UNIVERSIDADE D
COIMBRA

Daniela Catarina Simões Lopes

Relatórios de Estágio e Monografia intitulada "Exosome 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

Daniela Catarina Simões Lopes

Relatórios de Estágio e Monografia intitulada "Exosome 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, Daniela Catarina Simões Lopes, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2016228645, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Exosome 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.

Daniela Catarina Simões Lopes

(Daniela Catarina Simões Lopes)

Agradecimentos

À minha orientadora, Professora Doutora Ana Cláudia Santos, pela disponibilidade, apoio e orientação ao longo da construção da presente monografia.

A toda a equipa da Farmácia Medeiros, e em especial à Dra. Carolina Marques, pela oportunidade de estágio, disponibilidade e por todos os ensinamentos e valores transmitidos ao longo do período de estágio, proporcionando-me a melhor experiência em Farmácia Comunitária que podia ter tido.

A toda a equipa dos Serviços Farmacêuticos do CHUC, e em especial à Doutora Marília João Rocha, pela excelente integração, disponibilidade, apoio, orientação e ensinamentos transmitidos ao longo do estágio curricular.

Aos meus pais, irmãos, irmã, avós e restantes familiares pela força e apoio incondicional que me deram ao longo desta caminhada, por estarem sempre do meu lado e por me incentivarem a dar o meu melhor em todos os momentos.

Aos amigos incríveis que Coimbra me deu, que com certeza levarei para o resto da minha vida, por todos os momentos vividos e por partilharem comigo esta experiência única de vida.

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

A ti, Coimbra, por me teres proporcionado a melhor experiência académica que podia ter vivido.

A todos aqueles que me acompanharam ao longo destes 5 anos, e que de alguma forma contribuíram para que fosse possível a concretização desta etapa.

Muito obrigada!

Índice

Parte I - Relatório de Estágio em Farmácia Comunitária

Parte II - Relatório de Estágio em Farmácia Hospitalar

<u>Parte III</u> - Monografia: "Exosome Membrane-Coated Nanosystems for Biomedical Applications"

Parte I

Relatório de Estágio em Farmácia Comunitária

Farmácia Medeiros

Farmácia
Medeiros

Abreviaturas

FFUC - Faculdade de Farmácia da Universidade de Coimbra

MICF - Mestrado Integrado em Ciências Farmacêuticas

PIM - Preparação Individualizada da Medicação

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-se como um local privilegiado de prestação de cuidados de saúde à população, sendo que o farmacêutico comunitário é muitas vezes o primeiro profissional de saúde a quem os utentes recorrem para resolver os seus problemas de saúde. O farmacêutico comunitário, enquanto um profissional de saúde especialista do medicamento e agente de saúde pública, assume um papel imprescindível para garantir o uso seguro, eficaz e racional do medicamento e promover a saúde e o bem-estar da população, através da prestação de um aconselhamento farmacológico e não farmacológico personalizado e adequado a cada situação. Assim, o farmacêutico tem o dever de prestar os melhores cuidados de saúde aos utentes e de exercer a sua atividade profissional de forma ética, responsável e acompanhada de uma permanente atualização técnica e científica [1].

O plano curricular do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) culmina com a realização de um estágio curricular obrigatório em Farmácia Comunitária, visando aproximar os estudantes da realidade profissional, e experienciar na prática, uma das profissões do setor farmacêutico. O estágio curricular em Farmácia Comunitária oferece aos estudantes a oportunidade de aplicar e consolidar os conhecimentos teóricos adquiridos ao longo do percurso académico, e adquirir competências e experiência profissional fundamentais para o exercício da profissão.

Neste contexto, o meu estágio curricular em Farmácia Comunitária foi realizado na Farmácia Medeiros, tendo o mesmo sido iniciado em janeiro e terminado em abril de 2021, com a duração total de 648h, sob orientação da Dra. Carolina Marques. A escolha da Farmácia Medeiros para a realização do estágio curricular em Farmácia Comunitária teve em conta um conjunto de fatores, nomeadamente o facto de ter realizado anteriormente um estágio extracurricular nesta farmácia, o que me permitiu ter um conhecimento prévio da equipa técnica e dos procedimentos de funcionamento interno da farmácia, e também a proximidade da mesma à minha zona de residência.

O presente relatório de estágio compreende uma análise SWOT (*Strenghts*, *Weaknesses*, *Opportunities*, *Threats*), na qual pretendo descrever numa perspetiva interna os pontos fortes (*Strenghts*) e os pontos fracos (*Weaknesses*), e numa perspetiva externa as oportunidades (*Opportunities*) e as ameaças (*Threats*) com que me deparei ao longo do estágio.

2. Farmácia Medeiros

Fundada em 1905, a Farmácia Medeiros localizada na vila do Avelar, na Praça Costa Rego n.º 130, pertencente ao concelho de Ansião e distrito de Leiria, é uma farmácia histórica que conta com mais de um século de existência, sendo que ao longo do tempo a Farmácia Medeiros foi adquirindo um número considerável de utentes fidelizados, que reconhecem esta farmácia como um local de eleição para dar resposta às suas necessidades.

Em comparação com outras farmácias, a Farmácia Medeiros apresenta um horário de funcionamento alargado, dado que nos dias úteis o horário de funcionamento da farmácia é das 9h às 20h, e nos fins de semana e feriados é das 9h às 19h. Para além disso, a farmácia encontra-se em serviço de disponibilidade após o fecho. O horário de funcionamento alargado permite responder de forma mais adequada às necessidades da população e à sua conveniência, permitindo assim, uma maior afluência de utentes à farmácia.

A Farmácia Medeiros é propriedade da Dra. Maria Alice David Abreu Figueiredo Medeiros, sendo também a diretora técnica da farmácia. A Farmácia Medeiros dispõe de uma restante equipa técnica dotada de uma enorme experiência, competência, profissionalismo e dedicação, sendo constituída por cinco farmacêuticos e três técnicas de farmácia.

3. Análise SWOT

3.1. Pontos Fortes

3.1.1. Localização

A Farmácia Medeiros é a única farmácia localizada na vila do Avelar, sendo que para além da inexistência de concorrência nas redondezas, a farmácia encontra-se localizada na proximidade do Hospital Fundação Nossa Senhora da Guia, e de diversos estabelecimentos comerciais, de trabalho e de ensino. Desta forma, a sua localização privilegiada permite que, para além dos utentes habituais e fidelizados, um perfil bastante diversificado de utentes frequente a farmácia, incluindo trabalhadores e residentes das redondezas, e também turistas e pessoas de passagem. De salientar que na vila do Avelar reside um número considerável de emigrantes oriundos da Inglaterra que são clientes habituais da farmácia, sendo que a interação com estes utentes permitiu-me colocar em prática os meus conhecimentos da língua inglesa.

Por estas razões, reconheço a localização da farmácia como um ponto forte do estágio, na medida em que me permitiu contactar com uma grande diversidade de utentes de diferentes faixas etárias, com diferentes graus de literacia em saúde, de diferentes classes profissionais e sociais, e de diferentes nacionalidades, permitindo-me contactar com diferentes realidades e experienciar situações muito diversas.

3.1.2. Integração na equipa técnica

A equipa técnica que integra a Farmácia Medeiros foi claramente um dos pontos mais fortes do estágio, na medida em que todos os elementos da equipa técnica contribuíram para que me sentisse integrada na equipa e sempre se mostraram disponíveis para esclarecer eventuais dúvidas que surgissem com o decorrer do estágio, e transmitir-me o maior número de conhecimentos possível, fornecendo-me todo o apoio e as ferramentas necessárias para o meu crescimento pessoal e profissional.

Devido ao espírito de entreajuda e à constante partilha de conhecimentos entre os vários elementos que integram a equipa técnica da farmácia, sinto que tive a oportunidade de aplicar na prática os conhecimentos teóricos adquiridos ao longo do meu percurso académico, mas também de enriquecer e complementar os meus conhecimentos e desenvolver competências práticas e experiência profissional essenciais ao futuro exercício da profissão.

3.1.3. Plano de estágio

Relativamente ao plano de estágio considero que o mesmo foi muito bem estruturado, na medida em que me permitiu adquirir gradualmente conhecimentos e competências nas diferentes atividades do quotidiano da farmácia comunitária, tendo sido todas as atividades desenvolvidas de acordo com as Boas Práticas Farmacêuticas para a farmácia comunitária [2].

Nas fases iniciais do estágio tive a oportunidade de participar na realização de todas as tarefas que envolvem o *back office,* nomeadamente na receção de encomendas e armazenamento dos produtos, fecho e envio dos contentores do Valormed para os fornecedores, medição semanal das condições de temperatura e humidade com recurso a termohigrómetros, verificação de prazos de validade, gestão de *stocks* e de reservas, gestão de devoluções e respetiva regularização, gestão mensal de estupefacientes e psicotrópicos, transferência de produtos entre farmácias, preparação individualizada da medicação (PIM), dispensa de medicamentos para lares de idosos e divulgação de campanhas promocionais.

A receção das encomendas e posterior armazenamento dos produtos de acordo com o princípio de "*first expired, first out"* foi uma tarefa com a qual tive um contacto bastante próximo durante todo o estágio, a qual implica a conferência dos produtos recebidos, da respetiva quantidade, estado da embalagem, prazo de validade, preço de venda ao público (PVP) e preço de venda à farmácia (PVF). Considero que foi extremamente benefício começar por realizar esta tarefa, pois permitiu-me uma maior familiarização com os produtos da farmácia e respetivas embalagens, nomes comerciais e princípio(s) ativo(s), bem como do seu local de armazenamento na farmácia, o que agilizou posteriormente a etapa do atendimento.

Numa fase posterior do estágio comecei gradualmente a integrar as atividades do *front office* o que me permitiu ter um contacto mais próximo com os utentes. Numa fase inicial os atendimentos por mim realizados, foram sempre acompanhados e supervisionados pelos vários elementos da equipa, que sempre se mostraram disponíveis para me ajudar e esclarecer qualquer questão que surgisse no decorrer do atendimento, sendo que posteriormente, comecei a realizar os atendimentos de forma mais autónoma e independente. De salientar que desde o início do estágio foi-me dada total autorização para observar os atendimentos realizados pelos elementos da equipa técnica da farmácia, o que considero que foi extremamente importante para melhorar a qualidade dos meus atendimentos. De facto, a possibilidade de assistir aos atendimentos antes de os efetuar de forma independente foi fundamental para a familiarização com o sistema informático Sifarma 2000® e para aprender técnicas para uma interação e comunicação mais eficaz com os utentes.

3.1.4. Maior proximidade com os utentes

Devido à sua simpatia, competência, dedicação e profissionalismo, ao longo dos seus 116 anos de existência a Farmácia Medeiros foi criando um vínculo com a população através do estabelecimento de relações de maior confiança com os utentes. A maior proximidade entre o farmacêutico e o utente é essencial para assegurar um melhor acompanhamento dos utentes e a prestação de um aconselhamento farmacêutico mais personalizado e direcionado, tendo como principal foco o utente e as suas necessidades.

A Farmácia Medeiros é uma farmácia bastante reconhecida pela população, apresentando um grande leque de utentes fidelizados que constituem a grande maioria dos utentes da farmácia. A existência de utentes fidelizados facilita a gestão dos *stocks* e o processo de identificação dos utentes, permitindo fazer uma melhor gestão do tempo de atendimento. Enquanto estagiária considero que o contacto com utentes fidelizados foi um ponto forte neste processo de aprendizagem, na medida em que me foi possível estabelecer uma relação de maior confiança e criar uma empatia com alguns dos utentes habituais, permitindo-me ter mais à vontade no atendimento e aconselhamento levando a uma maior satisfação dos utentes.

3.1.5. Prestação de serviços de saúde

Na Farmácia Medeiros a oferta de serviços aos utentes é bastante diversificada, incluindo consultas de nutrição, rasteiros de audição, administração de vacinas e injetavéis, determinação de parâmetros antropométricos como o peso, altura e índice de massa corporal, medição da pressão arterial, e ainda a determinação de parâmetros bioquímicos, nomeadamente dos níveis de glicémia, colesterol total e triglicerídeos. Outros serviços

diferenciados prestados pela Farmácia Medeiros incluem o fornecimento de medicamentos e outros produtos de saúde a diversas instituições, nomeadamente a lares de idosos, e ainda a PIM para os utentes que solicitem este serviço.

3.1.5.1. Determinação da pressão arterial e parâmetros bioquímicos

A determinação da pressão arterial e de parâmetros bioquímicos é um serviço que se releva de extrema importância para aferir sobre a efetividade da terapêutica e para a deteção precoce de situações de risco. Estes serviços permitem estabelecer uma relação de confiança com os utentes e a sua fidelização, e constituem um momento oportuno para promover um conjunto de medidas não farmacológicas e hábitos de vida saudáveis junto da população.

Apesar de não ter sido muito regular, durante o meu estágio tive a oportunidade de observar e participar em algumas medições de pressão arterial e de parâmetros bioquímicos, sendo estas medições efetuadas no gabinete de atendimento ao utente. O contacto com estes serviços permitiu-me aperfeiçoar a minha capacidade para manusear os aparelhos necessários a estas medições e aplicar os meus conhecimentos para a interpretação dos resultados.

3.1.5.2. Colaboração com diversas instituições

A Farmácia Medeiros possui um protocolo com os lares de idosos do Avelar e de Chão de Couce, sendo a farmácia responsável pelo fornecimento de medicamentos e produtos de saúde a estes estabelecimentos, um serviço que se releva de extrema importância tanto para a farmácia como para estas instituições. Todos os utentes dos lares possuem uma ficha de utente no Sifarma 2000® com todos os seus dados pessoais e a medicação habitual, sendo que este serviço implica a consulta prévia da ficha do utente para verificar qual a sua medicação habitual e a realização de uma venda a crédito. Durante o estágio pude participar na dispensa da medicação e produtos de saúde para os lares de idosos, o que reconheço como um ponto forte do estágio, na medida em que esta tarefa contribuiu para uma maior familiarização com os produtos da farmácia e respetivas embalagens, nomes comerciais e princípio(s) ativo(s).

3.1.5.3. Preparação individualizada da medicação

A PIM consiste na organização da medicação do utente ao longo dos principais momentos do dia, tendo por base o perfil farmacoterapêutico e o regime posológico específico de cada utente, com o intuito de auxiliar o utente a fazer uma melhor gestão da sua medicação. Este é um serviço que se revela de extrema importância para auxiliar o utente na correta utilização dos medicamentos, com o objetivo de aumentar a adesão à terapêutica instituída a fim de garantir a sua eficácia e segurança [3]. Durante o período de estágio tive a oportunidade de efetuar a organização semanal da medicação dos utentes em caixas

organizadoras da medicação (*pill-boxes*), tendo por base a posologia prescrita, um serviço solicitado maioritariamente por utentes idosos polimedicados com regimes terapêuticos complexos, devido à dificuldade na gestão da sua medicação.

3.1.6. Estratégias de venda na farmácia e posicionamento *online*

Enquanto estagiária na Farmácia Medeiros assisti a inúmeras estratégias de *merchandising* implementadas na farmácia ao longo de cada mês, desde a celebração de dias temáticos à divulgação de campanhas promocionais na farmácia, com o objetivo de divulgar os produtos junto da população e potenciar as vendas. Para tal, a reorganização dos produtos nos lineares e a disposição dos produtos nas zonas quentes e de maior destaque da farmácia, de forma a potenciar as compras por impulso e aumentar a rotatividade dos produtos foi uma tarefa com a qual tive um contacto bastante próximo durante o estágio.

Com o objetivo de sensibilizar a população para as campanhas promocionais em vigor na farmácia e aumentar a proximidade da farmácia com os utentes, a Farmácia Medeiros dispõe de uma página de *Facebook*, sendo uma prática comum a realização mensal de uma *Newsletter* que é publicada nas redes sociais da farmácia, que para além de incluir informação relevante sobre diversos temas, contém também sugestões de produtos existentes na farmácia para cada situação em particular e as campanhas promocionais em vigor na farmácia para cada mês. A minha participação na elaboração das *Newsletters* permitiu-me desenvolver capacidades de comunicação e de promoção dos produtos junto da população.

3.2. Pontos Fracos

3.2.1. Reduzido contacto com medicamentos manipulados

Os medicamentos manipulados constituem uma alternativa terapêutica aos medicamentos industrializados permitindo uma personalização da terapêutica tendo em conta o perfil fisiopatológico específico de cada utente. A preparação de medicamentos manipulados é uma atividade que assume uma extrema importância para assegurar um tratamento personalizado e ajustado às necessidades individuais de cada utente.

Por ser um serviço cada vez menos solicitado pelos utentes, na Farmácia Medeiros a preparação de medicamentos manipulados é uma prática pouco frequente. Por esta razão, durante o período de estágio o meu contacto com os medicamentos manipulados foi bastante reduzido, o que reconheço como um ponto fraco do meu estágio, na medida em que não tive a oportunidade de aplicar na prática os conteúdos e as técnicas de manipulação adquiridas ao longo do plano de estudos do MICF. No entanto, durante o período de estágio usufrui da

oportunidade de participar na preparação, rotulagem e na definição do preço final de um medicamento manipulado, mais concretamente de uma pomada de ácido salicílico e ureia.

3.2.2. Existência de períodos de menor movimento de utentes

Outro aspeto que reconheço como um ponto fraco do meu estágio foi a existência de alguns períodos de ausência de utentes na farmácia, sendo que o menor movimento de utentes contribuiu inevitalmente para limitar o meu tempo de observação e realização de atendimentos. No entanto, os períodos mais calmos e de menor movimento de utentes foram utilizados para a partilha de informações sobre técnicas de abordagem e comunicação com os utentes e sobre os produtos disponíveis na farmácia, para a realização de formações disponíveis em plataformas *online*, para a disposição e reorganização dos produtos nos lineares, divulgação de campanhas promocionais na farmácia, elaboração das *Newsletters* e também para explorar as potencialidades do sistema informático Sifarma 2000®.

3.3. Oportunidades

3.3.1. Formações

A constante atualização técnica e científica é um dever deontológico do farmacêutico comunitário que deve procurar desenvolver uma formação contínua em permanente atualização de forma a estar a par da inovação técnica e científica. As formações conduzidas quer externamente quer internamente à própria farmácia, constituem uma excelente oportunidade para complementar e aprofundar conhecimentos sobre várias patologias e as especificidades dos produtos de forma a conseguir aconselhá-los de forma mais adequada.

Durante o período de estágio na Farmácia Medeiros tive a oportunidade de assistir a diversas formações externas, quer pela visita dos Delegados de Informação Médica à farmácia, quer formações em contexto *online* para as quais a farmácia era convidada, entre as quais destaco algumas formações sobre várias temáticas no âmbito da veterinária organizadas pelo Espaço Animal, e também algumas formações na área da dermocosmética, organizadas pela *Bioderma* e pelo *Laboratoire* Native Portugal, que contempla as marcas *Jowaé, Lierac, Phyto* e *Roger&Gallet*. Para além destas, tive também a oportunidade de estar presente em outras formações sobre a diarreia, obstipação, contraceção oral de emergência, higiene íntima feminina, produtos homeopáticos do laboratório *Boiron*, entre outras.

3.3.2. Sifarma 2000® e contacto com o novo módulo de atendimento

O sistema informático utilizado na Farmácia Medeiros é o Sifarma 2000®, uma ferramenta extremamente intuitiva que facilita bastante a execução das tarefas associadas ao

quotidiano da farmácia e o trabalho diário. No entanto, para além do sistema informático Sifarma 2000®, a Farmácia Medeiros tem implementado o novo módulo de atendimento do Sifarma®, uma ferramenta que foi desenvolvida no sentido de colmatar algumas lacunas do programa informático antigo, tornando-o mais intuitivo e dinâmico.

Apesar de ter tido um contacto mais notório e evidente com o Sifarma 2000®, durante o estágio também tive contacto com o novo módulo de atendimento, através da receção de encomendas e realização de alguns atendimentos, o que me permitiu constatar que existem algumas funções que não são possíveis de realizar no novo módulo de atendimento, nomeadamente a consulta e o somatório de vendas, e a realização de vendas suspensas.

A possibilidade de contactar com as duas modalidades de atendimento em paralelo foi uma grande mais-valia do estágio, pois permitiu-me uma maior familiarização com ambos os programas informáticos, o que considero que será muito benéfico no meu futuro profissional.

3.3.3. Dermocosmética e produtos de uso veterinário

Na Farmácia Medeiros a aposta nas áreas da dermocosmética e da veterinária é notória, sendo duas áreas bastante exploradas e que se relevam como uma excelente oportunidade de diferenciação para a farmácia.

Relativamente à componente da dermocosmética, a Farmácia Medeiros disponibiliza uma variedade considerável de marcas e gamas, o que aliado aos diversos momentos formativos em que tive a oportunidade de estar presente, se traduziu numa excelente oportunidade para a aquisição de conhecimentos nesta área. Durante o estágio tive ainda a oportunidade de presenciar a introdução de uma marca dermocosmética na farmácia, a *Bioderma*, o que reconheço como um aspeto positivo, pois no seguimento da introdução desta marca na farmácia foi-me dada a oportunidade de estar presente numa formação realizada pela Delegada da marca, que foi crucial para enriquecer os meus conhecimentos nesta matéria.

Também na área da veterinária a diversidade de produtos é evidente, sendo também este um campo de atuação fortemente explorado e de extrema importância para a Farmácia Medeiros. De facto, devido à sua localização rural, na Farmácia Medeiros os produtos de uso veterinário são bastante solicitados pela população, sendo o farmacêutico comunitário frequentemente procurado no sentido de solucionar diversos problemas relacionados com a saúde animal. Assim, por forma a responder de forma célebre e eficaz às necessidades dos utentes, a Farmácia Medeiros possui uma variedade considerável de produtos de veterinária, com os quais tive a oportunidade de contactar de forma próxima durante o período de estágio, o que me permitiu adquirir um conhecimento mais pormenorizado dos produtos de veterinária disponíveis na farmácia e das suas particularidades.

3.4. Ameaças

3.4.1. Interpretação de receitas manuais

Apesar da grande maioria das receitas médicas que aparecem na farmácia serem em formato eletrónico, a utilização das receitas manuais, ainda que seja cada vez menos frequente, ainda é permitida apenas em situações excecionais, como falência informática, inadaptação do prescritor, prescrição ao domicílio, e se o número de receitas for inferior ou igual a 40 receitas por mês. Este modelo de prescrição médica requer uma maior atenção por parte do farmacêutico, sendo necessário um cuidado especial para a verificação do preenchimento correto de todos os campos e para a interpretação da prescrição, tornando o processo de atendimento mais moroso e demorado. Ao longo do período de estágio deparei-me com algumas receitas manuais em que a caligrafia do médico prescritor era pouco percetível e ilegível, o que dificultava a leitura e interpretação da prescrição. Estas situações propiciam a ocorrência de erros na interpretação do nome, da forma farmacêutica e da dosagem do medicamento podendo levar a erros de dispensa do medicamento, e, portanto, para minimizar a ocorrência de erros a dupla leitura da prescrição foi uma prática bastante frequente.

3.4.2. Alteração do preço dos medicamentos

A constante alteração do PVP dos medicamentos gera alguma desconfiança e indignação por parte dos utentes, que por assumirem que estas alterações de preço são inerentes à própria farmácia, acabam por criar um sentimento de desconfiança junto do farmacêutico, traduzindo-se numa ameaça para as farmácias. Durante o meu estágio deparei-me com algumas situações em que face a uma alteração do PVP do medicamento, senti alguma desconfiança por parte dos utentes, mesmo após explicar que a origem destas alterações de preço são extrínsecas à farmácia e que não são responsabilidade da mesma. Esta desconfiança por parte dos utentes em relação ao preço dos medicamentos foi mais evidente nas situações em que os utentes se faziam acompanhar da guia de tratamento, na qual vêm mencionado o preço do medicamento genérico mais barato. Nestas situações, quando era apresentado o medicamento com uma discrepância de preço em relação ao mencionado na guia de tratamento os utentes ficavam um pouco indignados e desconfiados.

3.4.3. Medicamentos esgotados

A existência de medicamentos esgotados constitui uma ameaça tanto para a farmácia como para os utentes, pois quando o utente tem uma ideia predefinida do produto, a inexistência do mesmo e a incapacidade de satisfazer as suas necessidades gera o seu descontentamento e indignação. Por outro lado, quando não existem alternativas terapêuticas

igualmente eficazes, a existência de medicamentos esgotados pode privar o utente de ter acesso à sua medicação habitual, comprometendo o estado de saúde e o bem-estar dos utentes. Para além disso, a existência de medicamentos esgotados constitui também uma ameaça para a farmácia na medida em que, por não compreenderem que a responsabilidade desta situação não é da farmácia, os utentes acabam por perder a confiança na farmácia e nos seus profissionais levando à perda de utentes habituais e fidelizados.

4. Conclusão

O estágio curricular em farmácia comunitária constitui uma excelente oportunidade para os estudantes do MICF vivenciarem e experienciarem a realidade do mercado de trabalho no âmbito da farmácia comunitária, uma das áreas de maior destaque do setor farmacêutico. Reconheço este período de estágio como uma etapa fundamental do percurso académico para uma maior sedimentação e consolidação dos conhecimentos teóricos e científicos adquiridos até então, e a aquisição de competências práticas e experiência profissional.

