figure 6 -** A) Schematics of macrophage-biomimetic nanosystem (DOX-MPK@MDL) preparation by a self-assembly technique to actively target metastatic 4T1 tumor cells and suppress lung metastasis from breast cancer. B) *In vivo* distribution of the nanosystems assessed by fluorescence imaging. C) *Ex 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. E) Photographs of mice lungs (metastatic nodules are delimitated by yellow circles) and H&E staining imagens of lung tissue (metastatic lung lesions are indicated by black arrows). F) Histological analysis of the major organs (heart, liver, spleen, lung and kidney) after each treatment. Reproduced with permission from reference [61]. Copyright Royal Society of Chemistry (2019).
**Abbreviations:** DOX, doxorubicin; DOX-MPK, DOX prodrug.

## Appendix V

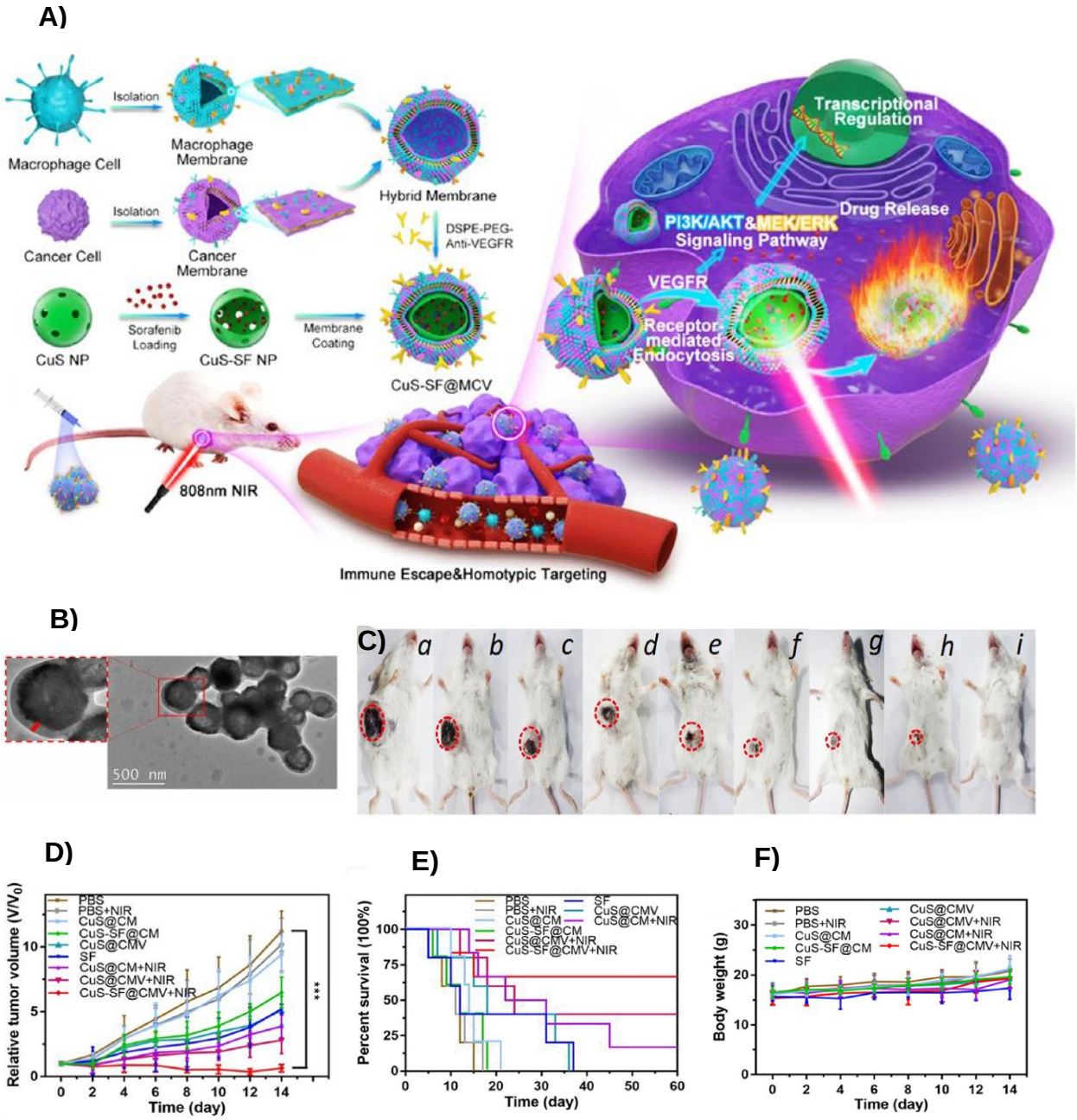


**Figure 7 -** A) Schematics for the preparation of CuS-SF@MCV for synergistic chemo-PTT in a mouse model of hepatocellular carcinoma. B) TEM images of CuS-SF@MCV showing the core-shell nanostructure. C) Comparative images of tumor size in tumor-bearing mice after different treatments, with CuS-SF@MCV 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 from reference [76]. Copyright Elsevier (2020).