O conceito de que o farmacêutico não é apenas um especialista do medicamento, mas sim um agente de saúde publica, preocupado com a saúde, bem-estar e qualidade de vida da população é uma noção que retiro desta experiência. Com este estágio consegui perceber a importância do papel do farmacêutico comunitário na sociedade, que engloba muito mais do que a dispensa de medicamentos e outros produtos de saúde. De facto, o farmacêutico comunitário possui uma posição privilegiada na sociedade para a promoção e educação para a saúde face à sua maior proximidade com a população, o que facilita o acompanhamento dos utentes e a prestação de um aconselhamento farmacêutico personalizado, tendo como foco não o medicamento dispensado, mas sim o utente e as suas necessidades.

Reconheço o estágio curricular na Farmácia Medeiros como uma experiência bastante enriquecedora no culminar do meu percurso académico, na medida em que me permitiu complementar e sedimentar os conhecimentos teóricos adquiridos ao longo do plano de estudos do MICF, e adquirir experiência profissional, competências sociais e humanas, que são essenciais ao exercício da profissão.

5. Referências Bibliográficas

1. ORDEM DOS FARMACÊUTICOS - **Código Deontológico da Ordem dos Farmacêuticos.** [Acedido a 9 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.** [Acedido a 14 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).** [Acedido a 20 de abril de 2021]. Disponível em:https://www.ordemfarmaceuticos.pt/fotos/documentos/norma_pim_vfinal_30_nge_00_010_02_1834827175bf58d479434f.pdf
4. INFOMED - **Resumo das Caraterísticas do Medicamento - Telfast 120 mg.** [Acedido a 30 de abril de 2021]. Disponível em: https://extranet.infarmed.pt/INFOMED-fo/detalhes-medicamento.xhtml
5. ASSOCIAÇÃO NACIONAL DE FARMÁCIAS - **Fluxograma de Indicação Farmacêutica para alívio dos sintomas associados à rinite alérgica.** [Acedido a 30 de abril de 2021]. Disponível em: http://www.anfonline.pt
6. ASSOCIAÇÃO NACIONAL DE FARMÁCIAS - **Fluxograma de Indicação Farmacêutica para alívio da candidíase vaginal.** [Acedido a 11 de maio de 2021]. Disponível em: http://www.anfonline.pt
7. INFOMED - **Resumo das Caraterísticas do Medicamento - UL-250.** [Acedido a 20 de maio de 2021]. Disponível em: https://extranet.infarmed.pt/INFOMED-fo/detalhes-medicamento.xhtml
8. ASSOCIAÇÃO NACIONAL DE FARMÁCIAS - **Fluxograma de Indicação Farmacêutica para alívio dos sintomas associados à diarreia aguda.** [Acedido a 20 de maio de 2021]. Disponível em: http://www.anfonline.pt

Anexos - Casos Práticos

Caso Prático 1 - Uma senhora de cerca de 50 anos deslocou-se à farmácia com queixas de espirros bastante frequentes e corrimento nasal bastante acentuado, referindo que neste momento " não aguenta mais" e que precisa de algo que lhe alivie esta sintomatologia.

Perante esta situação comecei por questionar a utente quando tinha aparecido a sintomatologia e se apresentava outros sintomas para além dos reportados, ao que a senhora respondeu que os sintomas tinham aparecido no dia anterior e que para além dos espirros e do corrimento nasal predominantes, tinha alguma comichão no nariz e nos olhos, que se apresentavam ligeiramente lacrimejantes. Perguntei ainda se era frequente manifestar esta sintomatologia e se tinha historial de rinite alérgica, uma vez que se o quadro apresentado pela utente era típico de alergia, ao que a utente respondeu que era bastante frequente manifestar esta sintomatologia e que todos os anos na primavera tem estes sintomas.

Nesta situação acabei por sugerir a administração de um anti-histamínico oral, nomeadamente 1 comprimido de Telfast® 120 mg por dia, de preferência antes de uma refeição, que é constituído pelo cloridrato de fexofenadina, um anti-histamínico H1 de 2ª geração sem efeito sedativo, que está indicado para o alívio dos sintomas associados à rinite alérgica [4]. Visto que os sintomas nasais como os espirros, rinorreia e prurido nasal eram bastante exacerbados, aconselhei ainda a administração do Vibrocil Anti-Alergias®, que é constituído por propionato de fluticasona, um corticosteroide intranasal indicado para o alívio dos sintomas nasais de rinite alérgica. Inicialmente, recomendei a administração de 2 pulverizações em cada narina, 1 vez por dia preferencialmente de manhã, devendo a administração ser reduzida para apenas 1 pulverização em cada narina após a melhoria dos sintomas, alertando que a utilização deve ser feita de forma regular durante 2 semanas [5].

Caso Prático 2 - Uma senhora de cerca de 30 anos deslocou-se à farmácia com queixas de comichão e prurido vulvar intenso que tiveram início no dia anterior, referindo que se sente bastante desconfortável e incomodada com esta situação.

Perante os dados reportados comecei por questionar a utente se para além da comichão e prurido vaginal, apresentava outros sintomas, como ardor ao urinar, dor, vermelhidão, corrimento vaginal anormal e odor desagradável, ao que a utente respondeu que, embora não tenha um odor desagradável, tinha um corrimento anormal espesso e esbranquiçado e ardor acompanhado por um prurido intenso. Após analisar a sintomatologia reportada pela utente, expliquei que a causa mais provável para esta situação seria uma infeção fúngica vaginal causada pela *Candida albicans*, e que o tratamento para a candidíase vaginal passaria pela administração de antifúngicos, como o clotrimazol. Desta forma, aconselhei o

Gino-Canesten® creme, explicando que deve ser aplicado internamente na vagina com o auxílio de aplicadores durante 6 dias consecutivos à noite, sendo que como a senhora apresentava sintomas externos muito exacerbados, como o prurido intenso e ardor, aconselhei também a aplicação do creme externamente na zona vulvar para aliviar estes sintomas. Adicionalmente para reduzir o desconforto e aliviar os sintomas externos de comichão e irritação vulvar, aconselhei ainda o Gino-Canesfresh® Calm, um gel de higiene íntima indicado para reduzir a irritação e desconforto da zona íntima associados à candidíase vaginal devido ao seu pH alcalino e propriedades calmantes.

Como medidas não farmacológicas, aconselhei a utilização de roupa interior de algodão devendo evitar a utilização de roupa interior demasiado justa, evitar fazer a higiene íntima com produtos de limpeza irritantes e secar bem a área genital, e após a ida à casa de banho aconselhei a fazer a limpeza da área genital de frente para trás [6].

Caso Prático 3 - Um senhor deslocou-se à farmácia, referindo que está bastante preocupado com o seu filho de 11 anos que está com diarreia desde o dia anterior, tendo tido dejeções líquidas bastantes frequentes.

Perante esta situação comecei por questionar o senhor se para além das dejeções diarreicas, o seu filho apresenta outros sintomas, como febre alta, vómitos, dor e cólicas abdominais, se o seu filho tinha viajado recentemente ou se conseguia relacionar este episódio de diarreia a alguma alteração dos hábitos alimentares ou introdução de algum medicamento, ao que o utente respondeu que não. Assim, como as dejeções diarreicas eram bastantes frequentes, comecei por reforçar a importância da reidratação oral para a manutenção do equilíbrio hidroeletrolítico, tendo aconselhado para o efeito, a toma de uma saqueta de Dioralyte® (eletrólitos + glucose) após cada dejeção diarreica. Referi que a saqueta deve ser dissolvida em 200 ml de água podendo a solução preparada ser conservada no frigorífico durante 24h. De forma a acelerar a renovação da flora intestinal, aconselhei a toma de 1 cápsula de UL-250®, 3 vezes ao dia, durante um período de 5 dias, um probiótico que é constituído pela *Saccharomyces boulardii* CNCM I-745 [7].

Como medidas não farmacológicas, aconselhei a ingestão abundante de líquidos, devendo evitar a administração de leite e de produtos lácteos. Expliquei ainda que a diarreia aguda é normalmente de origem infeciosa e autolimitada, sendo que na maioria dos casos o trânsito intestinal é restabelecido ao fim de 24h a 48h, reforçando a importância de consultar o médico se os episódios de diarreia persistirem ao fim deste período, ou se a criança manifestar febre ou sinais de desidratação [8].

Parte II

Relatório de Estágio em Farmácia Hospitalar

Centro Hospitalar e Universitário de Coimbra

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

SF - Serviços Farmacêuticos

SNS - Serviço Nacional de Saúde

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

UMIV - Unidade de Misturas Intravenosas

UPC - Unidade de Preparação de Citotóxicos

1. Introdução

Os Serviços Farmacêuticos (SF) Hospitalares compreendem todas as atividades desempenhadas por farmacêuticos em organismos hospitalares, cujo objetivo é o de garantir o uso seguro, racional e eficaz dos medicamentos e assegurar uma terapêutica medicamentosa eficaz, segura e de qualidade a todos os doentes. Os Farmacêuticos Hospitalares enquanto profissionais de saúde especialistas do medicamento integrados em equipas multidisciplinares, desempenham um papel crucial em todo o circuito do medicamento, de forma a otimizar os resultados em saúde e garantir a segurança e a qualidade dos cuidados de saúde prestados, através da utilização criteriosa, racional, eficaz e segura dos medicamentos [1,2].

O plano curricular do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) culmina com a realização de um estágio curricular, sendo que, apesar do estágio curricular em Farmácia Hospitalar ser facultativo, considero que a sua realização é extremamente importante para conhecer a realidade hospitalar. Assim, como esta área sempre me suscitou interesse e curiosidade, optei pela realização do estágio em Farmácia Hospitalar no Centro Hospitalar e Universitário de Coimbra, E.P.E. (CHUC), tendo o mesmo sido iniciado em maio e terminado em junho de 2021, com a duração total de 280h, sob orientação da Doutora Marília João Rocha.

O presente relatório de estágio compreende uma análise SWOT (*Strenghts*, *Weaknesses*, *Opportunities*, *Threats*), na qual pretendo descrever numa perspetiva interna os pontos fortes (*Strenghts*) e os pontos fracos (*Weaknesses*), e numa perspetiva externa as oportunidades (*Opportunities*) e as ameaças (*Threats*) com que me deparei ao longo do estágio.

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

O CHUC, E.P.E. é um centro hospitalar geral, central e universitário, que foi criado em 2011, na sequência do Decreto-Lei n.º 30/2011, de 2 de março, através da fusão de três unidades hospitalares: os Hospitais da Universidade de Coimbra (HUC), o Centro Hospitalar de Coimbra e o Centro Hospitalar Psiquiátrico de Coimbra. Atualmente, o CHUC é constituído por um conjunto de unidades hospitalares, incluindo os Hospitais da Universidade de Coimbra (HUC), o Hospital Pediátrico (HP), o Hospital Geral (HG), a Maternidade Bissaya Barreto (MBB), a Maternidade Daniel de Matos (MDD) e o Hospital Sobral Cid (HSC) [3].

O CHUC, E.P.E. é uma instituição integrada no Serviço Nacional de Saúde (SNS) considerada como uma referência a nível nacional e internacional, que tem como missão a prestação dos melhores cuidados de saúde aos doentes, mantendo presente uma forte componente formativa, de ensino e de investigação [3,4].

3. Análise SWOT

3.1. Pontos Fortes

3.1.1. Acolhimento e integração na equipa

A receção e o acolhimento dos estagiários pela equipa dos SF do CHUC foi claramente um dos pontos mais fortes do estágio, na medida em que todos os profissionais que integram a equipa dos SF contribuíram para me sentisse integrada na equipa promovendo a minha inclusão em todas as atividades e tarefas desenvolvidas em cada setor, e fornecendo-me todo o apoio, orientação e esclarecimentos necessários. Neste contexto, considero que o estágio em Farmácia Hospitalar se traduziu numa excelente oportunidade de aprendizagem nesta área, o que se deveu em grande parte aos profissionais com quem contactei durante o estágio, que sempre se mostraram disponíveis para me ajudar e esclarecer eventuais dúvidas que surgissem.

3.1.2. Plano de estágio previamente estruturado

A existência de um plano de estágio previamente estruturado foi sem dúvida um dos pontos mais fortes do estágio, na medida em que esta planificação e organização prévia permitiu um melhor funcionamento do período de estágio e a aquisição gradual de conhecimentos e competências nas diferentes atividades da Farmácia Hospitalar. De facto, no primeiro dia de estágio decorreu uma reunião de apresentação com a Doutora Marília João Rocha, na qual foram disponibilizadas todas as informações necessárias à realização do estágio e foi entregue a cada estagiário o respetivo plano de estágio individual. Também foi disponibilizado um caderno de estagiário, onde estavam descritas todas as atividades e os objetivos a desenvolver em cada setor, sendo que esta orientação prévia foi um grande auxílio, pois permitiu uma melhor compreensão dos objetivos a atingir durante o estágio, facilitando imenso a minha integração e adaptação posterior em cada um dos setores.

3.1.3. Rotatividade por vários setores

O estágio foi estruturado de forma rotativa entre os vários estagiários permitindo que em cada semana de estágio diferentes setores dos SF fossem abordados. Neste contexto, durante o estágio tive a possibilidade de contactar com quatro setores dos SF, nomeadamente com o setor dos Ensaios Clínicos, Gestão e Aprovisionamento, Farmacotecnia e Distribuição, sendo que os dois últimos assumiram um especial destaque, não só pela importância que assumem no hospital, mas também por terem sido os setores onde permaneci mais tempo.

Considero que a rotatividade e a possibilidade de contactar com diferentes setores foi um ponto forte neste processo de aprendizagem, na medida em que tornou o estágio mais dinâmico, proporcionando-me uma melhor compreensão da organização, funcionamento

interno e da logística de cada setor e uma visão mais completa e abrangente das funções e atividades desenvolvidas pelos Farmacêuticos Hospitalares em cada um dos setores.

No entanto, para além de ter contactado com diferentes setores dos SF nos HUC, durante o estágio também pude contactar com os SF do HP durante uma semana, sobretudo com a preparação de citotóxicos, sendo que a possibilidade de estagiar nos dois polos do CHUC foi uma grande mais-valia pois tornou esta experiência mais completa e diversificada.

3.1.4. Cedência de medicamentos em ambulatório

Durante o período de estágio usufrui da possibilidade de experienciar a cedência de medicamentos em dois ambulatórios diferentes, nomeadamente no ambulatório da unidade central dos HUC e no ambulatório do Edifício de São Jerónimo localizado no Hospital de Dia de oncologia, o que reconheço como um ponto forte do estágio, na medida em que pude contactar com realidades bastante diferentes e experienciar situações muito diversas.

No ambulatório da unidade central dos HUC pude constatar que a afluência e a diversidade de doentes é bastante elevada, o que me permitiu contactar com uma maior diversidade de patologias e medicamentos. Pelo contrário, no ambulatório do Edifício de São Jerónimo a diversidade de doentes é mais reduzida, uma vez que o mesmo se destina à cedência de medicamentos aos doentes do Hospital de Dia de oncologia, nomeadamente citotóxicos orais e fármacos adjuvantes, como antieméticos. Pude constatar que no ambulatório do São Jerónimo o atendimento é efetuado de forma mais personalizada em gabinetes próprios de atendimento de forma a salvaguardar a privacidade dos doentes.

Em ambos os ambulatórios tive a oportunidade de acompanhar todas as etapas do atendimento e de colaborar com o farmacêutico na preparação da medicação a ceder, o que reconheço como um ponto forte, na medida em que esta tarefa me permitiu uma maior familiarização com alguns medicamentos de uso exclusivo hospitalar e respetivas patologias.

3.1.5. Medicamentos sujeitos a legislação especial

Os hemoderivados, estupefacientes e psicotrópicos são medicamentos sujeitos a legislação especial, sendo que por estarem sujeitos a um controlo mais rigoroso possuem circuitos de distribuição próprios, com os quais pude contactar durante o estágio, o que me permitiu uma maior familiarização com estes fármacos e com os seus circuitos de distribuição.

A requisição clínica, distribuição aos serviços e administração dos hemoderivados em meio hospitalar requer um modelo próprio (modelo n.º 1804), que é constituído por duas vias: a Via Farmácia que é arquivada nos SF e a Via Serviço que é anexada ao processo clínico do doente [5]. A Via Farmácia possui três quadros (A, B e C), sendo os quadros A e B

preenchidos pelo médico e o quadro C pelo farmacêutico através do registo do nome do hemoderivado cedido, da quantidade cedida, do lote, do laboratório, 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 D adicional que é preenchido pelo enfermeiro responsável pela administração [5].

Tal como os hemoderivados, também os estupefacientes e psicotrópicos possuem um circuito de distribuição especial em ambiente hospitalar, com o qual pude contactar durante o estágio, tendo tido a oportunidade de colaborar com o farmacêutico responsável na preparação da medicação a ceder, o que me permitiu adquirir uma maior consciencialização do controlo rigoroso a que os estupefacientes e psicotrópicos estão sujeitos.

3.1.6. Contacto com diferentes áreas funcionais da Farmacotecnia

Outro aspeto que reconheço como um ponto forte do estágio foi a possibilidade de contactar com diferentes áreas funcionais do setor da farmacotecnia, nomeadamente com a Radiofarmácia, com a Unidade de Misturas Intravenosas (UMIV), com a unidade de preparação de medicamentos não estéreis e ainda com a Unidade de Preparação de Citotóxicos (UPC).

Na Radiofarmácia, que se encontra localizada no serviço de Medicina Nuclear, pude contactar com a eluição dos geradores, a preparação de alguns radiofármacos e o controlo de qualidade dos geradores e das preparações radiofarmacêuticas, o que me permitiu conhecer melhor as atividades desenvolvidas nesta área e adquirir conhecimentos mais consolidados.

Na UMIV pude observar a validação farmacêutica das prescrições e a preparação de alguns anticorpos monoclonais (nomeadamente do ustecinumab para o tratamento da doença de Chron e do ocrelizumab para a esclerose múltipla) e da nutrição parentérica na câmara de fluxo laminar horizontal, e ainda a preparação de soro autólogo na câmara de fluxo laminar vertical. Para além desta, durante o estágio também pude contactar com a unidade de preparação de medicamentos não estéreis onde pude colaborar na preparação de alguns manipulados, nomeadamente de uma suspensão oral de nistatina + lidocaína + bicarbonato de sódio indicada para mucosites graves em doentes submetidos a quimioterapia, e ainda de uma solução oral de cloreto de potássio indicada para situações de deficiência em potássio, o que me permitiu aplicar na prática as técnicas de manipulação adquiridas em contexto académico.

Por fim, na UPC pude contactar com todo o circuito de preparação dos citotóxicos, desde a validação farmacêutica dos protocolos oncológicos até à preparação dos citotóxicos na câmara de fluxo laminar vertical, tendo tido a oportunidade de entrar na sala limpa onde pude observar a preparação de alguns citotóxicos, o que me permitiu adquirir uma maior consciencialização dos procedimentos inerentes à manipulação e preparação dos citotóxicos.

3.2. Pontos Fracos

3.2.1. Curta duração do estágio

Um aspeto que reconheço como um ponto fraco do estágio é a curta duração do mesmo, na medida em que dois meses é um período de tempo muito reduzido para interiorizar todos os conceitos e procedimentos inerentes às várias áreas de atuação do farmacêutico no âmbito da Farmácia Hospitalar, atendendo à diversidade e complexidade de tarefas que o farmacêutico pode executar em cada setor dos SF.

Apesar de ter tido a oportunidade de contactar com vários setores durante o estágio, considero que a duração de apenas uma semana em cada setor não é suficiente para interiorizar as especificidades e a complexidade de todas as tarefas desempenhadas, e, portanto, penso que seria benéfico usufruir de um período de estágio mais alargado de forma a conseguir alcançar uma experiência mais completa nos diferentes setores dos SF.

3.3. Oportunidades

3.3.1. Contacto com medicamentos de uso exclusivo hospitalar

Como referido no ponto 3.1.4, o estágio em Farmácia Hospitalar proporcionou-me a oportunidade de contactar com alguns medicamentos de uso exclusivo hospitalar cedidos em ambulatório, permitindo-me estabelecer uma associação dos mesmos com as respetivas patologias, sendo alguns exemplos os medicamentos biológicos (anticorpos monoclonais), imunossupressores, citotóxicos, fármacos antivirais, fármacos para o tratamento da fibrose quística, esclerose múltipla, artrite reumatóide e insuficiência renal. A possibilidade de contactar com estes medicamentos revelou-se uma grande mais-valia, na medida em que me permitiu não só aplicar na prática os conhecimentos teóricos sobre os fármacos e respetivas patologias adquiridos em unidades curriculares como Farmacologia e Virologia, mas também porque me permitiu adquirir novos conhecimentos e complementar a formação académica.

3.3.2. Realização de um trabalho

Durante o período de estágio foi-me proposto a realização de um trabalho com o objetivo de analisar as variações de consumo de antibióticos no CHUC durante um período de três anos (2018-2020) e identificar as classes de antibióticos mais consumidas. Para a concretização do trabalho foi necessário recorrer ao programa *Antimicrobial Consumption Tool (*AMC *Tool)*, uma ferramenta especialmente desenvolvida para estimar o consumo de antibióticos através do cálculo da Dose Diária Definida (DDD), e ao Microsoft Excel® para o tratamento e análise dos dados obtidos. Assim, considero que a realização deste trabalho foi

uma grande mais-valia, não só por se ter traduzido numa excelente oportunidade para a aquisição de competências a nível informático, mas também porque me permitiu adquirir um maior conhecimento sobre o consumo de antibióticos a nível hospitalar, tendo sido bastante interessante observar as oscilações do consumo de antibióticos durante o período em estudo.

3.4. Ameaças

3.4.1. Plano curricular do MICF

O MICF é um curso extremamente abrangente e multidisciplinar que contempla um conjunto diversificado de unidades curriculares de forma a dotar os estudantes de competências e conhecimentos imprescindíveis ao sucesso nos diferentes ramos profissionais do setor farmacêutico**.** Durante o estágio curricular em Farmácia Hospitalar senti a aplicabilidade prática de algumas unidades curriculares do plano de estudos do MICF, entre as quais destaco as unidades curriculares de Farmácia Hospitalar, Farmacologia, Farmácia Clínica e Virologia. No entanto, apesar de reconhecer a importância dos conteúdos lecionados nestas unidades curriculares, considero que o plano de estudos do MICF está pouco orientado para esta área de atuação do farmacêutico, e, portanto, penso que seria benéfico reforçar o plano de estudos com mais unidades curriculares direcionadas para a área da Farmácia Hospitalar de forma a aproximar os estudantes desta possível saída profissional ao longo do curso.

4. Conclusão

A realização do estágio curricular em Farmácia Hospitalar no CHUC, o meu primeiro contacto com este ramo profissional do setor farmacêutico, foi uma experiência bastante enriquecedora no culminar do meu percurso académico, na medida em que me permitiu consolidar e sedimentar os conhecimentos teóricos adquiridos em contexto académico, e adquirir novos conhecimentos e competências pessoais e profissionais.

Neste estágio curricular tive a oportunidade de integrar vários setores dos SF e contactar com as diversas funções e atividades desenvolvidas pelos Farmacêuticos Hospitalares em cada setor, o que me permitiu adquirir uma maior consciencialização da dinâmica de funcionamento dos SF e da importância da intervenção do farmacêutico em meio hospitalar, que envolve todo o circuito do medicamento, de forma a garantir o seu uso racional, seguro e eficaz, assegurando a prestação dos melhores cuidados de saúde aos utentes.

Assim, com a conclusão do estágio curricular no CHUC, considero que esta etapa do meu percurso académico se traduziu numa excelente oportunidade de aprendizagem nesta área, permitindo-me contactar de forma próxima com a realidade da Farmácia Hospitalar.

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. [Acedido a 20 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.** [Acedido a 20 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). [Acedido a 22 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. [Acedido a 23 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.** [Acedido a 30 de junho de 2021]. Disponível em: https://www.infarmed.pt/documents/15786/1068535/despacho_1051-2000.pdf

Parte III

Monografia

"Exosome Membrane-Coated Nanosystems for Biomedical Applications"

Orientado pela Professora Doutora Ana Cláudia Santos

Resumo

As nanopartículas (NPs) têm sido amplamente exploradas como nanossistemas promissores para a libertação vetorizada de fármacos e de agentes de imagem para locais específicos, sendo utilizadas para uma variedade de aplicações teranósticas. Os sistemas de libertação de fármacos baseados em NPs oferecem vantagens consideráveis em termos de eficácia e segurança quando comparadas com as terapias convencionais, sendo reconhecidos como nanossistemas promissores para o diagnóstico e tratamento de várias doenças humanas.

Nos últimos anos, numa tentativa de aumentar as propriedades de "*biointerface"* das NPs e dotá-las de uma maior capacidade de vetorização celular, de evasão imunitária e de prolongamento do tempo de circulação sistémica, foi desenvolvida uma estratégia biomimética baseada no revestimento de NPs com membranas celulares. Esta abordagem inovadora visa a conceção de nanotransportadores biomiméticos, nos quais as NPs são revestidas com uma camada de uma membrana celular através de abordagens *"top-down"*, de forma a que tanto a composição complexa das membranas celulares como as suas funções biológicas possam ser rigorosamente preservadas. Até à data, um vasto repertório de membranas derivadas de células tem sido explorado para revestir as NPs, incluindo as membranas derivadas de glóbulos vermelhos, glóbulos brancos, plaquetas, células estaminais e células cancerígenas, sendo que cada tipo de membrana celular fornece propriedades específicas relacionadas com as células de origem.

Para além das membranas celulares, estudos recentes têm-se focado no revestimento de NPs com membranas derivadas de vesículas extracelulares, entre as quais os exossomas se destacam como os mais promissores desses revestimentos, no sentido de serem combinadas as vantagens de ambos os componentes, e dotar as NPs de uma capacidade superior de vetorização homotípica, de evasão imunitária, bem como de aumentar o tempo de circulação sistémica. As NPs revestidas com a membrana de exossomas têm demonstrado ser nanossistemas biomiméticos eficientes para a libertação vetorizada de fármacos e agentes de imagem para locais de interesse, devido à capacidade intrínseca dos exossomas de se dirigem para as células-alvo, evidenciando uma larga diversidade de aplicações no diagnóstico, tratamento bem como no teranóstico de uma vasta gama de doenças humanas, incluindo o cancro, as doenças neurodegenerativas, a regeneração da pele, e a cicatrização de feridas.

Esta revisão resume os avanços recentes referentes às NPs revestidas com membrana de vesículas extracelulares, com particular ênfase no revestimento das NPs com a membrana de exossomas, na qual o processo de preparação, a caracterização e as principais aplicações biomédicas destas NPs biomiméticas, bem como os desafios atuais para uma implementação

clínica bem-sucedida, e, por fim, as perspetivas futuras desta tecnologia biomimética inovadora na área biomédica são discutidos.

Palavras-chave: Biomimetismo, exossoma, vesícula extracelular, nanopartícula biomimética, nanopartícula revestida com membrana de vesícula extracelular, revestimento com membrana celular.

Abstract

Nanoparticles (NPs) have been widely explored as promising nanosystems for targeted delivery of drug molecules and imaging agents to specific sites, being used for a variety of theranostic applications. NP-based drug delivery systems offer considerable advantages in terms of efficacy and safety compared to conventional therapies, being recognized as promising nanosystems for the diagnosis and treatment of various human diseases.

In the last few years, in an attempt to increase the biointerfacing properties of NPs, and to endow them with superior cell targeting, immune evasion and prolonged systemic circulation capabilities, a biomimetic strategy based on coating NPs with natural cell membranes has been developed. This innovative approach aims the design of biomimetic nanocarriers, in which a NP core is surrounded by a natural cell membrane layer *via* top-down approaches, so that the complex composition of natural cell membranes and their biological functions can be faithfully preserved. To date, a large repertoire of natural cell-derived membranes has been explored to cover and thereby camouflage NPs, including those derived from red blood cells, white blood cells, platelets, stem cells and cancer cells, in which each cell membrane type provides specific donor cell-related properties.

In addition to cell membranes, recent studies have been focusing on coating NPs with the membrane of cell-derived extracellular vesicles (EVs), among which exosomes stand out as the most promising, with the aim of combining the advantages of both components, and endowing NPs with improved homotypic targeting, immune evasion and increased systemic circulation abilities. Nanosized delivery systems coated with the membrane of exosomes have been shown to be efficient biomimetic nanosystems for targeted delivery, not only of drug molecules but also imaging agents to sites of interest, due to the intrinsic cell-specific targeting features of exosomes, presenting a diversity of applications in the diagnosis, treatment and theranostics of a wide range of human diseases, including cancer, neurodegenerative diseases, skin regeneration and wounds repair.

This review summarizes the recent advances in the field of EV membrane-coated NPs, with particular emphasis on coating NPs with the membrane of exosomes, in which the preparation process, the characterization, and the main biomedical applications of these biomimetic NPs are critically assessed, as well as the current challenges for successful clinical implementation and the future perspectives of this innovative biomimetic technology in the biomedical field.

Keywords: Biomimicry, exosome, extracellular vesicle, biomimetic nanoparticle, extracellular vesicle membrane-coated nanoparticle, cell membrane coating.

Abbreviations

Alix - Apoptosis-linked-gene-2 interacting protein

Anti-miR-21- Antisense miRNA targeting miRNA-21

BBB - Blood-brain barrier

BCD - ^{10}B boron-containing carbon dot

BNCT - Boron neutron capture therapy

CBSA - Cationic bovine serum albumin

c-Met - Mesenchymal epithelial transition factor

CSC - Cancer stem cell

CTC - Circulating tumor cell

CuB - Cucurbitacin B

DC - Dendritic cell

DOX - Doxorubicin

EGFR - Epidermal growth factor receptor

ESCRT - Endosomal Sorting Complex Required for Transport

EV - Extracellular vesicle

HCQ - Hydroxychloroquine

HGN - Hollow gold nanoparticle

HSA - Human serum albumin

Hsp - Heat shock protein

ICD - Immunogenic cell death

ICG - Indocyanine green

ILV - Intraluminal vesicle

imDC - Immature dendritic cell

Lamp2b - Lysosome-associated membrane glycoprotein 2b

MHC - Major histocompatibility complex

miRNA - microRNA

MOF - Metal organic framework

MPS - Mononuclear phagocyte system

MRI - Magnetic resonance imaging

mRNA - Messenger RNA

MSC - Mesenchymal stem cell

MSN - Mesoporous silica nanoparticle

MVB - Multivesicular body

NIR - Near-infrared

NK - Natural killer

NP - Nanoparticle

PCL - Poly (ε-caprolactone)

PDT - Photodynamic therapy

PEG - Polyethylene glycol

PLA - Poly-lactic acid

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

PSiNP - Porous silicon nanoparticle

PTT - Photothermal therapy

PTX - Paclitaxel

ROS - Reactive oxygen species

RVG - Rabies virus glycoprotein

SBHA - Suberoyl bis-hydroxamic acid

SDS-PAGE - Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

siRNA - Small interfering RNA

SNA - Spherical nucleic acid

SNARE - Soluble NSF attachment protein receptor

SNCA - Synuclein alpha gene

SPION - Superparamagnetic iron oxide nanoparticle

TEM - Transmission electron microscopy

TME - Tumor microenvironment

TNBC - Triple-negative breast cancer

Tsg101 - Tumor susceptibility gene 101

VEGF - Vascular endothelial growth factor

ZnS - Hollow zinc sulphide

α-syn - α-synuclein

1. Introduction

Nanotechnology holds a great potential for the diagnosis and treatment of various human diseases, owing to the unique ability of nanoscale materials to deliver therapeutic and imaging agents to target sites, improving efficacy and safety over conventional therapies [1]. Some advantages of nanoparticles (NPs) include their excellent drug loading ability, easy scalability, greater flexibility to undergo surface modification and controlled drug release, protecting the cargo from degradation and premature leakage [2-4].

However, despite the long clinical success of NP-based drug delivery systems, their use in clinical practice is limited due to the low ability to cross biological barriers, reduced biocompatibility and rapid clearance by the mononuclear phagocyte system (MPS) due to their foreign nature, which ultimately decreases the systemic circulation time and the delivery efficiency to the target site [2-4]. In an attempt to increase the selectivity of delivery, current research is focused on active targeting strategies that requires the surface functionalization of NPs with targeting ligands, such as peptides, aptamers, antibodies or other small molecules, to enable the specific binding to receptors overexpressed by target cells [5, 6]. To reduce immune clearance of NPs and increase their systemic circulation time, the functionalization with polyethylene glycol (PEG), a hydrophilic polymer, remain the most widely used strategy, as PEGylated NPs are less likely to be recognized by the MPS [7].

In an attempt to overcome the limitations of NPs and enhance their biointerfacial properties, recent studies have been focusing on cloaking NPs with naturally derived cell membranes to allow NPs to target specific cells. This cell membrane-coating nanotechnology approach requires the surface functionalization of NPs with a layer of a natural cell membrane *via* top-down approaches, in a way that cell membrane-coated NPs are composed of two parts: a NP inner core and a layer of the natural cell membrane, to form a core-shell nanostructure, with the NP being the inner core and the layer of the natural cell membrane the shell [8-10].

This cell membrane-coating nanotechnology approach has emerged as a promising biomimetic strategy to enable the transfer of the inherent biological properties of natural cell membranes to the surface of NPs, imparting them with the unique complex composition of donor cells. By covering nanomaterials with a layer of a natural cell membrane, the antigenic profile and the biointerfacial properties of natural cell membranes can be faithfully preserved and directly transfer onto the surface of NPs, endowing the coated NPs with desirable biological properties, including higher biocompatibility, longer systemic circulation, immune evasion abilities and active targeting capabilities [11-16]. In recent years, several types of cell-derived membranes have been used to camouflage NPs, such as the membranes derived from

red blood cells, platelets, white blood cells, stem cells and cancer cells, with each cell membrane type providing specific donor cell-related properties [9, 11].

Recently, a new type of membrane has received much attention, one that is not cellular, but derived from extracellular vesicles (EVs), which are naturally secreted cell-derived nanovesicles that perform an important role in mediating short and long-distance cell-cell communication through the delivery of biological content between neighboring and distant cells [17]. In mammalian cells, these cell-derived vesicles are normally categorized into three different subtypes based on their mechanisms of biogenesis and size, namely apoptotic bodies, microvesicles and exosomes [18, 19]. Apoptotic bodies are the largest in size (50 nm to 5000 nm), that are released during programmed cell death (apoptosis) through direct outward budding of apoptotic cell membrane, while microvesicles (also called ectosomes) are usually smaller with a size ranging from 50 nm to 1000 nm and are originated through direct outward budding of the plasma membrane [20]. Regarding the three subtypes of EVs, exosomes, which are lipid bilayer nanovesicles naturally secreted by eukaryotic cells, are the smallest with a size usually ranging from 30 nm to 150 nm, and are originated from the endocytic recycling pathway through fusion of multivesicular bodies (MVBs) with the plasma membrane [21].

Among the several types of EVs, current research is primarily focused on exosomes for coating nanomaterials. Owing to their excellent biocompatibility and stability, non-immunogenicity, immune evasion ability, prolonged systemic circulation, ability to cross biological barriers and intrinsic homologous targeting abilities, the membrane of naturally secreted exosomes has attracted considerable interest for coating nanomaterials [22, 23]. This biomimetic coating strategy can endow NPs with higher biocompatibility, immune evasion abilities, prolonged systemic circulation and homotypic targeting abilities, thereby improving therapeutic efficacy and reducing off-target toxicity in healthy tissues [22].

In this review, the most recent research on EV membrane-coated NPs, with particular emphasis on exosome membrane-coated NPs, for biomedical applications are summarized. To this end, the mechanisms of biogenesis, composition and biological functions of natural exosomes will be first clarified. Secondly, the preparation process of exosome membrane-coated NPs and their characterization will be discussed, and then, the most recent research on the biomedical applications of these biomimetic core-shell NPs, including cancer therapy and imaging, neuroimaging, Parkinson´s disease treatment and wound repair will be presented. Finally, the major challenges for successful implementation in clinical practice and the future prospects on this emerging biomimetic coating approach will be discussed.

2. Exosomes at the interface of cell-cell communication

EVs are a heterogenous group of nanosized cell-derived vesicles enclosed by a lipid bilayer membrane that are released by nearly all types of cells from all domains of life into extracellular environment under both physiological and pathological conditions [24, 25].

Exosomes are small cell-derived EVs recognized as important mediators for cell-cell communication by delivering a wide range of biological content, such as proteins, lipids, and nucleic acids to neighboring and distant cells, acting as important intercellular communication messengers. Owing to their intrinsic ability to transfer biomolecules between surrounding and distant cells, exosomes can mediate short and long-distance cell-cell communication and influence various physiological and pathological functions of recipient cells [26].

2.1. Structure and physiology of exosomes

2.1.1. Biogenesis of exosomes

Exosomes are cell-derived nanovesicles whose biogenesis typically encompasses a few steps: (1) invagination of the plasma membrane by inward budding, (2) accumulation of intraluminal vesicles (ILVs) within MVBs by inward budding of the MVB membrane, (3) fusion of MVBs with the plasma membrane, and (4) release of ILVs as exosomes into extracellular space upon the fusion of MVB with the plasma membrane [18, 22].

Exosome formation begins with invagination of the plasma membrane by inward budding forming early endosomes, which then experience a sequence of alterations to become late endosomes or MVBs, which are characterized by the presence of several ILVs in their luminal space, which are formed by inward budding of the MVB membrane [27]. Once MVBs containing several ILVs are formed, they can undergo different destinations: (1) degradation by fusion of the MVB with lysosomes, or (2) exocytosis through fusion of the MVB with the plasma membrane, leading to the release of ILVs as exosomes into extracellular space [19, 27]. Several studies have suggested that the secretion of exosomes into the extracellular environment through exocytosis is dependent on soluble NSF attachment protein receptors (SNAREs) and Rab GTPases, such as RAB27A, RAB11, and RAB31 [19].

2.1.2. Mechanisms of exosome biogenesis

The most reported mechanism for the formation and cargo sorting of ILVs into MVBs, involves the Endosomal Sorting Complex Required for Transport (ESCRT), which is composed of four protein complexes (ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III) that function cooperatively to promote exosome biogenesis [18, 19, 22]. The ESCRT-dependent mechanism is initiated through the sequestration of ubiquitinated proteins by ESCRT-0, which

subsequently recruits the ESCRT-I and ESCRT-II, both responsible for the invagination of the MVB membrane, and then the ESCRT-III causes the scission of the inward budding vesicles, leading to the formation of ILVs within the lumen of MVBs [27, 28].

However, the existence of some ESCRT- independent mechanisms for MVB formation and exosome biogenesis have been reported. Actually, it was found that the concomitant inhibition of all four ESCRT complexes did not suppress the formation of MVBs, suggesting the existence of alternative mechanisms to the ESCRT pathway [26]. One of the proposed mechanisms to generate ILVs without the ESCRT machinery is dependent on ceramide, as a study conducted in mouse oligodendroglial cell lines showed that the secretion of exosomes does not require the ESCRT machinery, but was dependent on sphingomyelinase, an enzyme that catalyzes the production of ceramide [19, 26, 29]. The ESCRT- independent mechanism also seems to be dependent on tetraspanin CD63, which is abundantly found in exosomes, and is known to play an important role in mediating ILV formation [29, 30].

2.1.3. Composition of exosomes

Exosomes are natural cell-derived EVs characterized by an amphiphilic structure similar to that of synthetic liposomes, consisting of a hydrophilic core surrounded by a phospholipidic bilayer membrane [31, 32]. As shown in Figure 1, exosomes are normally enriched in proteins, lipids and nucleic acids, which are the main components, and are derived from their parent cells [19, 28]. Indeed, it has been confirmed that the biological content of exosomes resembles the composition of the donor cells that secrete them, which means that the composition of exosomes is directly related to the physiopathological status of their progenitor cells and may change in response to physiological and pathological conditions [26, 27].

Regarding the protein composition, exosomes are typically enriched in multiple proteins both inside and on the surface membrane, including adhesion molecules (e.g. integrins), proteins responsible for membrane transport and fusion (annexins and Rab GTPases), cytoskeletal proteins (like actin and tubulin), heat shock proteins (Hsp70 and Hsp90), proteins involved in MVB biogenesis, including the apoptosis-linked-gene-2 interacting protein X (Alix) and tumor susceptibility gene 101 (Tsg101), lysosomal proteins, such as lysosome-associated membrane glycoprotein 2b (Lamp2b), and surface tetraspanins, such as CD9, CD63, CD81 and CD82 [19, 27, 30]. The tetraspanins CD9 and CD81 can facilitate the direct membrane fusion between exosomes and target cells, the tetraspanins CD55 and CD59 can offer protection against complement attack, while the expression of the "self-marker" CD47 , a "don´t eat me" signal, can avoid immune phagocytic clearance, increasing the stability of exosomes in circulation and their systemic circulation time [33, 34]. Additionally, exosomes

may also contain major histocompatibility complex (MHC) class I and II proteins responsible for antigen presentation [19, 27, 30].

Regarding the lipid composition, exosomes contain a lipid bilayer membrane whose composition resembles that of their parent cells that secrete them, and appears to be abundant in lipid rafts, such as ceramide, cholesterol, sphingolipids and phosphoglycerides. The lipid composition of exosomes not only allows the direct fusion of exosomes with the plasma membrane of recipient cells, but the phospholipidic bilayer membrane structure also increases the physicochemical stability of exosomes, which may contribute to protect the encapsulated cargo from degradation, ensuring its integrity until its distribution to target cells [18, 22].

Regarding the nucleic acid composition, exosomes are known to be transporters of a wide range of genetic material that can be transmitted to neighboring and distant cells, including RNA molecules, namely messenger RNA (mRNA) and microRNA (miRNA), and also DNA molecules, such as mitochondrial DNA and chromosomal DNA [18, 22].

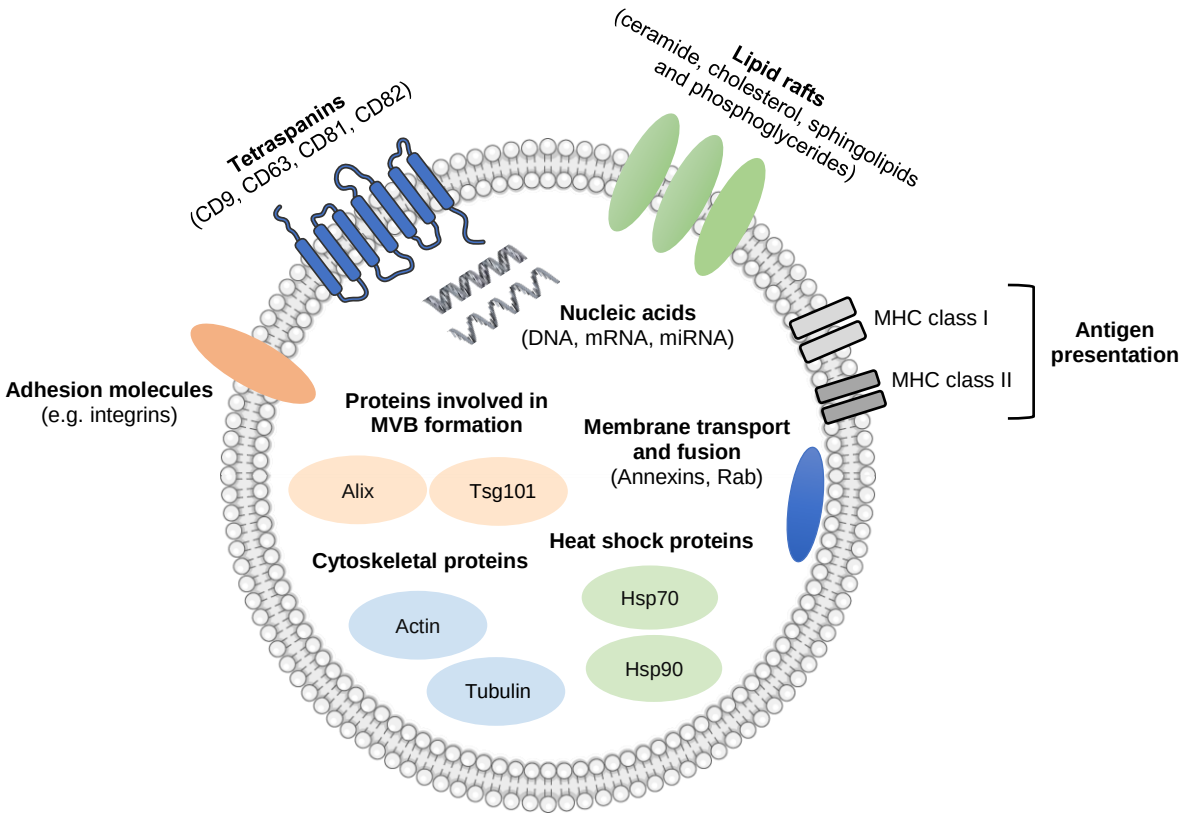

Figure 1. Schematic of the composition of exosomes. Exosomes are normally enriched in lipid rafts, nucleic acids and proteins, including adhesion molecules, tetraspanins (CD9, CD63, CD81 and CD82), proteins responsible for membrane transport and fusion (annexins and Rab GTPases), proteins involved in MVB biogenesis (Alix and Tsg101), heat shock proteins (Hsp70 and Hsp90), cytoskeletal proteins (actin and tubulin) and MHC class I and II proteins.
Abbreviations: Alix, apoptosis-linked-gene-2 interacting protein X; Hsp, heat shock protein; MHC, major histocompatibility complex; miRNA, microRNA; mRNA, messenger RNA; MVB, multivesicular body; Tsg101, tumor susceptibility gene 101.

2.2. Cell-cell communication and physiological / pathophysiological processes

Cell-cell communication is crucial for homeostasis and can occur through a variety of signaling mechanisms, mainly by exosomes that are released by almost all healthy and diseased cells serving as important mediators for intercellular communication, since the biomolecules enclosed in exosomes can be delivered to neighboring and distant cells [22, 31]. It was originally believed that exosomes were only a way for cells to discharge unwanted or unnecessary material, with exosomes being initially recognized as cellular waste. However, nowadays its widely accepted that exosomes can communicate with proximal and distal cells to reprogram them [19, 33].

Once exosomes are released into extracellular space, through fusion of MVBs with the plasma membrane, they can be internalized by recipient cells leading to the modification of target cells´ phenotypes and behaviour. There are three mechanisms proposed for cellular internalization of exosomes, including (1) direct fusion of exosomes with the cell membrane, (2) interaction with cell surface receptors (ligand-receptor interactions), and (3) exosome uptake through endocytosis, including caveolin-mediated endocytosis, clathrin-mediated endocytosis, lipid-raft-mediated endocytosis, phagocytosis and macropinocytosis [35, 36].

Exosomes have been recognized as a crucial mechanism for short and long-distance cell communication performing a key role in several physiological processes, namely in tissue repair, cell proliferation, blood coagulation and immune surveillance [19, 22]. In recent years, several studies have reported the important role of exosomes in immunomodulation, as immune cell-derived exosomes can trigger potent immune responses because of their antigen-presentation capacity. As a matter of fact, previous studies have shown that because of the expression of MHC molecules on their surface, exosomes derived from B lymphocytes can present antigens to $CD4^+$ T cells and $CD8^+$ T cells inducing strong immune responses [37].

Besides physiological functions, exosomes can also play a vital role in various pathological processes. Several studies have emerged in recent years documenting the important contribution of exosomes to the spread and progression of various diseases, such as neurodegenerative and cardiovascular diseases, and particularly for cancer, where the pathological functions of exosomes are widely investigated. Previous studies have shown that tumor cell-derived exosomes exhibit similar properties to those of their parent cells and can transport tumor antigens, which can modulate the tumor microenvironment (TME) and facilitate dissemination of tumors [24, 38]. As a matter of fact, exosomes derived from tumor cells are known to be involved in several mechanisms that induce tumor development, growth

and metastasis, such as cell proliferation, generation of pre-metastatic niches, promotion of tumor angiogenesis, and tumor immunosuppression, by suppressing the activity of natural killer (NK) cells, the differentiation of dendritic cells (DCs) and the activation of T cells [38].

3. Exosomes as nanocarriers for diagnostics and therapeutics

Quite recently, considerable attention has been paid to exosomes for diagnostic and therapeutic applications. As mentioned earlier, exosomes contain a complex and diverse content, and previous studies have shown that exosome cargo is highly influenced by the physiopathological status of the progenitor cells, which highlights the interest of using exosomes as biomarkers of pathological conditions [24]. Recently, exosomes have been used as a non-invasive approach for the diagnosis of a wide range of human diseases, since exosomes can be found in various biological fluids, such as blood, saliva, urine and ascites [23, 39].

The discovery of the important role of exosomes in mediating intercellular communication led to the hypothesis of harnessing exosomes as promising drug delivery systems for therapeutic applications. Compared to synthetic nanomaterials, natural exosomes have some remarkable attributes that make them an interesting approach for drug delivery, such as high biocompatibility, non-immunogenicity, extraordinary stability in biological fluids, and intrinsic targeting properties, since exosomes can preserve the surface protein composition of the donating cell' plasma membrane and the intrinsic targeting properties of the donor cells that secrete them [22, 23, 40]. As a matter of fact, previous studies had suggested that exosomes secreted by specific cell types exhibit an intrinsic cell tropism that favors the selective uptake by homologous cells, due to the presence of homotypic adhesion molecules on the exosome membrane [34]. Another advantages of exosomes as drug delivery systems include their nanoscale size, which facilitates their penetration through biological barriers, such as the blood-brain barrier (BBB), and their ability to avoid immune clearance, due to the presence of "self-marker" CD47 on their surface, a "don´t eat me" signal, increasing the systemic circulation time and protecting the cargo from degradation [22, 34].

Due to the unique structure of exosomes and their excellent delivery properties, much research has been done on exosomes as nanocarriers for delivery of a wide variety of therapeutic cargo, including anticancer drugs, therapeutic proteins, nucleic acids and nanomaterials [41]. In recent years, research on loading exosomes with chemotherapeutic molecules has become very popular, especially with doxorubicin (DOX), which is the most investigated chemotherapeutic agent loaded in exosomes [22]. For example, Tian *et al.* loaded exosomes derived from immature dendritic cells (imDCs) with DOX through electroporation to deliver the chemotherapeutic drug to breast cancer cells [42]. To further enhance the

ability to actively target tumor sites, imDCs were genetically modificated to express Lamp2b, an exosomal surface protein, combined with the iRGD peptide, which may interact with the αV integrin, overexpressed by tumor cells. Indeed, to achieve greater accumulation at tumor sites, exosomes can be engineered to express surface ligands that can bind to specific molecules overexpressed in tumor cells. The engineered DOX-loaded exosomes showed to efficiently deliver the chemotherapeutic drug to breast cancer cells, exhibiting efficient antitumor effects by reducing tumor growth and progression [42].