**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; VEGFR, vascular endothelial growth factor receptor.

## Appendix VI

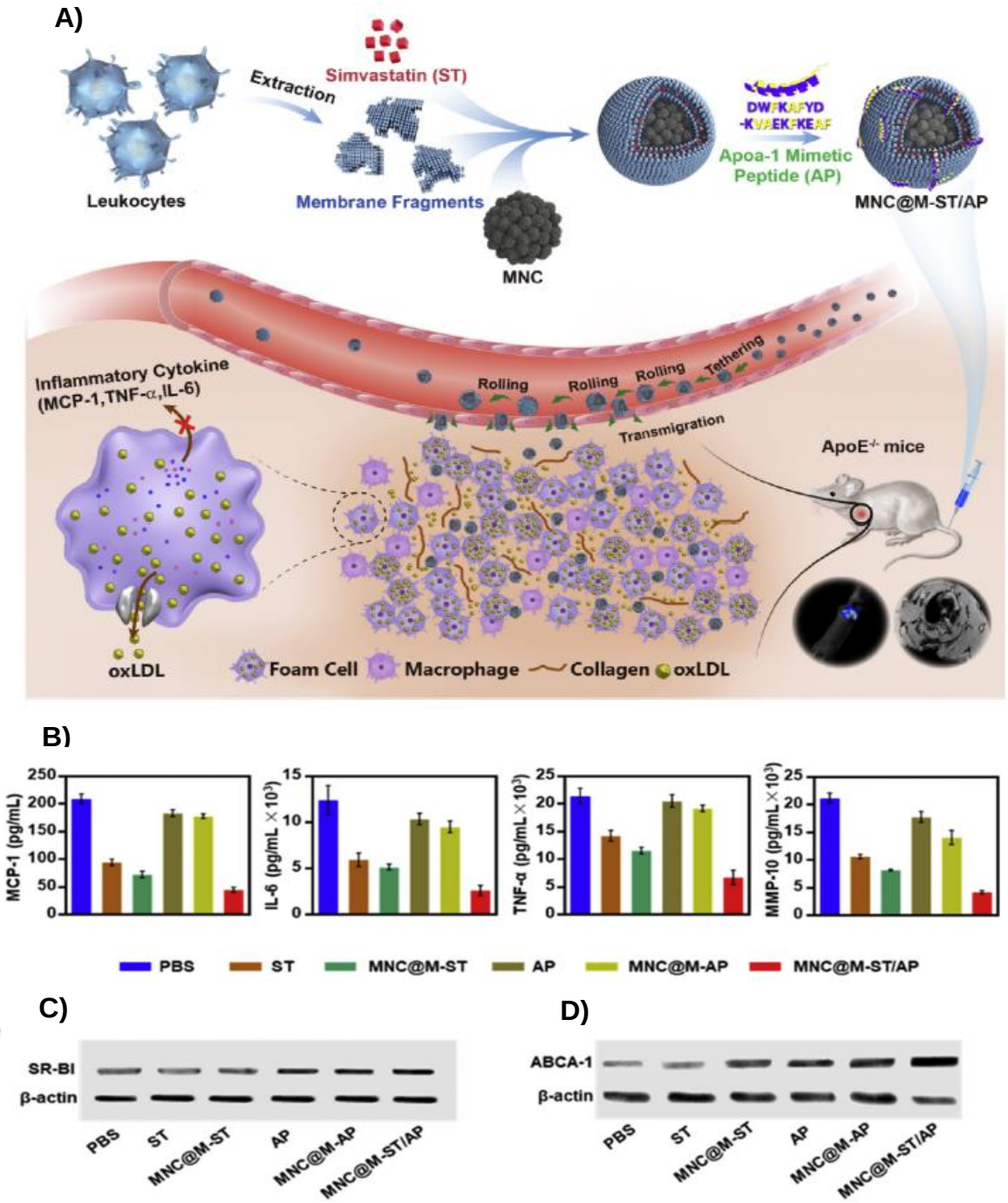


**Figure 8 -** A) Schematics for the preparation of MNC@M-ST/AP for early detection of atherosclerosis *via* MRI and targeted therapy by simvastatin and AP. The anti-inflammatory drug simvastatin alleviates inflammation by reducing the levels of inflammatory cytokines whereas AP induces oxLDL efflux from foam cells. B) Levels of inflammatory cytokines (MCP-1, IL-6, TNF-α and MMP-10) in an ApoE- mice model with early atherosclorotic lesions for different treatments. C-D) Levels of the cholesterol-efflux receptors (SR-BI and ABCA-1) in an ApoE- mice model with early atherosclorotic lesions for different treatments. Reproduced with permission from reference[87]. Copyright Elsevier (2020). **Abbreviations:** MNC, magnetic nanocluster; MRI, magnetic resonance imaging; oxLDL, oxidized low-density lipoprotein.