Recently, it has been shown that natural cell-derived exosomes are also capable of carrying NPs. By loading NPs in naturally secreted exosomes, the problems often associated with NP-based drug delivery systems such as lack of targeting specificity, poor ability to overcome biological barriers, reduced biocompatibility, high cytotoxicity and rapid clearance by the MPS can be avoided. However, in addition to loading NPs in exosomes, their attachment to the exosome membrane surface has also been investigated [41].

3.1. Biomimetic exosome-like nanoparticles

Despite the promising potential of endogenously produced exosomes as drug delivery systems, their use in clinical practice has been hindered by the reduced number of exosomes naturally secreted by most cells, poor production and isolation yields, lack of standardized methods for exosome isolation and purification, and low encapsulation efficiency into natural exosomes [22, 43]. In fact, previous studies have shown that the encapsulation efficiency of therapeutic cargo into endogenous exosomes is usually less than 30 %, which is considerably lower compared to the loading efficiency of NPs (up to about 94 %) [22].

To overcome these limitations of natural exosomes, great effort has been devoted to the study of bioinspired exosome-like NPs, including exosome-mimetic nanovesicles, artificial exosomes, hybrid exosomes and exosome membrane-coated NPs, as depicted in Figure 2. The goal of creating biomimetic exosome-like NPs is to decrease the gap between natural exosomes and nanomaterials by combining the advantages of both components and overcoming their limitations [22, 23]. Bioinspired exosome-like NPs have been recognized as a promising alternative to natural exosomes, as biomimetic exosome-like NPs have proven to possess similar physicochemical properties to those of natural exosomes, but exhibit superior loading efficiency, higher production yield and greater scalability [23].

Although significant progress has been made, scalability still represents a major challenge for clinical application of natural exosomes. To address the low production yield of natural exosomes, the generation of exosome-mimetic nanovesicles has proven to be an efficient approach for stable and clinical-scale production of exosomes. Exosome-mimetic

nanovesicles are cell-derived nanovesicles that can be produced by extruding source cells through porous membranes, which is the most widely used approach, or by forcing donor cells to move through the microchannels of microfluidic devices, and it is known that exosome-mimetic nanovesicles can maintain the intrinsic features and targeting abilities of natural exosomes [22, 23]. As an example, Jang *et al.* generated DOX-loaded exosome-mimetic nanovesicles by extruding DOX-loaded monocytes/macrophages through membrane filters, and showed that the resulting exosome-mimetic nanovesicles had a similar size, morphology and surface protein markers to those of natural exosomes, but showed a 100-fold higher production yield. After *in vivo* administration, the exosome-mimetic nanovesicles showed to efficiently accumulate in tumor tissues and inhibit tumor growth [44].

To address the complexity, heterogeneity and safety concerns of natural exosomes, the preparation of artificial exosomes that include only essential components of natural exosomes has been investigated. Artificial exosomes are a synthetic construction inspired by endogenous exosomes whose preparation involves the assembly of synthetic lipid bilayers mimicking the lipid composition of natural exosomes, which are subsequently functionalized with only a few surface membrane proteins of natural exosomes by various methods, such as the cell-free protein synthesis technique [22, 23, 43]. As an example, Lu *et al.* synthesized connexin 43 (Cx43)-embedded lipid-coated chitosan NPs by using exosome-mimicking lipid bilayers, aiming the delivery of small interfering RNA (siRNA) targeting vascular endothelial growth factor (VEGF) (VEGF siRNA) to glioblastoma target cells [45]. To do so, chitosan NPs were loaded with the VEGF siRNA through electrostatic interactions, and then camouflaged with synthetic lipid bilayers mimicking natural exosomes. The integration of protein Cx43 into the synthetic lipid bilayers was achieved by using an *in vitro* cell-free protein synthesis system with plasmids encoding protein Cx43. The resulting exosome-like NPs demonstrated enhanced delivery efficiency, with the Cx43 protein favoring the cytosolic delivery of VEGF siRNA to glioblastoma cells *via* Cx43-mediated gap-function channels [45].

In an effort to combine the biological functions of natural exosomes with the pharmaceutical benefits of nanomaterials, the hybridization of exosomes with synthetic liposomes to originate hybrid exosomes has been investigated. The fabrication of hybrid exosomes involves the fusion of the exosome membrane with synthetic liposomes, in a way that the benefits of natural exosomes and liposomes can be combined [22, 46, 47]. To be exemplified, Sato and coworkers developed exosome-liposome hybrids through membrane fusion of Raw 264.7 cell-derived exosomes with synthetic liposomes using a freeze-thaw method. The results offered by this study suggested that exosomes can be successfully engineered through membrane fusion with liposomes, leading to the modification of exosome

surface and properties. It was also shown that Raw 264.7 cell-derived exosome-liposome hybrids had greater cell internalization efficiency compared to natural exosomes [47].

In another attempt to combine the advantages of endogenous exosomes with the benefits of nanomaterials while overcoming their limitations, the assembly of exosome membrane-coated NPs has been reported. Exosome membrane-coated NPs are generated by coating a NP inner core with the exosome membrane *via* top-down approaches, in a way that the surface composition and the inherent biological features of the exosome membrane can be faithfully preserved and directly transferred onto the surface of NPs. Therefore, exosome membrane-coated NPs may combine the biointerfacial features of the exosome membrane, such as higher biocompatibility, non-immunogenicity, reduced immune uptake by the MPS, prolonged systemic circulation, homotypic targeting abilities and increased cell-specific uptake, with the pharmaceutical benefits and outstanding delivery properties of nanomaterials [22,23].

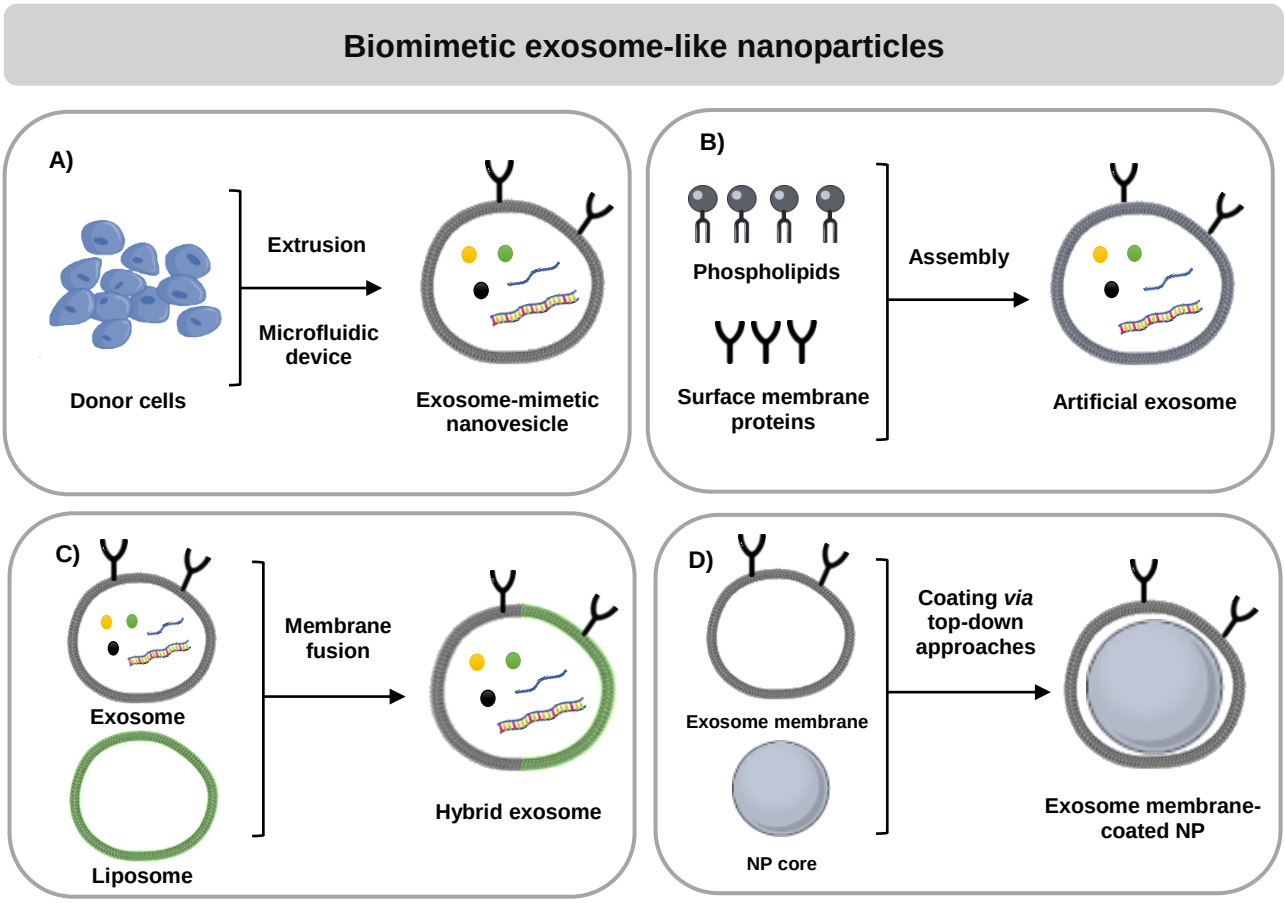

Figure 2. Schematics of biomimetic exosome-like NPs as promising alternatives to endogenous exosomes, including A) generation of exosome-mimetic nanovesicles by directly extruding donor cells or by forcing them to move through microfluidic devices, B) preparation of artificial exosomes using a synthetic strategy inspired by natural exosomes, C) fabrication of hybrid exosomes by fusion of exosomes with synthetic liposomes, and D) generation of exosome membrane-coated NPs by coating NPs with the exosome membrane *via* top-down approaches. **Abbreviations:** NP, nanoparticle.

4. Exosome membrane coatings

As a result of the homotypic targeting and immune evasion abilities of natural exosomes, much research has been paid to the exosome membrane for NP coatings, as these biomimetic nanosystems offer the opportunity to protect the cargo from immune clearance and increase the selectivity of delivery to target cells. Indeed, the exosome membrane coating has shown to endow NPs with the unique ability to target homologous cells, offering the possibility of a targeted therapy, and to reduce immune recognition and clearance of the coated NPs, endowing them with sheath properties and prolonged systemic circulation [34].

4.1. Preparation of exosome membrane-coated nanoparticles

As illustrated in Figure 3, the preparation of exosome membrane-coated NPs typically comprises three steps: (1) extraction of the exosome membrane through a hypotonic treatment of exosomes, previously collected from the cell culture supernatant by ultracentrifugation, (2) selection and synthesis of the NP inner core, and (3) coating of the synthetized NP inner core with the extracted exosome membrane to form a core-shell nanostructure [22]. Each of these preparation steps will be described in detail below.

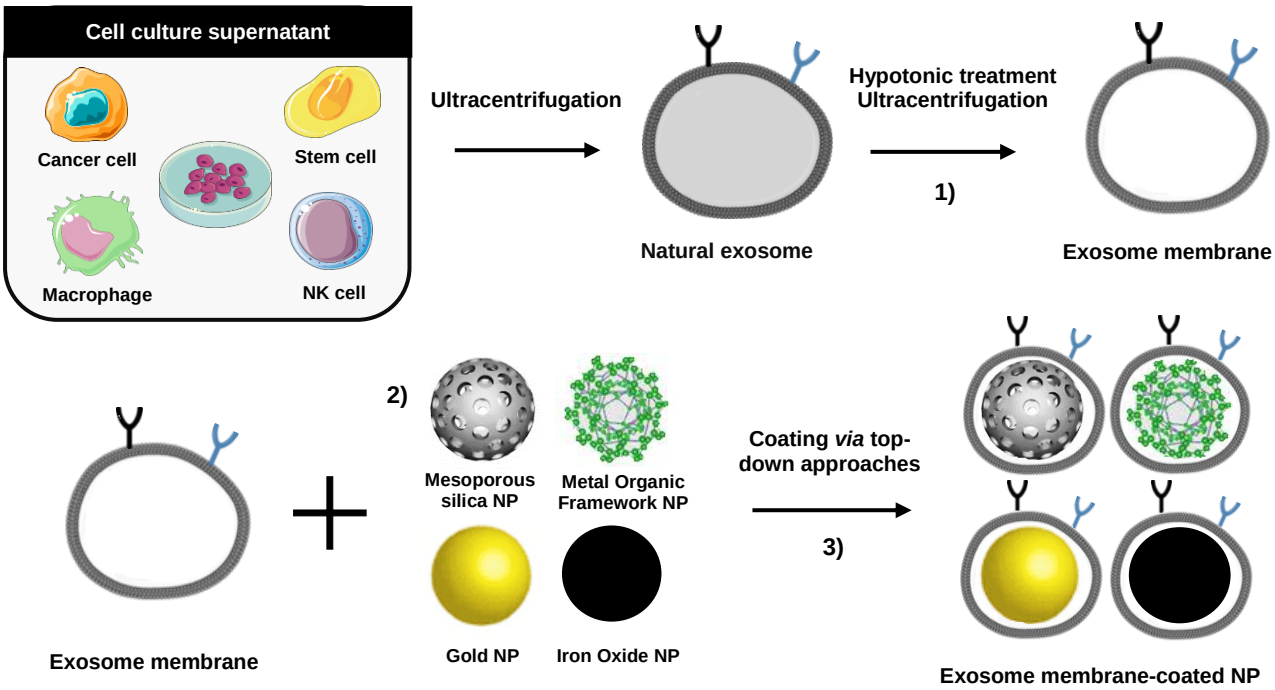

Figure 3. Schematic of the three steps to synthesize exosome membrane-coated NPs including 1) extraction of the exosome membrane through a hypotonic treatment of exosomes, previously isolated from the cell culture supernatant by ultracentrifugation, 2) selection and fabrication of the NP inner core, and 3) coating of the synthetized NP core with the extracted exosome membrane *via* top-down approaches to obtain a core-shell nanostructure. **Abbreviations:** NP, nanoparticle; NK, natural killer.

4.1.1. Extraction of the exosome membrane

The preparation of exosome membrane-coated NPs requires the extraction of the exosome membrane through a hypotonic treatment of exosomes, to remove intracellular components while leaving surface membrane proteins intact, which have been shown to play an important role in several biological functions, including cell recognition, signaling and communication [8, 40]. To do so, endogenously produced exosomes must be first collected from the cell culture supernatant by ultracentrifugation (differential centrifugation), which is the most commonly used method to isolate exosomes from the cell culture supernatant [19]. Then, the exosome membrane can be extracted by re-suspending the collected exosome pellets in a hypotonic lysis buffer, containing a protease inhibitor cocktail, followed by ultracentrifugation of the resulting lysate to remove the intracellular contents and isolate the exosome membrane. Finally, the membrane rich fraction should be washed with isotonic buffers, such as phosphate-buffered saline, to collect the purified exosome membrane [48, 49].

4.1.2. Synthesis of the nanoparticle inner core

Then, the next step involves the selection and preparation of the NP inner core, which will be later coated with the extracted exosome membrane. So far, according to the intended application, several types of NPs ranging from organic to inorganic cores have been coated with the exosome membrane. Among inorganic NPs, mesoporous silica NPs (MSNs), gold NPs, iron oxide NPs, gold-iron oxide NPs, and superparamagnetic iron oxide NPs (SPIONs) have been widely investigated, while the most utilized organic polymeric NPs include poly (lactic-*co*-glycolic acid) (PLGA) NPs, poly (ε-caprolactone) (PCL) NPs and human serum albumin (HSA) NPs [50, 51]. Independent of the inner core material, it is crucial to ensure that the synthetized NP inner core has a negative zeta potential, to promote the establishment of electrostatic repulsion interactions between the negatively charged NP surface and the negatively charged membrane components, and consequently, to facilitate the correct orientation of the biomembrane around the NP surface core [13, 52, 53].

4.1.3. Coating the nanoparticle core with the exosome membrane

Finally, to obtain exosome membrane-coated NPs, the extracted exosome membrane must be cloaked around the synthesized NP core by various coating methods. As a matter of fact, the process of coating the NP surface core with the exosome membrane to obtain exosome membrane-coated NPs can be performed by different coating methods, which are quite similar to those used to camouflage NPs with natural cell membranes [54].

4.1.3.1. Co-extrusion/ sonication

Among the different coating methods for assembling exosome membrane-coated NPs, co-extrusion through porous membranes and sonication stand out as the most widely used approaches. Previous studies have shown that the disruptive forces provided by physical extrusion and sonication can disrupt the exosome membrane structure, allowing it to reassemble around the NP surface core to form a core-shell nanostructure [22, 34].

Physical extrusion was the first coating method reported and was commonly used to prepare synthetic liposomes. In this method, the NP inner core and the purified exosome membrane are combined and co-extruded through porous membranes to originate exosome membrane-coated NPs. It is believed that the mechanical forces induced by extrusion can disrupt the exosome membrane structure, enabling it to reassemble around the NP surface to form a core−shell nanostructure [9, 10]. Another approach widely used to camouflage the exosome membrane around the synthetized NP inner core is sonication. In this approach, exosome membrane-coated NPs are manufactured by exposing both the NP inner core and the purified exosome membrane to ultrasonic disruptive forces, provided by ultrasonic energy, leading to the spontaneous formation of a core−shell nanostructure, with the added advantage of losing less material compared to physical extrusion [9, 10].

4.1.3.2. Direct incubation of nanoparticles with cells or exosomes

Although physical extrusion and sonication are widely used to camouflage NPs with the exosome membrane, there are still some challenges associated with these approaches, including the labor-intensive and the time-consuming preparation, and the possibility of damaging the protein integrity of the exosome membrane [22]. Previous studies have shown that the surface membrane proteins are critical for the biological functions of exosomes, and thus, by damaging the protein integrity of the exosome membrane these approaches can adversely affect the biological properties of these biomimetic nanosystems.

Since the surface protein composition of exosomes is crucial to their biological functions, preserving the integrity of the exosome membrane is a very important concern [55]. Thus, to circumvent the possibility of affecting the protein integrity of the exosome membrane and preserve its biological functions, some nondisruptive coating techniques have been proposed to coat NPs with the exosome membrane. One of these approaches is based on direct incubation of NPs with living cells, allowing them to secrete exosomes containing exogenous NPs, taking advantage of the exosome biogenesis pathway to encapsulate NPs with the exosome membrane [22, 54]. In another approach, coating NPs with the exosome membrane can also be achieved by directly incubating NPs with pre-collected exosomes [54].

4.1.3.3. Microfluidic sonication method

To overcome the labor-intensive and time-consuming limitations of co-extrusion and sonication, recently Liu *et al.* developed an efficient strategy based on microfluidic sonication to construct core-shell PLGA NPs in a single continuous manner, using ultrasonication to coat PLGA NPs with several types of biomembranes, including a lipid membrane, cancer cell membrane, and a exosome membrane, in which the membranes of exosomes and cancer cells were isolated from A549 human lung carcinoma cells, and then coated around PLGA NPs using the microfluidic sonication approach. *In vivo* studies showed that the resulting exosome membrane-coated PLGA NPs possessed enhanced homotypic targeting abilities and superior systemic circulation time, compared to lipid membrane- and cancer cell membrane-coated PLGA NPs, due to the reduced immune uptake by monocytes/macrophages [34].

This microfluidic sonication approach was expanded in a subsequent study performed by Han *et al.*, in which PLGA NPs were coated with an MDA-MB-231 cell-derived exosome membrane functionalized with AS1411 aptamers. Because of the exosome membrane coating, the biomimetic nanosystem exhibited longer systemic circulation *in vivo*, and due to the specific binding of AS1411 aptamers to nucleolin, which is overexpressed on the membrane of some cancer cells, the resulting biomimetic NPs showed superior tumor-targeting abilities [56].

4.2. Characterization of exosome membrane-coated nanoparticles

After preparation of the biomimetic core-shell NPs, their characterization is a critical step to confirm that the cell membrane has been successfully wrapped around the NP surface. The characterization of the resulting biomimetic core-shell NPs is decisive to ensure the successful membrane coating and the formation of the core-shell nanostructure, which can be determined by the assessment of their physicochemical and biological properties, including morphology, size, zeta potential (surface charge), and surface protein composition [8, 10, 51].

The process of cell membrane coating often modifies the NP morphology, size and zeta potential, and thus, various techniques such as transmission electron microscopy (TEM), dynamic light scattering and NP tracking analysis can be employed to examine the morphology, and to measure the size distribution and the surface zeta potential of the resulting biomimetic core-shell NPs [8, 10, 57]. To confirm the successful membrane wrapping around the NP surface, a core-shell nanostructure should be observed on TEM images, and a 10-20 nm increase in particle size compared to non-coated NPs, regarding to the thickness of the membrane coating. Further, after the coating process, the coated NPs should have a negative zeta potential measurement with a similar charge to that of the purified membrane [8].

To further evaluate the successful membrane wrapping around the NP surface and ensure the effectiveness of the resulting biomimetic core-shell NPs, an evaluation of their biological properties must be done to confirm the preservation of the surface membrane proteins of natural exosomes and their associated functions [51, 58]. To this end, various techniques such as Western blotting and Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) can be used to investigate the surface protein composition of the coated NPs, which should be similar to that of the purified exosome membrane [8, 10].

5. Applications of exosome membrane-coated nanoparticles

In recent years much attention has been devoted to EV membrane-coated NPs as attractive biomimetic nanoplatforms for many clinical applications, on account of their excellent biocompatibility, non-immunogenicity, immune evasion abilities, prolonged systemic circulation, homotypic targeting abilities and cell-specific uptake [54]. As depicted in Figure 4, this biomimetic coating strategy has been exploited for many biomedical applications, including cancer therapy and imaging, neuroimaging, Parkinson´s disease treatment, bacterial infection treatment, skin regeneration and wound repair. A summary of the biomedical applications of EV membrane-coated NPs is summarized in Table 1 (Appendix 1) and Table 2 (Appendix II).

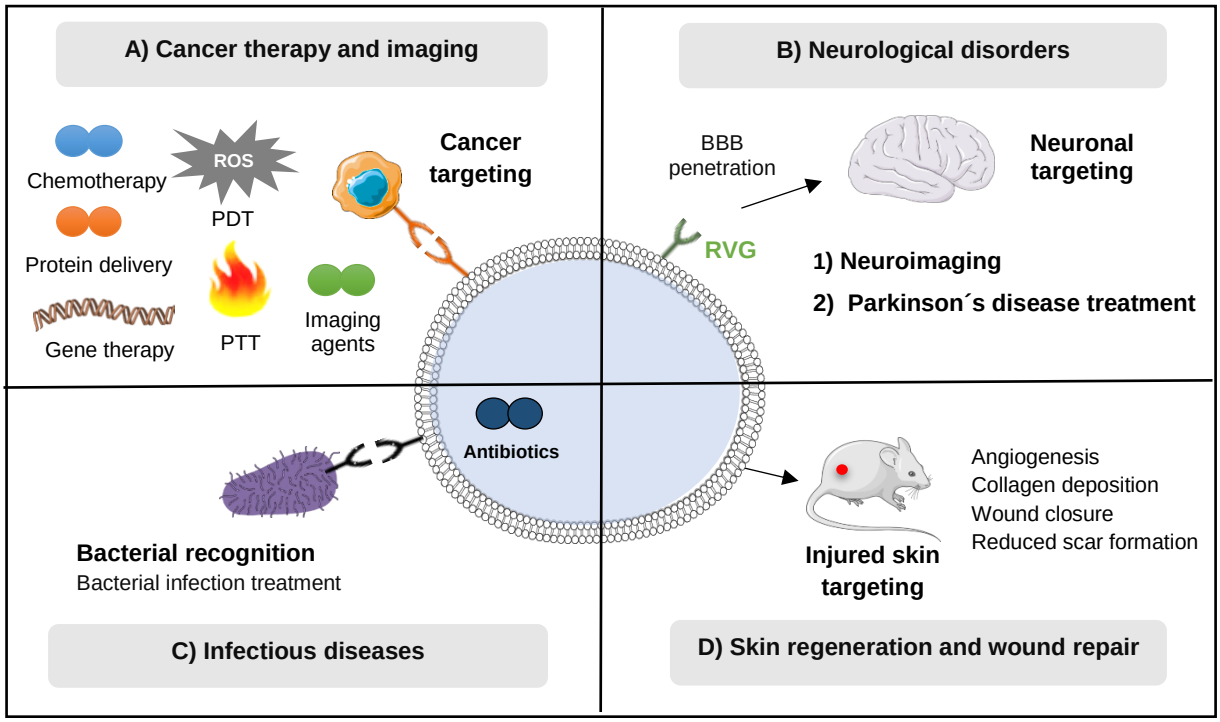

Figure 4. Overview of the main biomedical applications of extracellular vesicle membrane-coated nanoparticles, including A) cancer therapy and imaging, B) neuroimaging and Parkinson´s disease treatment, C) bacterial infection treatment, and D) skin regeneration and wound repair.
Abbreviations: BBB, blood-brain barrier; PDT, photodynamic therapy; PTT, photothermal therapy; ROS, reactive oxygen species; RVG, rabies virus glycoprotein.

5.1. Cancer applications

In recent years ongoing research in the field of cancer therapy has been exploiting the unique features of natural exosomes and the high drug loading capacity of NPs to achieve remarkable antitumor effects on both primary and metastatic tumor cells.

In this review, particular attention will be paid to exosome membrane-coated NPs for cancer therapy and imaging, particularly for chemotherapy delivery, protein delivery, gene delivery and gene silencing, antimetastatic therapy, phototherapy, chemodynamic therapy, radiotherapy, immunotherapy, tumor imaging and theranostics. Indeed, these biomimetic nanoplatforms have been designed to deliver not only therapeutic molecules, but also imaging agents to tumor sites, providing a synergistic approach for cancer therapy and diagnosis.

5.1.1. Chemotherapy delivery

Chemotherapy remains one of the most widely used therapeutic approaches for cancer eradication, however, this strategy is limited, mostly due to the low tumor specificity of chemotherapeutic drugs, which results in severe side effects and off-target toxicity in healthy cells [2, 59]. Thus, to circumvent the limitations of chemotherapy, the encapsulation of NPs with the exosome membrane has been reported to improve the targeted delivery of chemotherapeutic drugs to specific tumor sites, markedly reducing chemotherapy side effects and off-target toxicity in healthy cells, thus increasing therapeutic efficacy and safety.

For cancer applications, cancer cell-derived exosomes are the most widely investigated to camouflage around NPs, since exosomes derived from cancer cells can maintain the surface antigens from their progenitor cells, suggesting that cancer cell-derived exosomes can be used as effective NP coatings to selectively target homologous cancer cells [54, 60].

In this regard, in an effort to develop cancer cell-derived exosome membrane-coated NPs for targeted delivery of chemotherapeutic drugs to tumor sites while avoiding the possibility of damaging the protein integrity of the exosome membrane, Yong *et al.* coated DOX-loaded luminescent porous silicon NPs (PSiNPs), with an exosome membrane derived from different cancer cell lines by exocytosis, taking advantage of the exosome biogenesis pathway to generate exosome membrane-coated DOX-loaded PSiNPs as a drug vehicle for DOX, a chemotherapeutic agent [61]. After intravenous injection in subcutaneous, orthotopic and metastatic tumor-bearing mice models, the biomimetic nanosystem showed greater extravasation ability, enhanced accumulation and penetration into tumor tissues, being efficiently internalized by both cancer cells and cancer stem cells (CSCs), which are a small population of cells responsible for cancer proliferation and metastasis. Therefore, the

biomimetic nanosystem showed efficient antitumor and CSCs- killing activities *in vivo*, which resulted in excellent antitumor efficacy and greater suppression of tumor growth. Beyond that, the biomimetic nanosystem demonstrated cross-reactivity between different cancer cell types, since DOX-loaded PSiNPs coated with H22 cancer cell-derived exosome membrane showed increased uptake in B16-F10 cancer cells, and vice-versa. In summary, this study provides an innovative coating technique based on exocytosis from tumor cells to synthetize exosome membrane-coated NPs as promising nanocarriers to deliver anticancer drugs to tumor sites, without damaging the surface protein integrity of the exosome membrane [61].

In another example of using cancer cell-derived exosome membrane-coated NPs for tumor-targeted delivery of chemotherapeutic drugs, Iles *et al.* coated MIL-88A metal organic framework (MOF) NPs, which have been recognized as desirable drug nanocarriers due to their unique features, such as high biocompatibility, controlled drug release and high loading efficiency, with an exosome membrane derived from HeLa cells for targeted delivery of Suberoyl bis-hydroxamic acid (SBHA), a histone inhibitor and chemotherapeutic drug, to homotypic HeLa cells [62, 63]. The biomimetic nanosystem showed to be selectively taken up by homotypic HeLa cells *in vitro*, due to the homotypic tumor-targeting ability of the exosome membrane coating, enabling the targeted delivery of the chemotherapeutic drug without premature leakage of the therapeutic payload, offering an excellent opportunity to markedly reduce chemotherapy side effects and off-target toxicity [63].

Similarly, in another study, Qiao *et al.* enveloped Doxil, a chemotherapy drug that encapsulates DOX into liposomes, with an exosome membrane derived from HT1080 tumor cells, and showed that these biomimetic NPs could effectively deliver the chemotherapeutic drug to HT1080 tumors *in vivo*, where it inhibited tumor growth [64]. *In vivo* studies showed that HT1080 cell-derived exosomes homed to their source cells more efficiently than HeLa cell-derived exosomes, resulting in enhanced accumulation in homotypic HT1080 tumor cells. These results indicate the innate tropism of HT1080 cell-derived exosomes to accumulate in homotypic HT1080 tumor cells, which resulted in higher accumulation of DOX in tumor cells and greater suppression of tumor growth to practically undetected levels. Additionally, compared to free Doxil, Doxil encapsulation in exosomes induced significantly less DOX-induced cardiotoxicity, most likely due to the increased accumulation at tumor cells and reduced accumulation in the heart. In summary, the designed biomimetic nanosystem showed to efficiently deliver the chemotherapeutic drug to homotypic tumor cells, resulting in enhanced accumulation in tumor sites and reduced off-target toxicity [64].

However, in addition to using the cancer cell-derived exosome membrane for NP coatings, the exosome membrane derived from non-cancerous cells has also been explored for tumor-targeted delivery of chemotherapeutic drugs. For instance, Li *et al.* coated DOX-loaded PLGA NPs with an exosome membrane derived from macrophages for targeted delivery of DOX to triple-negative breast cancer (TNBC) cells, which is an aggressive subtype of breast cancer known for high proliferation rates and poor overall survival [65]. To further enhance the tumor-targeting ability, a c-Met targeting peptide was decorated on the surface of the biomimetic nanosystem, in order to increase its ability to target the mesenchymal epithelial transition factor (c-Met), which is highly expressed by TNBC cells. *In vivo* studies showed that the resulting biomimetic nanosystem had superior accumulation at targeted tumor sites and a pH-sensitive drug release in acid conditions, allowing the DOX delivery only in the acidic conditions of lysosomes for targeted therapy. Overall, the resulting exosome membrane-coated DOX-loaded PLGA NPs decorated with the c-Met binding peptide showed excellent immune evasion abilities, prolonged systemic circulation and enhanced tumor-targeting capabilities, which resulted in remarkable antitumor efficacy and greater suppression of tumor growth *in vivo*. Upon histological evaluation, no pathological malformations on major organs were observed, confirming the biocompatibility of the biomimetic nanoplatform [65].

5.1.2. Protein delivery

The intracellular delivery of therapeutic proteins *via* systemic administration is very challenging, mostly owing to the susceptibility of proteins to be degraded and denatured *in vivo* and the low transduction efficiency, which in turn can compromise its therapeutic efficiency. Therefore, to overcome the challenges of intracellular delivery of therapeutic proteins, the biomimetic approach of exosome membrane-coated NPs has been widely explored, as this biomimetic nanosystems have been proven to protect loaded proteins from degradation by protease *in vivo*, avoid phagocytic clearance and selectively target homotypic tumor sites, increasing cell-specific uptake and enhance the intracellular delivery of proteins [48].

In this regard, Cheng *et al.* camouflaged a protein-loaded ZIF-8 MOF inner core, named MP, with an exosome membrane derived from MDA-MB-231 cells, aiming the intracellular delivery of functional proteins to human breast adenocarcinoma MDA-MB-231 cells (Appendix III A) [48]. The resulting exosome membrane-coated MP, designated EMP, showed to be endowed with immune evasion abilities and prolonged systemic circulation, and to protect loaded proteins from degradation by protease *in vivo*, releasing the therapeutic cargoes only in the acidic conditions of the TME for localized drug release. Because of the homotypic tumor-targeting ability of the exosome membrane, the biomimetic nanosystem exhibited higher

uptake by homologous MDA-MB-231 cells, compared to 293T human embryonic kidney cells, 3T3 mouse embryo fibroblasts, CAD mouse central nervous system-derived cells, MCF7 human breast adenocarcinoma cells, and SH-SY5Y human neuroblastoma cells, indicating that tumor cell-derived exosomes possess a natural tropism to accumulate in homotypic tumor cells, from which exosomes are derived (Appendix III B and III C). *In vivo* studies showed superior accumulation of the biomimetic nanosystem at tumor sites, compared to non-coated NPs, resulting in greater transduction efficiency of gelonin, a protein that induces cell apoptosis by disturbing protein synthesis, and superior antitumor efficacy (Appendix III D - III F) [48].

5.1.3. Gene delivery and gene silencing

In recent years, gene therapy is becoming a promising approach for cancer therapy, by delivering therapeutic nucleic acids (mRNA, miRNA and siRNA) to target tumor cells, aiming to correct the abnormal gene expression and suppress tumor growth by regulating gene expression [66,67]. Due to the intrinsic ability of exosomes to transport nucleic acids between neighboring cells, exosome membrane-coated NPs have been recognized as promising gene delivery vehicles for targeted delivery of therapeutic nucleic acids to tumor sites [68].

For example, Alhasan *et al.* coated spherical nucleic acids (SNAs), which are composed of a gold core with a dense shell of oriented oligonucleotides, loaded with antisense miRNA targeting miRNA-21 (miR-21) (anti-miR-21), with an exosome membrane derived from PC-3 prostate cancer cells taking advantage of the exosome biogenesis pathway [69]. The resulting exosome membrane-coated SNAs exocytosed from tumor cells showed to efficiently deliver the anti-miR-21 to PC-3 prostate cancer cells, resulting in remarkable gene-silencing effects by downregulating the expression of oncogenic miR-21, which is significantly overexpressed in tumor tissues and is known to be involved in cancer proliferation and metastasis [69].

Over the years, there have been multiple studies indicating that exosomes secreted by specific types of cells may exhibit direct antitumor properties [22]. For example, previous studies have shown that NK cells can release exosomes capable of accumulating at specific tumor sites, exerting a strong antitumor activity, due to the presence of killer proteins, such as FASL and perforin [23, 38]. Therefore, several studies have emerged documenting the huge potential of NK cell-derived exosomes in the field of cancer therapy, since they can not only improve the tumor-targeting ability by guiding therapeutic agents to specific tumor sites, but can also act as direct antitumor agents due to their natural antitumor properties [70].

For instance, Wang *et al.* developed biomimetic core-shell NPs by coating tyrosine-coupled dendrimers, loaded with the therapeutic Let-7a miRNA, with a membrane of NK cell-derived exosomes, previously isolated from the NK cell culture supernatants by differential

centrifugation [70]. Because of the tumor-targeting ability of the NK cell-derived exosome membrane coating, which can act as a tumor-targeting navigator due to the specific binding of the C-X-C chemokine receptor type 4 (CXCR4) expressed on NK cell-derived exosomes, a chemokine receptor involved in leukocyte trafficking, to the stromal cell-derived factor-1 (SDF-1), which is released by some tumor cells, the biomimetic nanosystem showed to specifically target and accumulate at tumor sites, enabling the targeted delivery of the therapeutic Let-7a miRNA to neuroblastoma CHLA-255 cells. In summary, the resulting biomimetic nanosystem demonstrated excellent antitumor effects and greater suppression of tumor growth *in vitro* and *in vivo*, due to the synergistic antitumor effects of the therapeutic Let-7a miRNA and the intrinsic antitumor properties of NK cell-derived exosomes [70].

5.1.4. Antimetastatic therapy

Current research has been showing that, in addition to target primary tumor sites, these biomimetic core-shell NPs are also capable of targeting metastatic tumor sites. Ongoing developments on the biomimetic approach of exosome membrane-coated NPs have demonstrated the enormous potential of these biomimetic nanosystems as effective antitumor and antimetastatic agents, suppressing the growth of both primary and metastatic tumors [23].

For instance, inspired by the macrophage's ability to be efficiently recruited to inflamed/tumor sites, Xiong *et al.* coated an HSA inner core composed of a laurate-functionalized platinum (Pt (IV)) prodrug, named Pt (IV) HSA NPs, with the membrane of exosomes derived from murine RAW264.1 cells (Rex) to generate Rex-coated Pt (IV) HSA NPs (NPs/Rex), for targeted delivery of Pt (IV) to orthotopic breast tumors and lung metastatic nodules (Appendix IV A and IV B) [71]. After internalization of the biomimetic nanosystem by tumor cells, Pt (IV) was reduced to cisplatin, a well-known chemotherapeutic agent that causes DNA damage, inducing tumor cell death by triggering apoptotic signals and inhibiting the proliferation of breast cancer cells. After intravenous injection in a tumor-bearing mice model, the resulting NPs/Rex exhibited longer systemic circulation, excellent biocompatibility, and showed to be specifically recruited to orthotopic breast tumors and lung metastatic nodules, exhibiting remarkable antitumor and antimetastatic effects *in vivo*, by suppressing orthotopic breast tumor growth and reducing the number of lung metastatic nodules (Appendix IV C - IV F). Additionally, because of its ability to be specifically recruited to orthotopic breast tumors and lung metastatic nodules, the biomimetic nanosystem showed less uptake by the liver and kidneys, which resulted in considerably less hepatotoxicity and nephrotoxicity, which are common side effects of free cisplatin [71].

The high incidence of lung metastasis of TNBC after surgery is the most significant cause of death related to breast cancer [72]. In this regard, in an effort to suppress postoperative breast cancer lung metastasis through modulation of the lung pre-metastatic niche microenvironment, Zhao *et al.* designed a biomimetic core-shell nanoplatform by coating a cationic bovine serum albumin (CBSA) core conjugated with S100A4 siRNA (siS100A4), with an exosome membrane shell derived from autologous breast cancer cells [72]. The resulting biomimetic core-shell NPs exhibited outstanding biocompatibility and showed to efficiently protect the loaded siS100A4 from degradation, improving the delivery of the therapeutic siS100A4 to pre-metastatic niches in the lungs, which resulted in a remarkable suppression of lung metastasis by silencing the expression of the S100A4 metastasis-related protein, which is responsible for tumor growth and metastasis. In summary, the biomimetic nanosystem showed to effectively target and deliver the siS100A4 to pre-metastatic niches in the lungs, which resulted in remarkable gene-silencing effects and efficient suppression of postoperative breast cancer lung metastasis by downregulation of S100A4 expression [72].

Cancer metastasis appears to be widely dependent on the ability of circulating tumor cells (CTCs), which have been shown to play a critical role in tumor genesis, development, progression and metastasis, to travel and invade distant tissues. Consequently, in order to design an efficient antimetastatic approach capable of preventing the development of metastatic nodules, the successful recognition and capture of blood CTCs must be achieved, in order to prevent them from spreading and colonizing in distant tissues [73].

However, currently, the efficiency of the antimetastatic therapy remains unsatisfactory, mostly owing to the difficulty to recognize and capture the blood CTCs [49]. In this regard, to address this challenge, Wang *et al.* developed a biomimetic nanoplatform for breast cancer metastasis inhibition, by coating PEGylated-PCL (PEG-PCL) NPs, co-loaded with ROS-sensitive thioether-linked PTX-linoleic acid prodrug (PTX-S-LA) and cucurbitacin B (CuB), named PCNPs, with an exosome membrane derived from human breast adenocarcinoma MDA-MB-231 cells [49]. Because of the expression of adhesion molecules on the surface of the resulting exosome membrane-coated PCNPs (EMPCs), particularly the CD44, which is found on both the cancer cell membrane and on the cancer cell derived- exosome membrane, and is responsible for mediating homotypic cancer binding, the resulting biomimetic nanosystem showed to efficiently target not only primary tumor cells but also the blood CTCs during circulation. After cellular internalization of the biomimetic nanosystem, CuB was released first, which contributed not only to the release of PTX from the biomimetic NPs, due to the increased intracellular levels of ROS within tumor cells, but also contributed to an efficient suppression of tumor metastasis through downregulation of the FAK/MMP signaling pathway

(Appendix V A). *In vivo* studies suggested that the resulting biomimetic nanosystem possessed excellent immune evasion abilities, prolonged systemic circulation and enhanced tumor accumulation, which resulted in excellent antitumor efficacy and greater suppression of tumor growth (Appendix V B and V C). Additionally, because of its ability to recognize and capture the blood CTCs through the CD44-mediated interaction, the biomimetic nanosystem showed exceptional antimetastatic effects, which resulted in an efficient reduction of lung metastatic nodules (Appendix V D - V F). In summary, the biomimetic nanoplatform showed to improve the CTCs' capture ability and the antimetastatic efficacy against breast cancer cells [49].

5.1.5. Phototherapy

Phototherapy has emerged as a non-invasive and effective strategy to selectively destroy cancer cells without damaging healthy cells, reducing the side effects and increasing the therapeutic efficacy compared to traditional anticancer therapies. Because of its advantages, including non-invasiveness, specific tumor-targeting and low systemic toxicity, this approach has been recognized as a promising strategy for cancer treatment [74].

The main types of phototherapy include photothermal therapy (PTT) and photodynamic therapy (PDT), which are promising light-activated approaches that require tumor irradiation with external light to destroy cancer cells *via* thermal ablation and generation of reactive oxygen species (ROS), correspondingly [68, 75, 76].

5.1.5.1. Photothermal therapy

PTT is a phototherapy approach in which photothermal agents with light-absorbing properties are delivered to target tumor sites and then irradiated with near-infrared (NIR) light to produce heat capable of destroying cancer cells. This is possible due to the ability of the photoabsorbing agents to absorb light energy and convert it into cytotoxic heat capable of killing cancer cells through hyperthermia [74, 77]. Currently, the PTT approach has been showing to be a promising and minimally invasive strategy for cancer treatment, due to the lower susceptibility of heathy cells to heat compared to cancer cells [78].

Recently, the biomimetic approach of exosome membrane coated-NPs has been investigated for PTT, as these biomimetic core-shell NPs have been shown to enhance the delivery efficiency of photothermal agents to targeted tumor sites. However, in addition to being used alone, PTT can also be used in combination with other anticancer approaches, such as chemotherapy, with the goal of producing synergistic anticancer effects [79].

For instance, to achieve an efficient synergistic anticancer effect, Tian *et al.* reported a combined chemo-PTT therapy against 4T1 breast cancer cells by combining PTT with

chemotherapy. For this purpose, MSNs co-loaded with indocyanine green (ICG) and DOX, designated ID@MSNs, were camouflaged with a 4T1 breast cancer cell-derived exosome membrane to generate ID@E-MSNs (Appendix VI A) [79]. After intravenous injection in a tumor-bearing mice model, the resulting ID@E-MSNs showed enhanced uptake by homotypic 4T1 breast cancer cells, compared to non-coated NPs, resulting in higher accumulation in the tumor tissue and greater suppression of tumor growth *in vivo* (Appendix VI B - VI D). Closer examination of its chemo-PTT synergistic effects showed that after NIR irradiation, the ICG could efficiently convert light into cytotoxic heat to produce hyperthermia capable of killing tumor cells, which was also crucial to disrupt the ID@E-MSNs structure, allowing the release of DOX for chemotherapy, leading to combined chemo-PTT effects. Upon histological evaluation, no pathological malformations on major organs were observed, confirming the biosafety of this biomimetic nanoplatform (Appendix VI E). In summary, this study provides a chemo-PTT synergistic approach for efficient breast cancer treatment [79].

5.1.5.2. Photodynamic therapy

Another type of phototherapy is PDT, which is a non-invasive approach that requires photosensitizer agents that are delivered to tumor sites and then activated through irradiation with a specific wavelength of light energy, normally with laser irradiation. This causes the photosensitizer agents to transfer energy to the surrounding oxygen molecules, to produce a large amount of ROS, in particular singlet oxygen ($^1O_2$), capable of damaging tumor cells [74]. Consequently, elevated oxygen levels in TME are crucial to increase ROS production upon laser irradiation and ensure the efficiency of PDT, and thus, the effectiveness of this approach may be compromised in hypoxic solid tumors due to lack of oxygen [80].

Beyond the oxygen levels within the TME, another barrier that may compromise the efficiency of PDT is the high autophagic activity in cancer cells, which allow them to eliminate the damaged organelles generated by ROS and survive to PDT [81]. Consequently, in order to increase the antitumor efficacy of PDT against glioblastoma, a synergistic strategy based on suppression of the autophagic activity in glioblastoma cells was employed by Mo *et al.,* in which hollow zinc sulfide (ZnS) NPs loaded with hydroxychloroquine (HCQ), an autophagic inhibitor drug, were first covered with a U-87 glioblastoma cell-derived exosome membrane, and then decorated with iRGD-modified phosphatidylserine [81]. While ZnS acts as a photosensitizer producing ROS under light irradiation capable of inducing substantial cell damage, the loaded HCQ can inhibit the autophagic flux to enable the accumulation of damaged organelles in cancer cells, increasing the antitumor efficiency of PDT. The biomimetic nanosystem showed to efficiently cross the BBB and preferentially accumulate in glioblastoma cells *in vivo*, due to

the homotypic tumor-targeting ability of the exosome membrane and the ability of the iRGD peptide to target αvβ3 integrins and neuropilin-1 receptors expressed by glioblastoma cells, which resulted in greater suppression of tumor growth and extended overall survival of glioblastoma-bearing mice. In summary, this study provides an efficient strategy based on suppression of autophagy within cancer cells to increase the antitumor efficiency of PDT [81].

5.1.6. Chemodynamic therapy

Chemodynamic therapy is a promising strategy that employs Fenton reaction to convert hydrogen peroxide (H_2O_2) into cytotoxic hydroxyl (·OH) radicals capable of killing cancer cells. In this regard, very recently Pan *et al.* developed a biomimetic nanosystem for chemo/chemodynamic therapy against prostate cancer, by coating a DOX-loaded Fe_3O_4-HSA core (PMA/Fe-HSA@DOX), with a urinary exosome membrane isolated from the urine of prostate cancer patients, to originate exosome membrane-coated PMA/Fe-HSA@DOX (Exo-PMA/Fe-HSA@DOX) [82]. In this study, a novel electroporation-based coating technique was used to camouflage NPs with the exosome membrane, a strategy that employs an external electric field to open pores in the exosome membrane through which NPs can pass. After being internalized by tumor cells, the Fe_3O_4 core can decompose the H_2O_2 under the acidic conditions of the TME, producing ·OH radicals capable of inducing synergistic antitumor effects in combination with DOX (Appendix VII A). Additionally, the high intracellular levels of ·OH and DOX within tumor cells led to increased inhibition of the epidermal growth factor receptor (EGFR) and its AKT/ NF-κB/IkB signaling pathway, which is responsible for tumor growth and proliferation (Appendix VII B). In summary, because of the tumor-targeting ability of the urinary exosome membrane, the biomimetic nanosystem showed enhanced uptake by prostate cancer cells, leading to higher accumulation at tumor sites and enhanced chemo/chemodynamic effects, which resulted in excellent antitumor efficacy and greater suppression of tumor growth *in vivo* (Appendix VII C - VII E) [82].

5.1.7. Radiotherapy

Boron neutron capture therapy (BNCT) is a non-invasive and targeted radiation therapy capable of selectively destroying boron-accumulating tumor cells without damaging neighboring healthy tissues. This type of radiotherapy is based on the ability of the nonradioactive isotope boron-10 (^{10}B) to capture thermal neutrons and release highly-energetic particles, namely helium-4 (^{4}He) and lithium-7 (^{7}Li) nuclei, after irradiation with a precise dose of neutron radiation. In this following, the BNCT approach comprises two key

steps. First, ^{10}B boron-containing compounds are delivered to selectively accumulate in tumor sites followed by neutron irradiation to selectively destroy tumor cells [83, 84].

In this regard, recently, Li *et al.* developed a biomimetic nanosystem based on exosome membrane- coated ^{10}B boron-containing carbon dots (BCDs) with the aim of using the BNCT approach to treat brain glioma *in vivo* [84]. To do so, BCDs consisting of boron phenylalanine (BPA), a boron-containing compound, and D-glucose were coated with a macrophage-derived exosome membrane to originate exosome membrane-coated BCDs (BCD-Exos) (Appendix VIII A and VIII B). *In vivo* studies showed that the resulting BCD-Exos could efficiently cross the BBB exhibiting superior tumor accumulation 4 h after administration compared to non-coated BCDs (Appendix VIII C). Then, tumor-bearing mice were irradiated with precise dose of thermal neutrons, which resulted in greater suppression of tumor growth and extended overall survival of mice treated with BCD-Exos (Appendix VIII D). Additionality, upon histological evaluation, no pathological malformations were observed in the mice brain and major organs, suggesting a desirable biosafety profile (Appendix VIII E and VIII F). These findings indicate the potential of the biomimetic nanosystem as a promising boron delivery system to improve the efficiency of BNCT to treat brain glioma *in vivo*, mostly owing to its ability to cross the BBB and selectively accumulate at tumor sites [84].

5.1.8. Immunotherapy

Immunotherapy is a very promising area in the field of cancer therapy that can be applied not only for cancer treatment, but also for preventive cancer vaccination. The main principle of cancer immunotherapy is to stimulate the patient´s own immune system to suppress tumor progression and destroy malignant cells [85, 86].