## Appendix VII

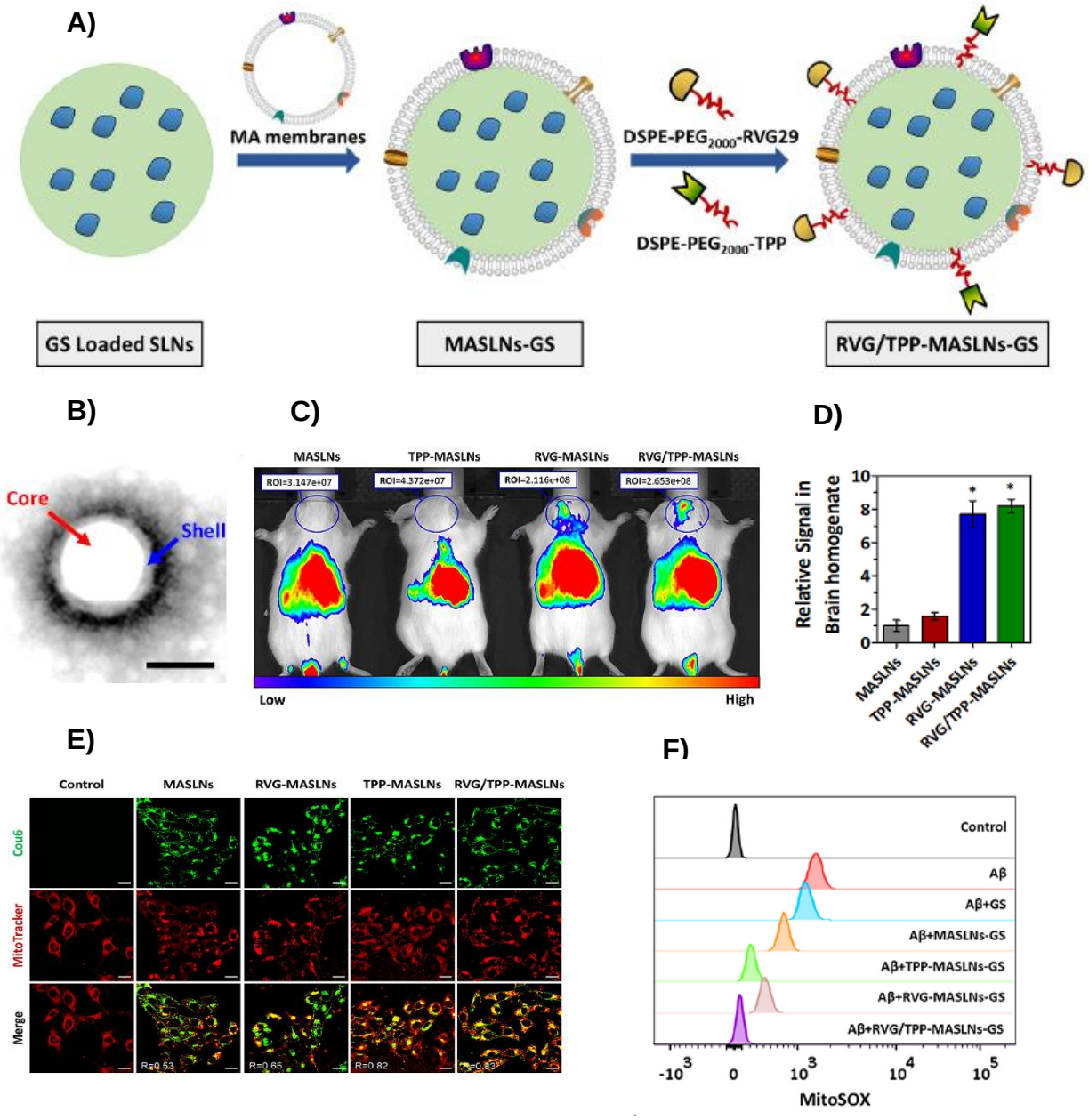


**Figure 9 -** A) Schematics for the preparation of RVG/TPP-MASLNs-GS for AD treatment. B) TEM images of RVG/TPP-MASLNs-GS showing the core-shell nanostructure. C) *In vivo* brain distribution of different formulations. D) Relative fluorescence signals of brain obtained for different treatments. E) Evaluation 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. Reproduced with permission from reference [88]. Copyright KeAi Publishing Communications Ltd. (2021). **Abbreviations:** Aβ, beta-amyloid; AD, Alzheimer's disease; 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 co-funcionalized with RVG29 and TPP; RVG29, rabies virus glycoprotein; SLN, solid lipid nanoparticle; TPP, triphenylphosphine.