Glioblastoma remains one of the most common brain tumors, for which effective treatments are still lacking, mostly owing to the BBB ability to block the penetration of anticancer drugs into glioblastoma cells [87]. Thus, to increase the BBB penetration ability and develop efficient treatments for glioblastoma, Zhang *et al.* coated DOX-loaded PEGylated-poly-lactic acid (PEG-PLA) NPs with an exosome membrane derived from bEnd.3 cells, a murine brain endothelial cell line, for immunogenic chemotherapy of glioblastoma cells, which relies on the ability of some chemotherapeutic drugs to simultaneously induce apoptosis and immunogenic cell death (ICD) of tumor cells, which is a particular type of cell death that triggers a potent antitumor immune response by stimulating the maturation of antigen-presenting cells and the infiltration of cytotoxic CD8+ T lymphocytes into tumor sites [87]. The resulting biomimetic core-shell NPs showed to be efficiently taken up by bEnd.3 cells *in vitro* and *in vivo*, resulting in greater BBB penetration ability and higher DOX accumulation in

glioma GL261 cells, inducing apoptosis and ICD of glioma cells. Indeed, in addition to inducing tumor cell apoptosis, DOX has also proven to be a potent ICD-inducer, capable of triggering a potent antitumor immune response by stimulating the maturation of DCs and the infiltration of cytotoxic CD8+ T lymphocytes in tumor sites. After intravenous injection in a glioblastoma-bearing mice model, the resulting exosome membrane-coated PEG-PLA NPs significantly enhanced DOX delivery to glioma cells, resulting in greater inhibition of tumor growth and extended survival of glioblastoma-bearing mice. These findings indicate the potential of the biomimetic nanosystem as a new avenue for immunogenic chemotherapy against cancer [87].

5.1.9. Tumor Imaging and Theranostics

In addition to serving as effective therapeutic drug delivery systems, exosome membrane-coated NPs have also been investigated for tumor imaging, which is a promising approach for cancer management, being widely used for early detection of cancer and to monitor tumor progression. For this purpose, several imaging modalities, including magnetic resonance imaging (MRI) and NIR fluorescence imaging have been used as promising cancer imaging tools [68, 88]. However, in recent years, much research has been devoted to theranostic nanoplatforms as promising tools for cancer management, combining both therapeutic and diagnostic features into a single nanoplatform [88]. In this regard, in recent years, exosome membrane-coated NPs have been recognized as promising theranostic nanoplatforms for simultaneous cancer imaging and therapy, as they shown to be suitable for the delivery of both therapeutic molecules and imaging agents to target tumor sites.

For theranostic applications, various types of NPs have been investigated, including gold-iron oxide NPs, which have proven to be endowed with unique properties for both imaging applications and PTT, since the iron oxide can act as a contrast agent for MRI, while the gold NP can act as a photothermal agent capable of converting NIR light into cytotoxic heat for thermal ablation of tumor cells through hyperthermia [78].

In this regard, Bose *et al.* coated gold-iron oxide NPs with a breast cancer cell-derived exosome membrane for targeted delivery of anti-miR-21 to tumor sites, photothermal ablation of tumor cells and MRI of cancer [78]. First, they generated anti-miR-21-loaded exosomes by direct transfection of 4T1 cells with anti-miR-21, and then coated the membrane of anti-miR-21-loaded exosomes onto gold-iron oxide NPs. The multifunctional biomimetic nanosystem showed remarkable photothermal effects under NIR radiation *in vitro* and demonstrated excellent results as an MRI contrast agent, both properties attributed to the gold-iron oxide core. *In vivo* studies demonstrated that the biomimetic nanosystem could specifically target and accumulate at homotypic 4T1 tumor cells, enabling the targeted delivery

of anti-miR-21 to tumor sites, which exhibited efficient antitumor effects in combination with DOX. Indeed, the co-delivery of anti-miR-21 and DOX showed to reduce DOX resistance in breast cancer cells, resulting in excellent antitumor efficacy and remarkable suppression of tumor growth, since anti-miR-21 can silence the expression of oncogenic miR-21, which is known to be responsible not only for tumor progression and metastasis, but also for resistance to chemotherapeutic drugs. In summary, this study provides a multifunctional theranostic nanoplatform that incorporate both imaging and photothermal agents for simultaneous tumor imaging, photothermal ablation of tumor cells and chemo-sensitizing anti-miR-21 [78].

Another multifunctional theranostic nanoplatform was developed by Sancho-Albero and coworkers, in which PEGylated-hollow gold NPs (PEG-HGNs) were coated with an exosome membrane derived from B16-F10 murine melanoma cells taking advantage of the exosome biogenesis pathway [89]. The resulting exosome membrane-coated PEG-HGNs showed to be efficiently taken up by homotypic B16-F10 murine melanoma cells *in vitro* and showed promising results as photothermal agents for thermal ablation of cancer cells upon NIR radiation, owing to its ability to convert the NIR light into cytotoxic heat capable of thermally damaging cancer cells. The authors used the intrinsic reflective optical properties of the exosome membrane-coated PEG-HGNs to monitor their accumulation in tumor tissues, and concluded that the biomimetic nanosystem could specifically accumulate in tumor cells, which indicates the tumor-targeting ability of the designed nanosystem [89].

5.2. Neuroimaging

Regarding to neuroimaging applications, Khongkow *et al.* functionalized gold NPs with neuron-targeted exosomes derived from genetically engineered human embryonic kidney cells (HEK293T), resulting in enhanced BBB penetration and improved accumulation in neuronal cells [90]. Previous studies have shown that the fusion of the exosome membrane with neuronal-targeting ligands, such as the rabies virus glycoprotein (RVG) peptide, can improve the brain-targeting ability, due to the specific binding of the RVG peptide to acetylcholine receptors expressed by neuronal cells. In light of this, exosome-producing HEK293T cells were transfected to produce exosomes with the RVG peptide on their surface which were first isolated from the cell culture supernatant and then coated around gold NPs. The ability of the resulting Lamp2b-RVG and glycosylation-stabilized peptides (GNSTM)- decorated gold NPs to penetrate the BBB and target brain cells was demonstrated *in vivo* after intravenous injection in a mouse model by bioluminescence imaging of the mouse brains. The results showed that compared to gold NPs coated with non-RVG-targeted exosomes, the gold NPs coated with RVG-targeted exosomes had a superior ability to cross the BBB showing greater

accumulation in brain cells. In conclusion, this study provides a promising approach to overcome the difficulty of crossing the BBB, accelerating the development of effective diagnostic and treatment strategies for a variety of brain diseases [90].

5.3. Parkinson´s disease treatment

Another application of exosome membrane-coated NPs is the treatment of neurodegenerative diseases, in which the most serious challenge is the difficulty of drug delivery systems to cross the BBB and target neuronal cells. Among neurodegenerative diseases, Parkinson´s disease is one of the most common diseases, which is characterized by progressive loss of dopaminergic neurons with consequent dopamine deficit and by the abnormal accumulation of α-synuclein (α-syn) on dopaminergic neurons, a protein encoded by the SNCA (synuclein alpha) gene that is known to be the main component of Lewy bodies, considered to be the most typical pathological disorder involved in this disease [91].

Recently, an effective approach based on exosome membrane-coated NPs was developed by Liu *et al.* with the aim of reducing the expression and cytotoxicity of α-syn aggregates in dopaminergic neurons and delay the progression of Parkinson´s disease [92]. In this study, a biomimetic core-shell nanosystem was developed by coating a phenylboronic acid-poly(2-(dimethylamino)ethyl acrylate) NP core, co-loaded with the drug curcumin and siRNA targeting SNCA (siSNCA), with an RVG-modified exosome membrane derived from imDCs (Appendix IX A). The biomimetic core-shell nanosystem showed to effectively cross the BBB and target dopaminergic neurons, releasing the loaded drugs in a ROS-responsive manner in the dopaminergic neurons to synergistically downregulate α-syn synthesis and reduce the existing α-syn aggregates, since siSNCA can prevent the α-syn aggregation by reducing α-syn synthesis, while curcumin can directly reduce the existing α-syn aggregates (Appendix IX B). Consequently, due to the synergistic effects of both drugs, the biomimetic nanosystem exhibited the most remarkable effect in clearing α-syn aggregates in the dopaminergic neurons, reducing the SNCA mRNA expression and the number of α-syn aggregates, markedly improving neuronal repair and motor behavior *in vivo*, thereby holding enormous potential for Parkinson´s disease treatment (Appendix IX C – IX F) [92].

5.4. Bacterial infection treatment

In an attempt to explore the potential of EV membrane-coated NPs for targeted delivery of antibiotics to specific infection sites, Gao *et al.* coated PLGA NPs loaded with antibiotics (vancomycin and rifampicin) with a bacterial EV membrane secreted by *Staphylococcus aureus (S. aureus)*, a Gram-positive bacterium [93]. The resulting PLGA NPs

coated with the *S. aureus*- derived EV membrane showed not only to target *S. aureus*-infected macrophages *in vitro*, but also to traffic to organs with higher bacterial burden of *S. aureus* infection *in vivo*. Indeed, *in vitro* experiments showed that the PLGA NPs coated with the *S. aureus*-derived EV membrane had preferential uptake by macrophages infected with *S. aureus*, while PLGA NPs coated with an *E. coli*-derived EV membrane had enhanced uptake by macrophages exposed to that type of bacterium. *In vivo* experiments showed that the PLGA NPs coated with the *S. aureus*-derived EV membrane could efficiently traffic to major organs of *S. aureus* infection, showing greater accumulation at these infectious organs. These findings demonstrate the targeting ability of the resulting PLGA NPs coated with the *S. aureus*-derived EV membrane, which significantly enabled the target delivery of antibiotics to specific *S. aureus* infection sites, resulting in a more efficient treatment of *S. aureus* infection [93].

5.5. Skin regeneration/ wound repair

Mesenchymal stem cells (MSCs) are multipotent cells that have been recognized as promising agents for various inflammatory diseases and cutaneous wound healing, due to their multipotent differentiation, immunosuppressive and regenerative properties [22, 94]. The beneficial therapeutic effects of MSCs in skin regeneration and wound repair are well-documented, and appear to be related to their ability to increase angiogenesis, enhance collagen synthesis and re-epithelialization, and accelerate skin regeneration and wound closure. Recently, MSC-derived exosomes have been investigated for skin regeneration and wound repair, as they can maintain the functional properties of their donor cells [95].

To investigate the wound healing effects of MSC-derived exosomes *in vivo*, Li *et al.* recently coated SPIONs (Fe_3O_4 NPs) with an MSC-derived exosome membrane by direct incubation of Fe_3O_4 NPs with MSCs, allowing them to secrete exosomes containing exogenous NPs, taking advantage of the exosome biogenesis pathway [95]. Due to the restricted ability of MSC-derived exosomes to target wounded skin sites, a magnetic guidance was employed to efficiently deliver the resulting exosome membrane-coated Fe_3O_4 NPs to the injured skin environment. Indeed, as a result of their magnetic properties, the Fe_3O_4 core showed to enhance the targeting abilities of MSC-derived exosomes under magnetic guidance, endowing them with excellent targeting properties for wounded skin sites after intravenous injection in a mouse model. In summary, the biomimetic nanosystem showed enormous potential along with magnetic guidance for wound repair and skin regeneration *in vivo*, *as* demonstrated by the increased proliferation of endothelial cells and angiogenesis, increased expression of injured skin healing-related proteins, increased collagen synthesis and re-epithelialization, faster wound closure and reduced scar formation [95].

6. Challenges and future prospects

The concept of using the exosome membrane to camouflage nanomaterials for biomedical applications is a very attractive and promising approach. However, although great progress has been made in the field of exosome membrane-coating nanotechnology, this is a relatively new approach and the investigation on this area is still in its infancy. Therefore, there are still some challenges that may hinder the implementation of exosome membrane-coated NPs in clinical practice, including their complexity, heterogeneity, reproducibility, the lack of standardized methods for exosome isolation and purification, the difficulty of large-scale manufacturing, the lack of agreement over the ideal coating method, and also the high risk that the coating techniques may compromise the biological functions of natural exosomes and their safety profile [22, 54]. In addition, another important challenge is the current lack of understanding of the biogenesis, composition and biological functions of natural exosomes, and therefore, in order to design exosome membrane-coated NPs more efficiently and safely, future research should be focused on clarifying the complex composition, the biological functionalities, and the intrinsic targeting abilities of natural exosomes [22].

Recently, considerable attention has been devoted to the study of exosome membrane-coated NPs, and for this purpose different coating methods have been investigated to coat the exosome membrane on NPs, including sonication and physical extrusion through porous membranes, which are two widely used approaches. In this regard, another challenge for clinical implementation of exosome membrane-coated NPs regards to the potential of the coating methods to damage the integrity of the exosome membrane structure and reduce the protein integrity, which may compromise the biological functions of natural exosomes and induce immunogenicity [22]. Indeed, exosomes contain a diverse set of proteins, some of which are responsible for their biological functions, while others may induce immune responses, and therefore, the manipulation of the exosome membrane can modify the surface composition and the orientation of proteins, which may trigger immune responses and induce immunogenicity [22, 23]. Thus, there is an urgent need to develop new nondisruptive coating techniques that do not negatively affect the protein integrity of the exosome membrane and thus, the efficacy and safety of these biomimetic nanoplatforms [22].

Still regarding to the coating methods for encapsulating NPs within the exosome membrane, another challenge is the lack of standardization among researchers over the best method to envelop the exosome membrane around NPs [54]. Despite the uncertainty on this subject, it is now accepted that the ideal coating method depends on the NP type and cell type, and thus, in order to assess which encapsulation method is the most favorable for a

particular scenario, several studies using different types of NPs, source cells and coating methods must be performed [54].

The reduced number of exosomes naturally secreted by most cells and the current lack of standardized protocols for exosome isolation and purification represents a major challenge for the successful implementation of natural exosomes in clinical practice, as it may hinder their clinical-scale production [54, 96]. Likewise, similar to natural exosomes, the clinical-scale production of exosome membrane-coated NPs remains a major obstacle, and thus, to circumvent the large-scale manufacturing challenges of these biomimetic NPs the approaches normally used to produce exosomes at large-scale, such as generation of cell-derived nanovesicles using extrusion through porous membranes, have recently been employed [22]. As an example, in an effort to prepare exosome-mimetic nanovesicles to encapsulate NPs within, Kim *et al.* recently prepared magnetic MSCs-derived nanovesicles camouflaged iron oxide NPs as a promising agent for ischemic stroke treatment. To achieve this, iron oxide NPs were encapsulated in MSCs-derived nanovesicles by extruding the MSCs treated with iron oxide NPs through porous membranes. The resulting exosome-mimetic nanovesicles exhibited 5.1-fold higher accumulation in the ischemic brain injury after intravenous injection in a mouse model under magnetic guidance, inducing angiogenesis, anti-apoptosis, and anti-inflammation. In summary, the resulting MSCs-derived nanovesicles camouflaged iron oxide NPs showed huge potential for ischemic stroke treatment producing a substantial reduction in infarct volume and enhancing motor function [97].

Finally, another concerning issue regards to the safety profile of these biomimetic nanosystems. As a matter of fact, since exosome membrane-coated NPs contain biological materials, the quality control of these biomimetic nanosystems is very demanding and their safety profile is a major concern. Therefore, a careful investigation of the immunogenicity and potential side effects of exosome membrane-coated NPs should be performed when translating these biomimetic nanoplatforms into clinical practice [22, 23, 54]. In the future, to reduce potential undesirable immune responses and ensure the biosafety of these biomimetic nanoplatforms, the development of personalized therapies that use the patient's own derived exosomes to camouflage around NPs should be further investigated [54].

In summary, it can be concluded that despite the enormous potential of exosome membrane-coated NPs for targeted delivery of therapeutic and imaging molecules to sites of interest, there are still many challenges for successful translation into the clinic. Thus, to develop exosome membrane-coated NPs more efficiently and improve the therapeutic efficacy

and safety of these biomimetic nanosystems, further research on this novel biomimetic approach is urgently needed to resolve these emerging challenges.

7. Conclusion

Cell membrane-coating nanotechnology is an emerging and promising biomimetic approach to combine the beneficial properties of both natural cell membranes and NPs, aiming to increase the biointerfacing properties of NPs and endow them with immune evasion, prolonged systemic circulation and cell-specific targeting abilities. To date, various types of cell membranes have been investigated for NP coatings, including those derived from red blood cells, platelets, white blood cells, stem cells and cancer cells, with each type of cell membrane coating providing specific donor cell-related properties.

In addition to natural cell membranes, the membrane derived from cell-derived EVs, particularly from natural exosomes, has been extensively investigated for NP coatings, since exosome membrane-coated NPs may combine the inherent biological features of endogenous exosomes, such as high biocompatibility, non-immunogenicity, immune evasion capabilities, prolonged systemic circulation, ability to cross biological barriers, and homotypic targeting abilities, with the biopharmaceutical benefits of nanomaterials. By covering NPs with an exosome membrane layer *via* top-down approaches, the surface membrane proteins responsible for evading immune clearance and the homotypic adhesion molecules responsible for the specific binding to target cells, can be faithfully preserved and directly transfer to the surface of NPs, which in turn contributes to prolong their systemic circulation time, protect the payload from immune clearance and increase the selectivity of delivery to target cells.

As a matter of fact, one of the most interesting features of these biomimetic nanosystems is that the intrinsic homotypic targeting abilities of the exosome membrane to selectively target homologous cells, from which exosomes are derived, can be maintained after the coating process. This way, the exosome membrane-coated NPs can be selectively homed to target cells *in vivo*, offering the possibility of a targeted and personalized therapy, which ultimately contributes to improve the therapeutic efficacy and reduce toxicity in healthy cells.

In the clinical field, the homologous targeting ability of exosome membrane-coated NPs has been harnessed to guide nanosized delivery systems to specific target sites, with the goal of delivering not only therapeutic molecules but also imaging agents to sites of particular interest, enabling the diagnosis, therapy and theranostics of a wide range of human diseases, including cancer - which is one of the most investigated areas in this field - but also for treatment of additional diseases, such as neurodegenerative diseases and wound healing applications.

In conclusion, the concept of exosome membrane-coating nanotechnology has emerged as a promising approach for diagnosis and treatment of various human diseases. However, despite the many advantages provided by exosome membrane-coated NPs, this is a relatively new technological approach, and some challenges for clinical translations still exist, and must be addressed in a near future. In the coming years, research on this biomimetic approach is expected to continue to grow, which will enable the development of promising bioinspired nanosystems for a variety of biomedical applications with the potential to revolutionize the diagnostics and treatment of a wide range of human diseases.

8. References

1. Jiang, Y., *et al.*, *Engineering biological interactions on the nanoscale.* Current opinion in biotechnology, 2019. **58**: p. 1-8.

2. 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.

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. 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).

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

6. 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.

7. Dash, P., *et al.*, *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.

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

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

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

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

12. Wang, H., *et al.*, *Cell membrane biomimetic nanoparticles for inflammation and cancer targeting in drug delivery.* Biomaterials science, 2020. **8**(2): p. 552-68.

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

14. Chai, Z., X. Hu, and W. Lu, *Cell membrane-coated nanoparticles for tumor-targeted drug delivery.* Science China Materials, 2017. **60**(6): p. 504-10.

15. Xuan, M., J. Shao, and J. Li, *Cell membrane-covered nanoparticles as biomaterials.* National Science Review, 2019. **6**(3): p. 551-61.

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

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

18. Gangadaran, P. and B.C. Ahn, *Extracellular Vesicle- and Extracellular Vesicle Mimetics-Based Drug Delivery Systems: New Perspectives, Challenges, and Clinical Developments.* Pharmaceutics, 2020. **12**(5).

19. Yang, B., Y. Chen, and J. Shi, *Exosome Biochemistry and Advanced Nanotechnology for Next-Generation Theranostic Platforms.* Advanced materials, 2019. **31**(2): p. e1802896.

20. Zhou, Y., *et al.*, *Fabrication of Cell-Derived Biomimetic Drug Delivery System.* Nanofabrication, 2019. **5**(1): p. 1-18.

21. Liu, L., H. He, and J. Liu, *Advances on Non-Genetic Cell Membrane Engineering for Biomedical Applications.* Polymers, 2019. **11**(12).

22. Lu, M. and Y. Huang, *Bioinspired exosome-like therapeutics and delivery nanoplatforms.* Biomaterials, 2020. **242**: p. 119925.

23. Zhang, X., *et al.*, *Engineered Extracellular Vesicles for Cancer Therapy.* Advanced materials, 2021. **33**(14): p. e2005709.

24. Fuhrmann, G., I.K. Herrmann, and M.M. Stevens, *Cell-derived vesicles for drug therapy and diagnostics: opportunities and challenges.* Nano today, 2015. **10**(3): p. 397-409.

25. Herrmann, I.K., M.J.A. Wood, and G. Fuhrmann, *Extracellular vesicles as a next-generation drug delivery platform.* Nature nanotechnology, 2021.

26. Li, S.P., *et al.*, *Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools.* Acta pharmacologica Sinica, 2018. **39**(4): p. 542-51.

27. Zhang, Y., *et al.*, E*xosomes: biogenesis, biologic function and clinical potential.* Cell & bioscience, 2019. **9**:19.

28. Sinha, D., *et al.*, *Trends in Research on Exosomes in Cancer Progression and Anticancer Therapy.* Cancers, 2021. **13**(2).

29. Schorey, J.S., *et al.*, *Exosomes and other extracellular vesicles in host-pathogen interactions.* EMBO reports, 2015. **16**(1): p. 24-43.

30. Liang, Y., *et al.*, *Engineering exosomes for targeted drug delivery.* Theranostics, 2021. **11**(7): p. 3183–95.

31. Kooijmans, S.A.A., O.G. de Jong, and R.M. Schiffelers, *Exploring interactions between extracellular vesicles and cells for innovative drug delivery system design*. Advanced Drug Delivery Reviews, 2021. **173**: p. 252-78.

32. Luan, X., *et al.*, *Engineering exosomes as refined biological nanoplatforms for drug delivery.* Acta pharmacologica Sinica, 2017. **38**(6): p. 754-63.

33. Datta, B., *et al.*, *Intriguing Biomedical Applications of Synthetic and Natural Cell-Derived Vesicles: A Comparative Overview.* ACS Applied Bio Materials, 2021. **4**(4): p. 2863-85.

34. Liu, C., *et al.*, *Microfluidic Sonication To Assemble Exosome Membrane-Coated Nanoparticles for Immune Evasion-Mediated Targeting.* Nano letters, 2019. **19**(11): p. 7836-44.

35. Peng, H., *et al.*, *Exosome: a significant nano-scale drug delivery carrier.* Journal of Materials Chemistry B, 2020. **6**(3): p. 7591-608.

36. Nam, G.H., *et al.*, *Emerging Prospects of Exosomes for Cancer Treatment: From Conventional Therapy to Immunotherapy.* Advanced materials, 2020. **32**(51): p. e2002440.

37. Tan, S., *et al.*, *Cell or cell membrane-based drug delivery systems.* Theranostics, 2015. **5**(8): p. 863-81.

38. Kim, H., *et al.*, *Exosomes: Cell-Derived Nanoplatforms for the Delivery of Cancer Therapeutics.* International journal of molecular sciences, 2020. **22**(1).

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

40. 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.

41. Sancho-Albero, M., A. Medel-Martínez, and P. Martín-Duque, *Use of exosomes as vectors to carry advanced therapies.* RSC Advances, 2020. **10**(40): p. 23975-87.

42. Tian, Y., *et al.*, *A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy.* Biomaterials, 2014. **35**(7): p. 2383-90.

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

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

45. Lu, M., *et al.*, *Cell-free synthesis of connexin 43-integrated exosome-mimetic nanoparticles for siRNA delivery.* Acta biomaterialia, 2019. **96**: p. 517-36.

46. 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.

47. Sato, Y.T., *et al.*, *Engineering hybrid exosomes by membrane fusion with liposomes.* Scientific reports, 2016. **6**: p. 21933.

48. Cheng, G., *et al.*, *Self-Assembly of Extracellular Vesicle-like Metal-Organic Framework Nanoparticles for Protection and Intracellular Delivery of Biofunctional Proteins.* Journal of the American Chemical Society, 2018. **140**(23): p. 7282-91.

49. Wang, K., *et al.*, *An exosome-like programmable-bioactivating paclitaxel prodrug nanoplatform for enhanced breast cancer metastasis inhibition.* Biomaterials, 2020. **257**: p. 120224.

50. 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.

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

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

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

54. Fathi, P., L. Rao, and X. Chen, *Extracellular vesicle-coated nanoparticles.* View, 2020. **2**(2): p. 20200187.

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

56. Han, Z., *et al.*, *Improving Tumor Targeting of Exosomal Membrane-Coated Polymeric Nanoparticles by Conjugation with Aptamers*. ACS Applied Bio Materials, 2020. **3**(5): p. 2666-73.

57. 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.

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

59. 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.

60. Park, J., *et al.*, *Tumor-Homing pH-Sensitive Extracellular Vesicles for Targeting Heterogeneous Tumors.* Pharmaceutics, 2020. **12**(4).

61. Yong, T., *et al.*, *Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy.* Nature communications, 2019. **10**(1): p. 3838.

62. Liu, W., *et al.*, *Recent advances in cell membrane coated metal-organic frameworks (MOFs) for tumor therapy.* Journal of materials chemistry B, 2021. **9**(22): p. 4459-74.

63. Illes, B., *et al.*, *Exosome-Coated Metal–Organic Framework Nanoparticles: An Efficient Drug Delivery Platform.* Chemistry of Materials, 2017. **29**(19): p. 8042-6.

64. Qiao, L., *et al.*, *Tumor cell-derived exosomes home to their cells of origin and can be used as Trojan horses to deliver cancer drugs.* Theranostics, 2020. **10**(8): p. 3474-87.

65. Li, S., *et al.*, *Engineering macrophage-derived exosomes for targeted chemotherapy of triple-negative breast cancer.* Nanoscale, 2020. **10**(4): p. 1549-2172.

66. Pereira-Silva, M., *et al.*, *Micelleplexes as nucleic acid delivery systems for cancer-targeted therapies.* Journal of controlled release : official journal of the Controlled Release Society, 2020. **323**: p. 442-62.

67. Pereira-Silva, M., *et al.*, *Micelleplex-based nucleic acid therapeutics: From targeted stimuli-responsiveness to nanotoxicity and regulation*. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences, 2020. **153**: p. 105461.

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

69. Alhasan, A.H., *et al.*, *Exosome encased spherical nucleic acid gold nanoparticle conjugates as potent microRNA regulation agents.* Small, 2014. **10**(1): p. 186-92.

70. Wang, G., *et al.*, *Cocktail Strategy Based on NK Cell-Derived Exosomes and Their Biomimetic Nanoparticles for Dual Tumor Therapy.* Cancers, 2019. **11**(10).

71. 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.

72. Zhao, L., *et al.*, *Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis.* Journal of controlled release: official journal of the Controlled Release Society, 2020. **318**: p. 1-15.

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

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

75. Luo, C., *et al.*, *Biomimetic Carriers Based on Giant Membrane Vesicles for Targeted Drug Delivery and Photodynamic/Photothermal Synergistic Therapy.* ACS applied materials & interfaces, 2019. **11**(47): p. 43811-9.

76. Fu, L.H., *et al.*, *Glucose Oxidase-Instructed Multimodal Synergistic Cancer Therapy.* Advanced materials, 2019. **31**(21): p. e1808325.

77. 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.

78. Bose, R.J.C., *et al.*, *Tumor Cell-Derived Extracellular Vesicle-Coated Nanocarriers: An Efficient Theranostic Platform for the Cancer-Specific Delivery of Anti-miR-21 and Imaging Agents.* ACS nano, 2018. **12**(11): p. 10817-32.

79. Tian, R., *et al.*, *Tumor Exosome Mimicking Nanoparticles for Tumor Combinatorial Chemo-Photothermal Therapy*. Frontiers in bioengineering and biotechnology, 2020. **8**: p. 1010.

80. Zhu, D., *et al.*, *Tumor-Exocytosed Exosome/Aggregation-Induced Emission Luminogen Hybrid Nanovesicles Facilitate Efficient Tumor Penetration and Photodynamic Therapy.* Angewandte Chemie, 2020. **59**(33): p. 13836-43.

81. Mo, J., *et al.*, *Zinc Sulfide-Based Hybrid Exosome-Coated Nanoplatform for Targeted Treatment of Glioblastoma Stem-Like Cells in an Orthotopic Mouse Glioblastoma Model.* bioRxiv, 2021.

82. Pan, S., *et al.*, *Urinary exosomes-based Engineered Nanovectors for Homologously Targeted Chemo-Chemodynamic Prostate Cancer Therapy via abrogating EGFR/AKT/NF-kB/IkB signaling.* Biomaterials, 2021. **275**: p. 120946.

83. Bortolussi, S., Y.H. Liu, and I. Porras, *Boron Neutron Capture Therapy: From Nuclear Physics to Biomedicine.* Biology, 2021. **10**(5).

84. Li, J., *et al.*, *Exosome-Coated ^{10}B Carbon Dots for Precise Boron Neutron Capture Therapy in a Mouse Model of Glioma In Situ.* Advanced Functional Materials, 2021. **31**(24): p. 2100969.

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

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

87. Zhang, C., *et al.*, *Doxorubicin-loaded nanoparticle coated with endothelial cells-derived exosomes for immunogenic chemotherapy of glioblastoma.* Bioengineering & Translational Medicine, 2020.

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

89. Sancho-Albero, M., *et al.*, *Efficient encapsulation of theranostic nanoparticles in cell-derived exosomes: leveraging the exosomal biogenesis pathway to obtain hollow gold nanoparticle-hybrids.* Nanoscale, 2019. **11**(40): p. 18825-36.

90. Khongkow, M., *et al.*, *Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood-brain barrier penetration.* Scientific reports, 2019. **9**(1): p. 8278.

91. Meade, R.M., D.P. Fairlie, and J.M. Mason, *Alpha-synuclein structure and Parkinson's disease - lessons and emerging principles*. Molecular neurodegeneration, 2019. **14**(1): p. 29.

92. Liu, L., *et al.*, *Targeted exosome coating gene-chem nanocomplex as "nanoscavenger" for clearing a-synuclein and immune activation of Parkinson's disease.* Science Advances, 2020. **6**(50).

93. Gao, F., *et al.*, *Kill the Real with the Fake: Eliminate Intracellular Staphylococcus aureus Using Nanoparticle Coated with Its Extracellular Vesicle Membrane as Active-Targeting Drug Carrier.* ACS infectious diseases, 2019. **5**(2): p. 218-27.

94. Harrell, C.R., *et al.*, *Therapeutic Use of Mesenchymal Stem Cell-Derived Exosomes: From Basic Science to Clinics.* Pharmaceutics, 2020. **12**(5).

95. Li, X., *et al.*, *Magnetic targeting enhances the cutaneous wound healing effects of human mesenchymal stem cell-derived iron oxide exosomes.* Journal of nanobiotechnology, 2020. **18**(1): p. 113.

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

97. Kim, H.Y., *et al.*, *Mesenchymal stem cell-derived magnetic extracellular nanovesicles for targeting and treatment of ischemic stroke.* Biomaterials, 2020. **243**: p. 119942.

9. Appendixes

Appendix I

Table 1. Overview of the studies employing extracellular vesicle (EV) membrane-coated nanosystems for cancer applications.

Application	Extracellular vesicle (EV) membrane source	Inner core	Drugs	Coating method	*In vivo* tumor model	Outcomes	Ref.
Chemotherapy delivery	H22, Bel7402, and B16-F10 cells -derived exosome membrane	Luminescent porous silicon nanoparticle (PSiNP)	Doxorubicin (DOX)	Direct incubation of target cells with NPs (exosomal biogenesis pathway)	Subcutaneous H22 tumor-bearing mice, orthotopic 4T1 tumor-bearing mice, and lung metastasis B16-F10 melanoma-bearing mice model	• Higher uptake in cancer cells and cancer stem cells (CSCs) • Reduction of the tumor size and the number of CSCs • Extended overall survival of tumor-bearing mice • Cross-reactivity between different cancer cells types	[61]
	HeLa cervical cancer cells- derived exosome membrane	MIL-88A metal organic framework nanoparticle (MOF NP)	Suberoyl bis-hydroxamic acid (SBHA)	Incubation of exosomes with MOF NPs	-	• Specific uptake by homotypic HeLa cells *in vitro* • Tumor-targeted drug delivery • Minimal premature leakage of the therapeutic payload	[63]
	HT1080 cells- derived exosome membrane	Doxil (DOX-loaded liposomes)		Co-extrusion through 200 nm porous membranes	Subcutaneous HT1080 tumor-bearing nude mice model	• Homotypic targeting ability • Higher uptake by HT1080 cells • 2,3-fold increase of DOX accumulation in tumor sites compared to non-coated Doxil • Superior anticancer effects • Tumor growth suppression to practically undetectable levels • Reduced cardiotoxicity	[64]
	Macrophage- derived exosome membrane	Poly (lactic-*co*-glycolic acid) (PLGA) NP	DOX	Co-extrusion through 100 nm porous membranes	Orthotopic MDA-MB-231 tumor-bearing nude mice model	• Longer systemic circulation • Enhanced tumor targeting ability provided by c-Met binding peptide • Tumor-targeted drug delivery • Superior antitumor effects • Inhibition of triple negative breast cancer (TNBC) growth	[65]

Protein delivery	Human breast adenocarcinoma MDA-MB-231 cells- derived exosome membrane	ZIF-8 MOF NP	Therapeutic proteins (gelonin)	Sonication and extrusion	Orthotopic MDA-MB-231 tumor-bearing mice model	• Coating efficiency of 97 % • Protection from protease degradation • Immune evasion • Longer systemic circulation • Homotypic targeting ability • Specific uptake by tumor cells • Higher transduction efficiency of protein gelonin • 14-fold increase in antitumor efficacy	[48]
Gene delivery and silencing	PC-3 prostate cancer cells-derived exosome membrane	Spherical nucleic acids (SNAs)	Antisense miRNA targeting miRNA-21 (Anti-miR-21)	Direct incubation of target cells with NPs (exosomal biogenesis pathway)	-	• <1% of SNAs were sorted in exosomes by leveraging exosomal biogenesis pathway • Gene-silencing effects *in vitro* • Downregulation of miRNA-21 (miR-21) expression with a knockdown efficiency of 50 %	[69]
	Natural killer (NK) cell- derived exosome membrane	Tyrosine-coupled dendrimers	Let-7-a miRNA	Incubation of purified exosomes with tyrosine-coupled dendrimers (24 h, 4 °C)	Neuroblastoma CHLA-255 tumor-bearing mice model	• Tumor-targeting ability • Enhanced accumulation at tumor sites *in vivo* and *in vitro* • Efficient delivery of Let-7-a miRNA to target tumor cells • Synergistic antitumor effects of the therapeutic Let-7-a miRNA and NK cell-derived exosomes • Tumor growth inhibition	[70]
Antimetastatic therapy	Murine RAW 264.7 cells- derived exosome membrane	Laurate-functionalized platinum (Pt (IV)) prodrug human serum albumin (HSA) NP	Platinum (Pt (IV))	Sonication	Balb 4T1 tumor-bearing mice model with lung metastasis	• Longer circulation time • Excellent biocompatibility • Higher accumulation in orthotopic breast tumors and lung metastatic nodules • Efficient antitumor and antimetastatic effects • Reduced hepatotoxicity and nephrotoxicity	[71]

	Autologous breast cancer cells- derived exosome membrane	Cationic bovine serum albumin (CBSA) NP	S100A4 siRNA (siS100A4)	Co-extrusion through 200 and 100 nm porous membranes (100 times)	Postoperative lung metastasis mice model	• Efficient delivery of siS100A4 to pre-metastatic niches in the lungs • Downregulation of S100A4 metastasis-related protein expression • Gene-silencing effects • Suppression of postoperative breast cancer lung metastasis	[72]
	Human breast adenocarcinoma MDA-MB-231 cells- derived exosome membrane	PEGylated-Poly (ε-caprolactone) (PEG-PCL) NP	Paclitaxel (PTX)-linoleic acid prodrug and cucurbitacin B (CuB)	Co-extrusion through 220 nm porous membranes	Orthotopic and xenograft MDA-MB-231 tumor-bearing mice model	• Immune evasion • Longer systemic circulation • Homotypic targeting ability • Efficient antitumor effects and tumor growth inhibition • Excellent circulating tumor cells (CTCs) capture ability and cancer metastasis suppression • Reduced number of metastatic tumor nodules in the lungs	[49]
Photothermal therapy	4T1 breast cancer cells- derived exosome membrane	Mesoporous silica NP (MSN)	Indocyanine green (ICG) and DOX	Co-extrusion through porous membranes	4T1 tumor-bearing mice model	• Homotypic targeting ability • Selective accumulation at homotypic 4T1 tumor sites • Effective near-infrared (NIR) light absorbance and targeted photothermal effects • Synergistic chemo-photothermal therapy (PTT) • Tumor growth suppression	[79]
Photodynamic therapy	U87 glioblastoma cells-derived exosome membrane	Hollow zinc sulfide (ZnS) NP	Hydroxy-chloroquine (HCQ)	Co-extrusion through 200 nm porous membranes	Intracranial Luc-U87 glioblastoma-bearing mice model	• Homotypic targeting ability • Suppression of autophagy • Synergistic antitumor effects of HCQ and ZnS • Higher antitumor efficiency of photodynamic therapy (PDT) • Suppression of tumor growth • Longer survival of mice with glioblastoma (up to 73 days)	[81]

Chemodynamic therapy	Urinary exosome membrane (isolated from the urine of prostate cancer patients)	Fe_3O_4 - HSA NP	DOX	Electroporation (250 V)	DU145 tumor-bearing BALB/C nude mice model	• Homotypic tumor-targeting ability • Enhanced uptake by prostate cancer cells • Synergistic chemo/chemodynamic effects and inhibition of EGFR/AKT/NF-κB/ IkB pathway • Suppression of tumor growth	[82]
Radiotherapy	Macrophage- derived exosome membrane	Carbon dots	Nonradioactive isotope boron-10 (^{10}B)	Incubation of purified exosomes with ^{10}B boron-containing carbon dots (BCDs) (37 °C, 2 h)	Orthotopic U-87-MG glioma tumor-bearing mice model	• Higher ability to cross the blood-brain barrier (BBB) • Selective accumulation at glioma tumor cells *in vivo* • Suppression of tumor growth • Longer survival of glioma-bearing mice (100 % at day 30)	[84]
Immunotherapy	bEnd.3 cells- derived exosome membrane	PEGylated-Poly-lactic acid (PEG-PLA) NP	DOX	Co-extrusion through 100 nm porous membranes	Orthotopic glioblastoma xenograft-bearing mice model	• Higher accumulation in homotypic bEnd.3 cells *in vivo* and *in vitro* • Maturation of dendritic cells and infiltration of cytotoxic CD8+ T lymphocytes in tumor sites • Suppression of tumor growth • Longer survival of glioblastoma-bearing mice	[87]
Tumor imaging and theranostics	4T1 murine breast cancer cells- derived exosome membrane	Gold-iron oxide NP	Anti-miR-21	Co-extrusion through 100 nm porous membranes	Syngeneic subcutaneous 4T1 tumor-bearing mice model	• Superior accumulation in homotypic 4T1 cells • Enhanced PTT effects *in vitro* • Magnetic resonance imaging (MRI) • Targeted delivery of anti-miR-21 and reduced DOX resistance • 3-fold higher cell killing efficiency of anti-miR-21 plus DOX compared to DOX alone	[78]
	B16-F10 murine melanoma cells- derived exosome membrane	PEGylated-hollow gold NP (PEG-HGN)	-	Direct incubation of target cells with PEG-HGNs (exosomal biogenesis pathway)	-	• Higher encapsulation efficiency (50 %) by taking advantage of exosome biogenesis pathway • Selective accumulation in homotypic B16-F10 cells • Strong absorbance at NIR region • Enhanced PTT effects *in vitro*	[89]

Appendix II

Table 2. Overview of the studies employing extracellular vesicle (EV) membrane-coated nanosystems for neuroimaging, Parkinson´s disease treatment, bacterial infection treatment and skin regeneration/ wound repair.

Application	Extracellular vesicle (EV) membrane source	Inner core	Drugs	Coating method	*In vivo* mice model	Outcomes	Ref.
Neuroimaging	Human embryonic kidney cells (HEK293T)- derived exosome membrane functionalized with rabies virus glycoprotein (RVG) peptide	Gold nanoparticle (NP)	-	Co-extrusion through 400 nm, 200 nm and 100 nm porous membranes (10 times in each)	Murine model	• Higher ability to cross the blood-brain barrier (BBB) • Selective accumulation at brain cells *in vivo* • Promising approach to overcome the difficulty of crossing the BBB for efficient diagnosis and treatment of a variety of brain diseases	[90]
Parkinson´s disease treatment	Immature dendritic cells (imDCs)-derived exosome membrane functionalized with RVG peptide	Phenylboronic acid-poly(2-(dimethylamino) ethyl acrylate) NP	siRNA targeting SNCA (siSNCA) and curcumin	Sonication	Parkinson's disease mice model	• Higher ability to cross the BBB • Selective accumulation at dopaminergic neurons • Enhanced α-synuclein (α-syn) clearance • Improved neuronal repair and motor behavior *in vivo*	[92]
Bacterial infection treatment	*Staphylococcus aureus*-derived EV membrane	Poly (lactic-*co*-glycolic acid) (PLGA) NP	Antibiotics (vancomycin and rifampicin)	Sonication	*S. aureus* bacteremia-bearing mice model	• Selective uptake in *S. aureus* infection sites • Targeted delivery of antibiotics to infection sites *in vivo* • Efficient reduction of the bacterial burden, particularly in the lungs and kidneys	[93]
Skin regeneration/ wound repair	Mesenchymal stem cells (MSCs)- derived exosome membrane	Superparamagnetic iron oxide NP (Fe_3O_4 NP)	-	Direct incubation of target cells with NPs (exosomal biogenesis pathway)	Mice model with skin injury	• Higher accumulation at injured skin sites • Increased expression of injured skin healing-related proteins • Enhanced angiogenesis • Increased collagen synthesis and re-epithelialization • Faster wound closure • Reduced scar formation	[95]

Appendix III

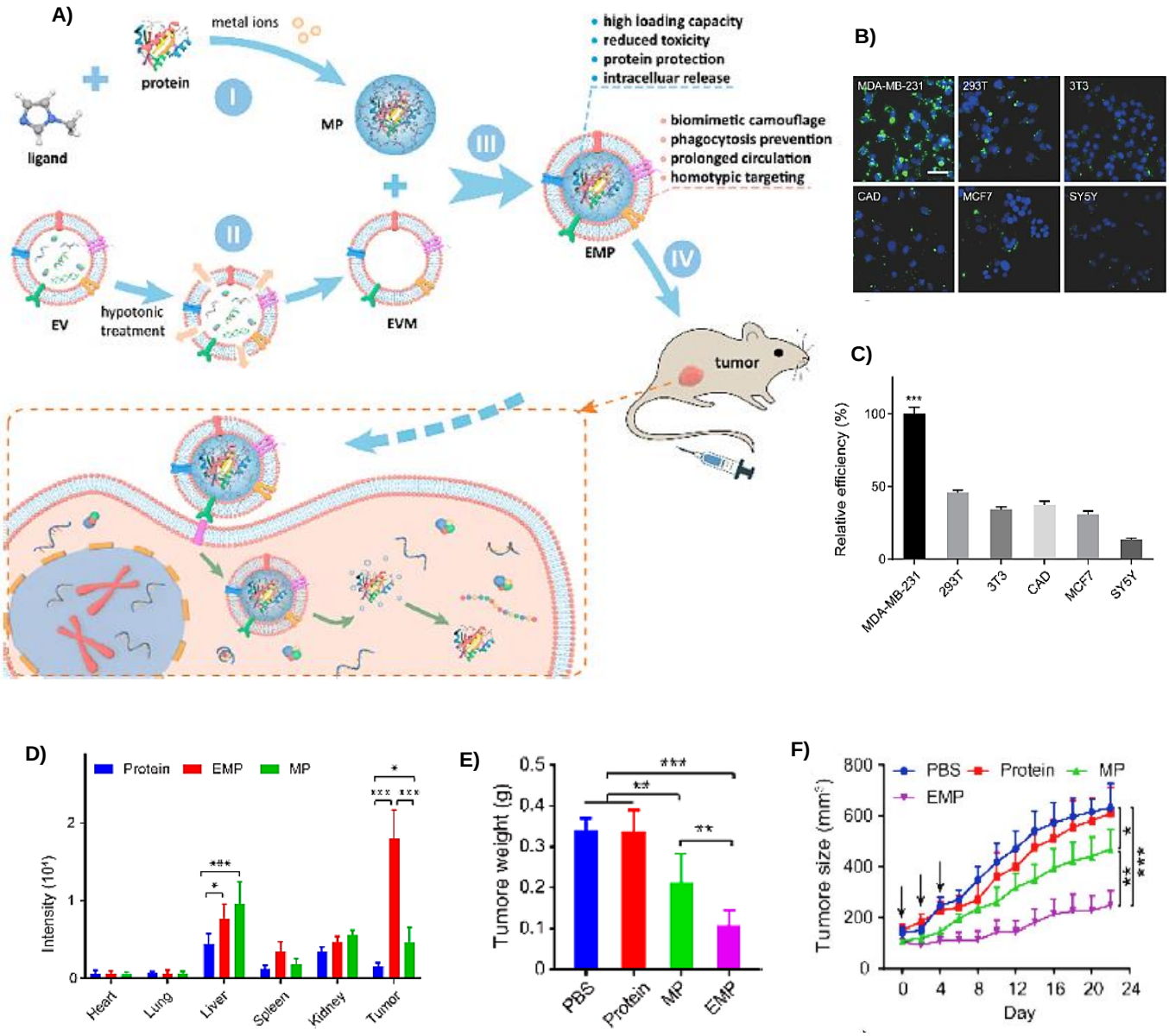

Figure 5. A) Schematic illustration of the preparation of EMP by coating MP with the exosome membrane derived from human breast adenocarcinoma MDA-MB-231 cells for intracellular delivery of functional proteins. B, C) Cellular uptake of EMP in MDA-MB-231 cells, 293T human embryonic kidney cells, 3T3 mouse embryo fibroblasts, CAD mouse central nervous system-derived cells, MCF7 human breast adenocarcinoma cells, and SH-SY5Y human neuroblastoma cells. D) Fluorescent intensity at major organs (heart, lung, liver, spleen, kidney) and tumor sites. E) Tumor weight changes after intravenous injection in MDA-MB-231 tumor-bearing mice with different treatments. F) Tumor size curves after intravenous injection in MDA-MB-231 tumor-bearing mice with different treatments. Reproduced with permission from reference [48]. Copyright American Chemical Society (2018).

Abbreviations: EMP, exosome membrane-coated MP; EV, extracellular vesicle; MP, protein-loaded metal organic framework nanoparticle.

Appendix IV

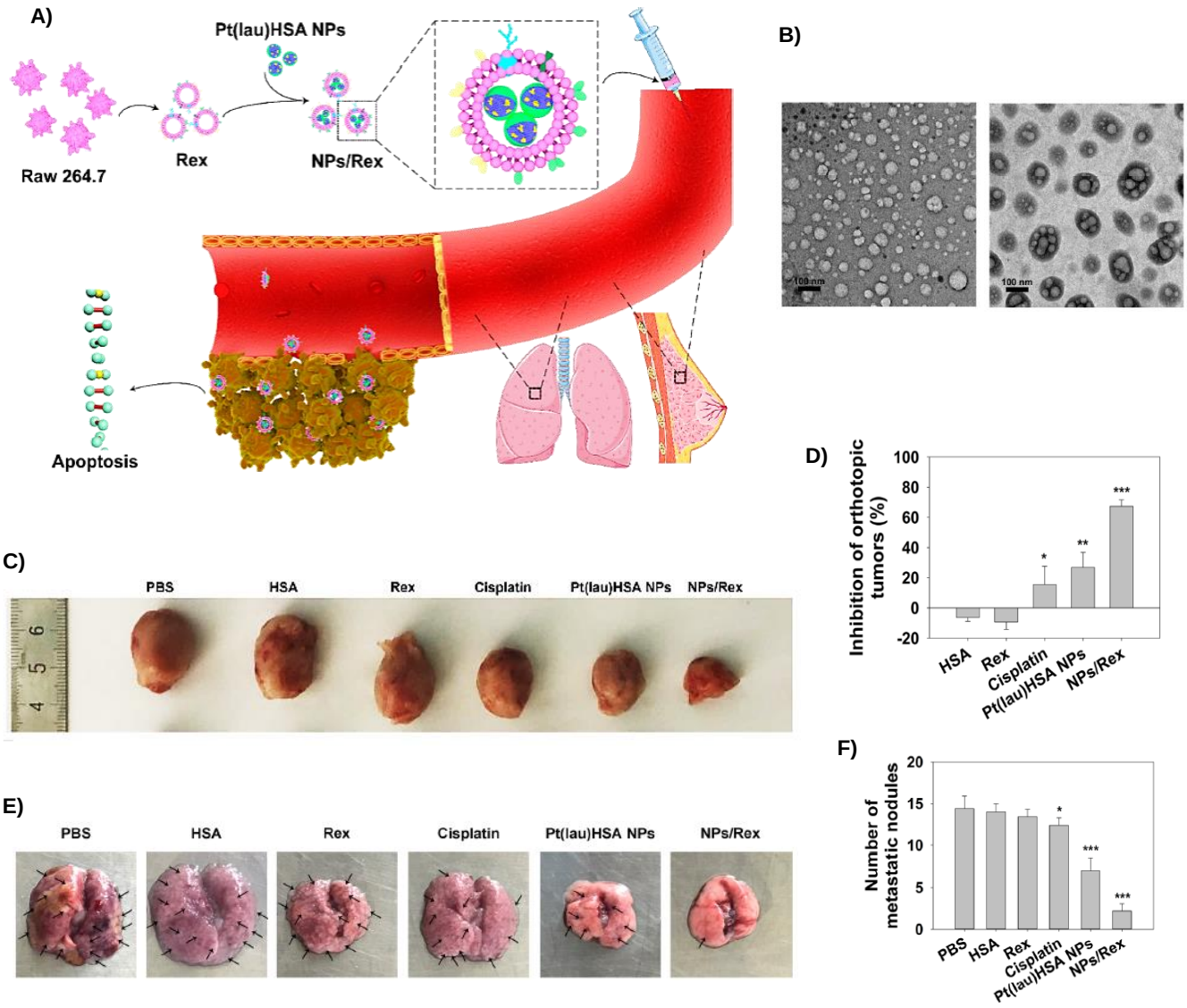

Figure 6. A) Schematic illustration of the preparation of NPs/Rex by coating Pt (IV) HSA NPs with the exosome membrane derived from Raw 264.7 cells for targeted delivery of Pt (IV) to orthotopic breast tumors and lung metastatic nodules. B) TEM imaging of Pt (IV) HSA NPs (left) and NPs/Rex (right). C) Photographs of orthotopic tumors after different treatments. D) Inhibition rate of 4T1 orthotopic tumors after different treatments. E) Photographs of lung tissues with visible metastatic nodules after different treatments (black arrows are meant to highlight visible metastatic lungs nodules). F) Number of metastatic lung nodules after different treatments. Reproduced with permission from reference [71]. Copyright American Chemical Society (2019).