## Appendix VIII

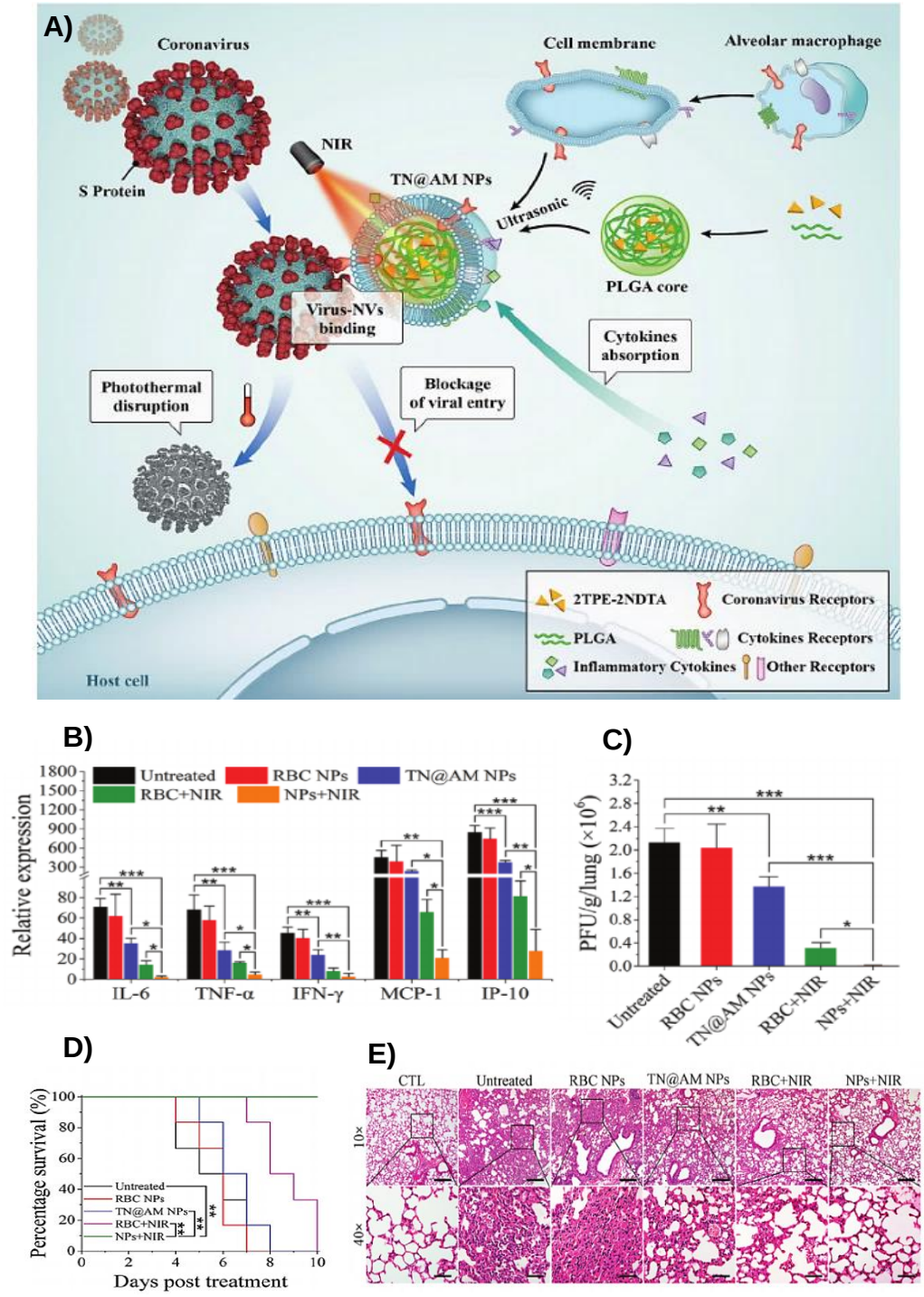


**Figure 10 -** A) Preparation of alveolar macrophage- like PLGA NPs (TN@AM NPs) for targeted PTT against coronavirus infection. B) Relative mRNA expression for IL-6, TNF-α, IFN- γ, MCP-1 and IP-10 (inflammatory cytokines) in the lungs. C) Representation of lung viral count after 5 days of different treatments. D) Analysis of mice survival (%) after each treatment. E) H&E staining imagens of pulmonary tissue recorder after 5 days of different treatments. Reproduced with permission from reference [94]. Copyright Wiley-VCH Verlag (2021). **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 2PTE-2NDTA.

## Appendix IX

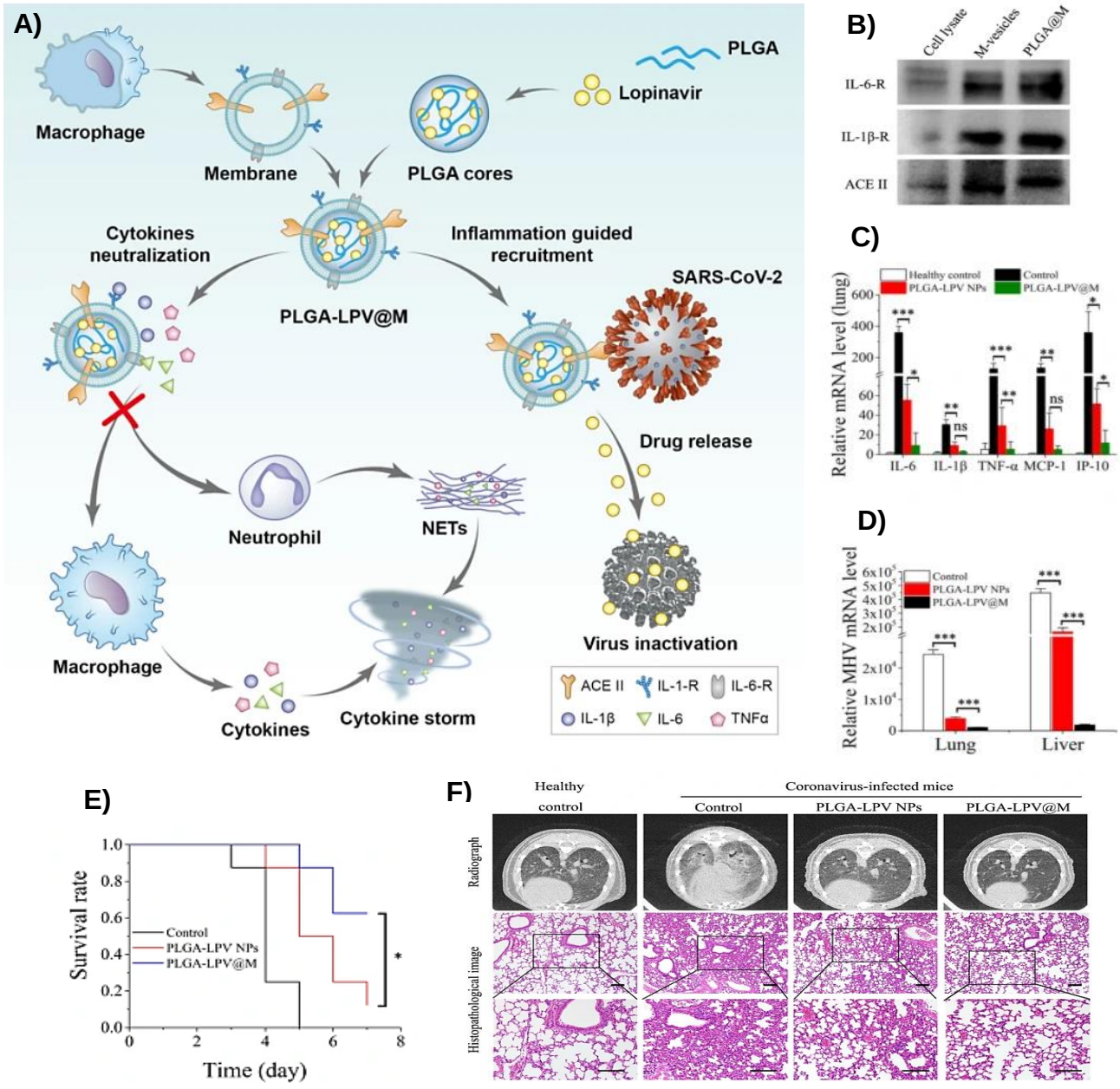


**Figure 11 -** A) Schematics for the preparation of PLGA-LPV@M for antiviral and anti-inflammatory therapy against coronavirus infection. B) Expression of three surface markers (IL-6R, IL-1β R and ACEII) assessed by western blotting. C) Relative mRNA expression for IL-6, IL-1β, TNF-α, MCP-1 and IP-10 (inflammatory 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 from reference [95]. Copyright BioMed Central Ltd. (2021). **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