Abbreviations: HSA, human serum albumin; NPs/Rex, Rex-coated Pt (IV) HSA NPs; Pt (IV) HSA NPs, HSA inner core composed of a laurate-functionalized platinum (Pt (IV)) prodrug; Rex, exosomes derived from murine RAW264.1 cells.

Appendix V

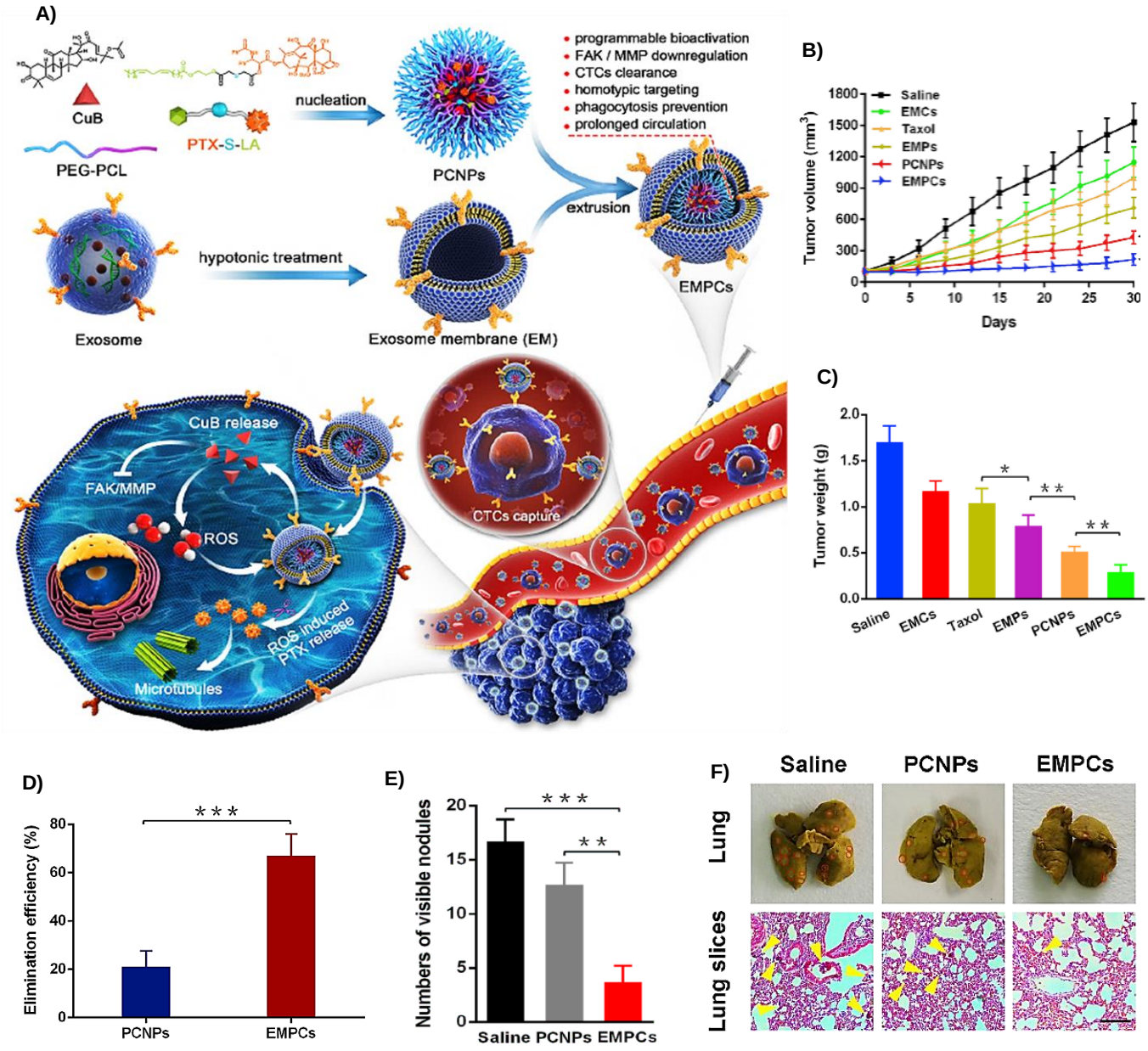

Figure 7. A) Schematic illustration of the preparation of EMPCs by coating PCNPs with the exosome membrane derived from human breast adenocarcinoma MDA-MB-231 cells for suppression of breast cancer metastasis. B) Tumor volume curves after intravenous injection in orthotopic MDA-MB-231 tumor-bearing mice. C) Tumor weight changes after intravenous injection in orthotopic MDA-MB-231 tumor-bearing mice. D) CTCs elimination efficiency of PCNPs and EMPCs. E) Numbers of visible metastatic lung nodules after intravenous injection. F) Photographs of mice lungs (top) and H&E staining of lung slices (down) after different treatments (red circles are meant to highlight visible metastatic lung nodules). Reproduced with permission from reference [49]. Copyright Elsevier (2020).

Abbreviations: CTC, circulating tumor cell; CuB, cucurbitacin B; EMPCs, exosome membrane-coated PCNPs; PCNPs, PEG-PCL nanoparticles co-loaded with CuB and PTX-S-LA; PEG-PCL, PEGylated- poly (ε-caprolactone) nanoparticle; PTX, paclitaxel; PTX-S-LA, paclitaxel-linoleic acid prodrug; ROS, reactive oxygen species

Appendix VI

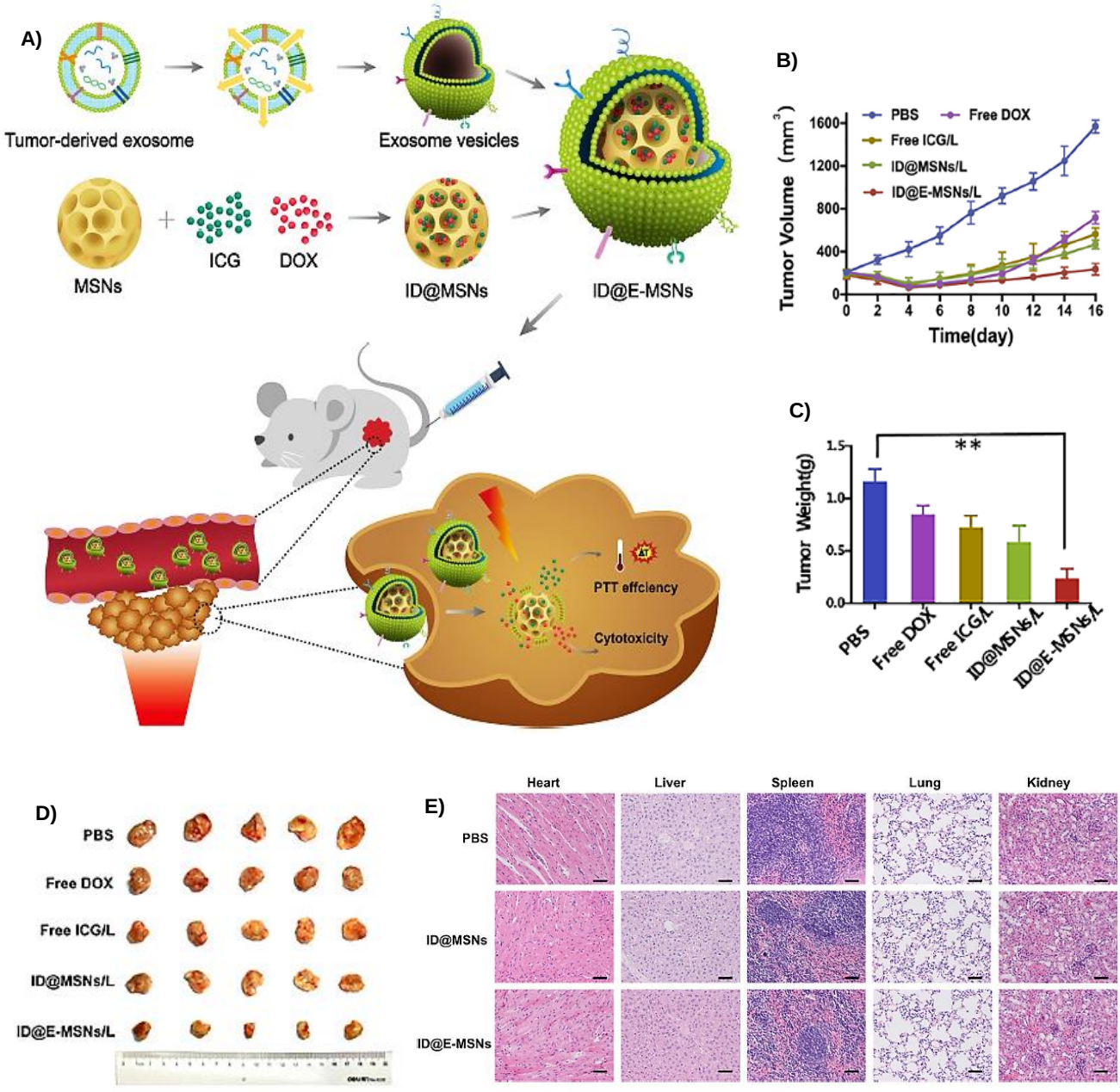

Figure 8. A) Schematic illustration of the preparation of ID@E-MSNPs by coating ID@MSNPs with the exosome membrane derived from 4T1 breast cancer cells for combined chemo-PTT against 4T1 breast cancer cells. B) Tumor volume curves after intravenous injection in 4T1 tumor-bearing mice after 16 days of treatment. C) Tumor weight changes after intravenous injection in 4T1 tumor-bearing mice. D) Comparative images of 4T1 tumors after different treatments. E) Histologic sections from major organs (heart, liver, lung, spleen, and kidney) after different treatments. Reproduced with permission from reference [79]. Copyright Frontiers Media S.A. (2020).

Abbreviations: DOX, doxorubicin; ICG, indocyanine green; ID@MSNs, mesoporous silica nanoparticles co-loaded with ICG and DOX; ID@E-MSNs, exosome membrane-coated ID@MSNs; MSNs, mesoporous silica nanoparticles; PTT, photothermal therapy.

Appendix VII

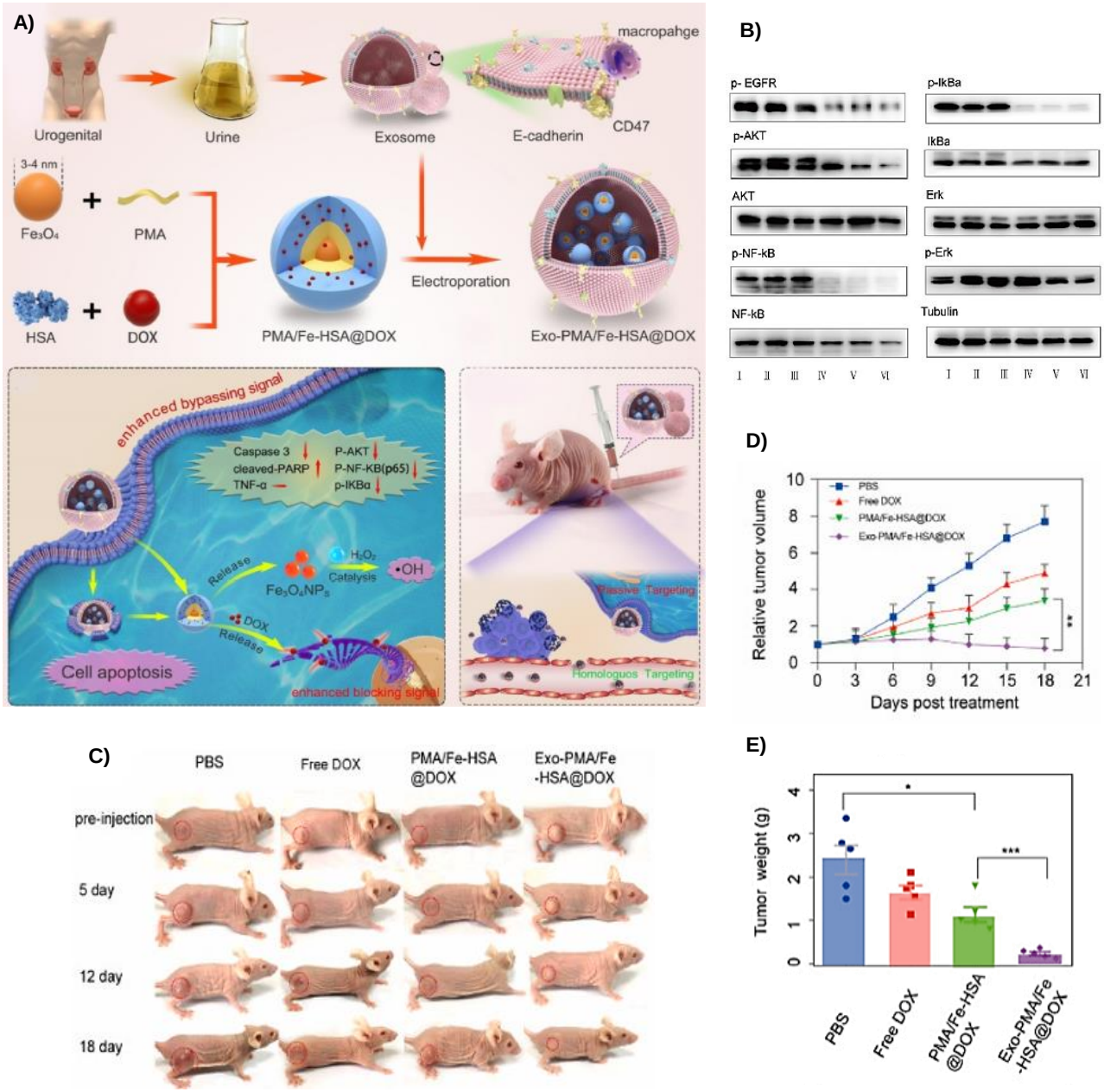

Figure 9. A) Schematic illustration of the preparation of Exo-PMA/Fe-HSA@DOX by coating PMA/Fe-HSA@DOX with a urinary exosome membrane for synergistic chemo/chemodynamic therapy against prostate cancer. B) Expression of EGFR and its downstream signaling proteins AKT, NF-κB, IkB and Erk after different treatments (I, II, III, IV, V and VI means PBS, PMA/Fe, Exosomes, DOX, PMA/Fe-HSA@DOX NPs and Exo-PMA/Fe-HSA@DOX). C) Photographs of mice tumors at pre-injection and 5, 12 and 18 days after intravenous injection with different treatments. D) Tumor volume curves after different treatments. E) Tumor weight changes after different treatments. Reproduced with permission from reference [82]. Copyright Elsevier (2021).

Abbreviations: DOX, doxorubicin; EGFR, epidermal growth factor receptor; Exo-PMA/Fe-HSA@DOX, exosome membrane-coated PMA/Fe-HSA@DOX; HSA, human serum albumin; PMA/Fe-HSA@DOX, DOX-loaded Fe_3O_4-HSA nanoparticle core.

Appendix VIII

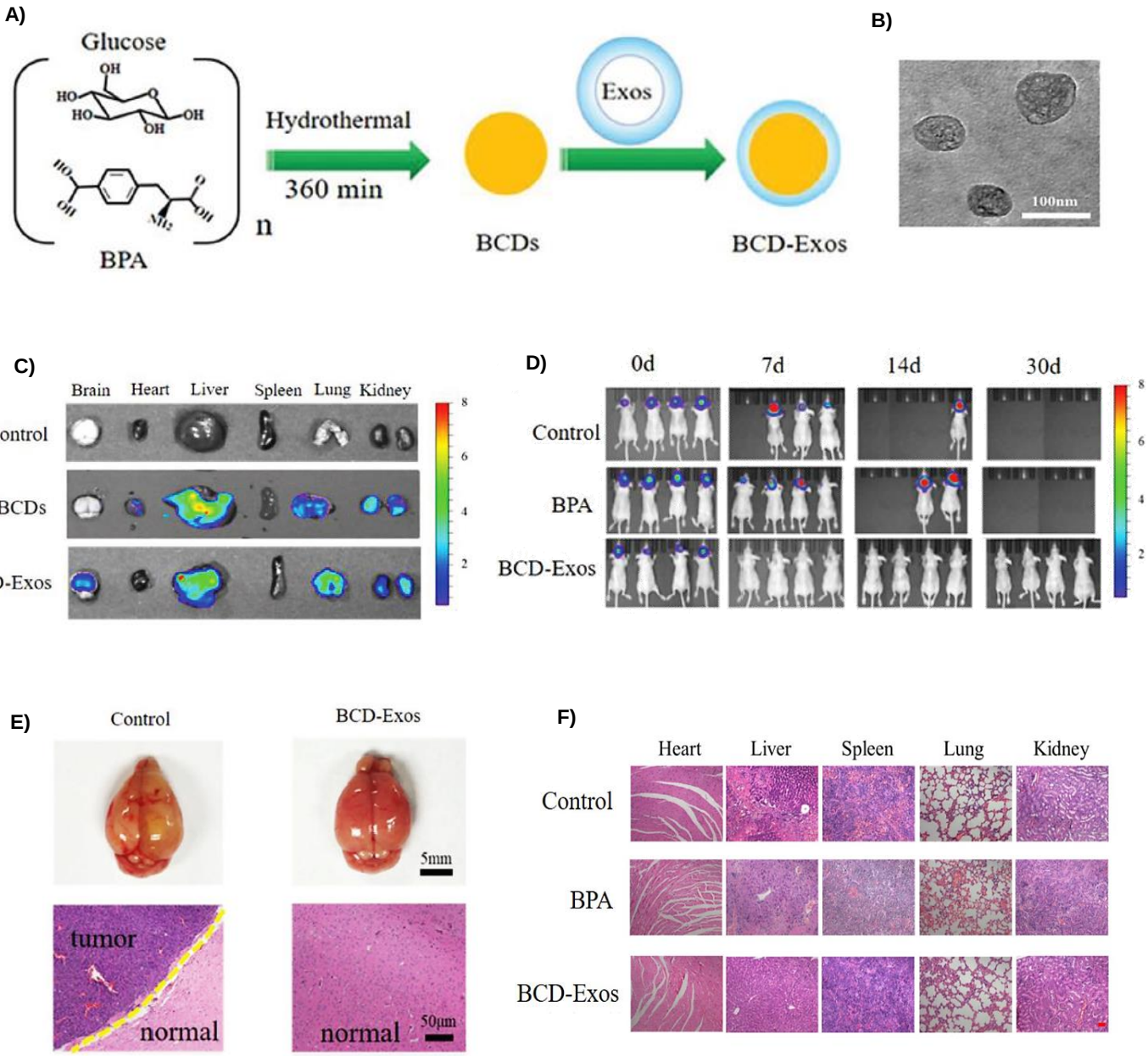

Figure 10. A) Schematic illustration of the preparation of BCD-Exos by coating BCDs consisted of BPA and D-glucose with the macrophage-derived exosome membrane for BNCT against brain glioma. B) TEM characterization of BCD-Exos in which a 100 nm core-shell nanostructure can be observed. C) Distribution of BCDs and BCD-Exos in main organs (brain, heart, liver, spleen, lung, and kidney) 4 h after administration. D) Overall survival rate of mice treated with control, BPA and BCD-Exos, 0, 7, 14 and 30 days after administration. E) Macroscopic (top) and microscopic (down) histologic evaluation of the mice brain tissue after different treatments. F) Histologic sections from major organs (heart, liver, spleen, lung and kidney) after different treatments. Reproduced with permission from reference [84]. Copyright Wiley-VCH Verlag (2021).

Abbreviations: BCD-Exos, exosome membrane-coated BCDs; BCDs, ^{10}B boron-containing carbon dots; BNCT, boron neutron capture therapy; BPA, boron phenylalanine.

Appendix IX

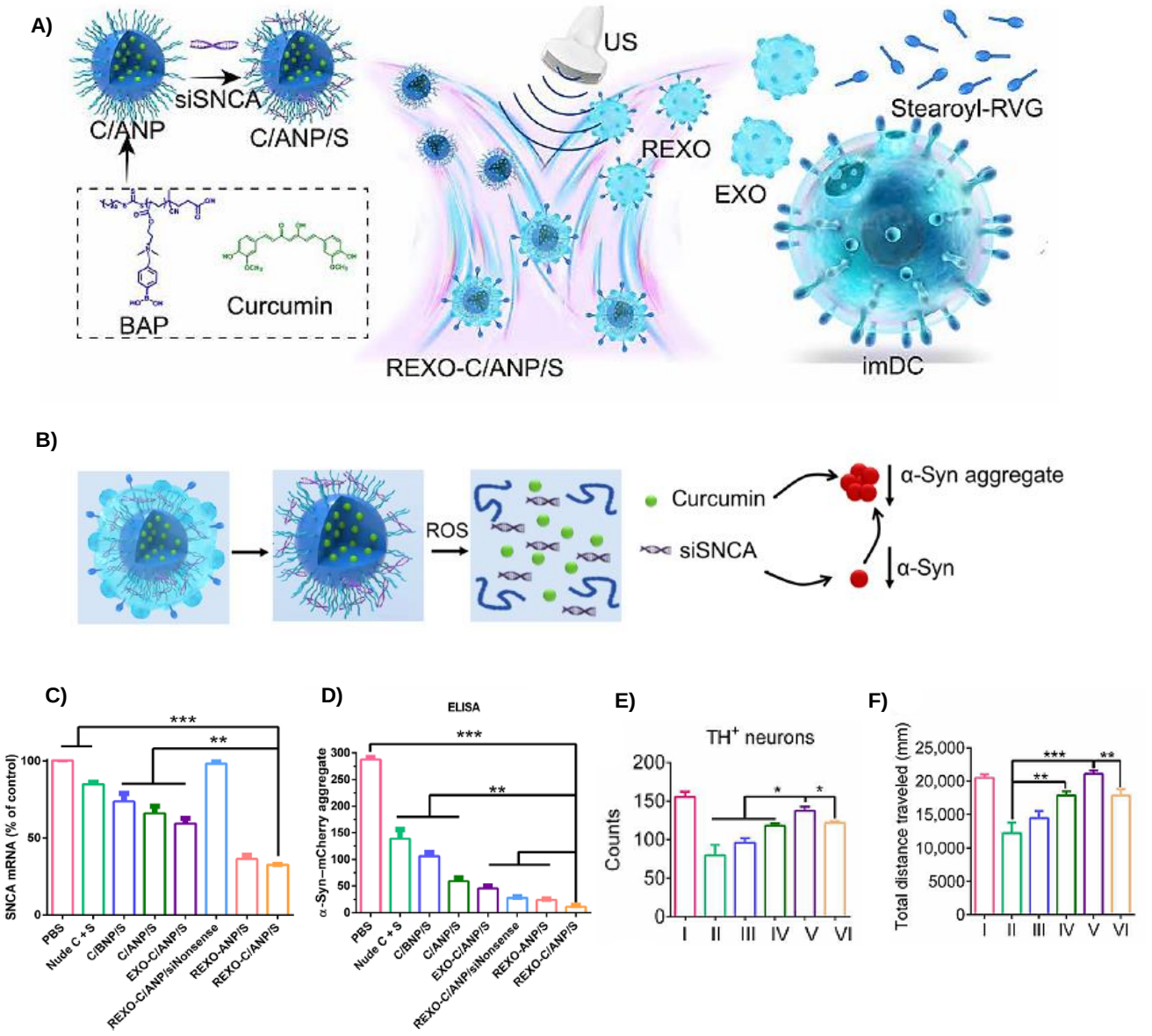

Figure 11. A) Schematic illustration of the preparation of REXO-C/ANP/S by coating C/ANP/S with the RVG-modified exosome membrane derived from imDCs. B) Synergistic effects of curcumin and siSNCA in clearing α-syn aggregates by downregulation of α-syn synthesis and reducing the existing α-syn aggregates. C) SNCA mRNA expression levels after different treatments. D) α-syn aggregates expression levels after different treatments. E) Number of TH^+ neurons in the mice brain sections after different treatments. F) Total distance traveled by mice after different treatments (I, II, III, IV, V and VI means normal mice, PD mice 5% glucose, PD mice C/ANP/S, PD mice EXO-C/ANP/S, PD mice REXO-C/ANP/S and PD mice REXO- C/ANP/siNonsense). Reproduced with permission from reference [92]. Copyright American Association for the Advancement of Science (2020).

Abbreviations: C/ANP/S, phenylboronic acid-poly(2-(dimethylamino)ethyl acrylate) nanoparticle core co-loaded with curcumin and siSNCA; imDC, immature dendritic cell; REXO-C/ANS/S, RVG-modified exosome membrane-coated C/ANP/S; ROS, reactive oxygen species; RVG, rabies virus glycoprotein; siSNCA, small interfering RNA targeting SNCA; SNCA, synuclein alpha gene; TH, tyrosine hydroxylase; α-syn, α-synuclein.