UNIVERSIDADE D
COIMBRA

Inês Ferreira Faria

Relatórios de Estágio e Monografia intitulada "Stem Cell Membrane-Coated Nanosystems for Biomedical Applications" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Ana Abrunheiro, 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.

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Coimbra, 15 de julho de 2021.

Inês Ferreira Faria

(Inês Ferreira Faria)

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Parte I

Relatório de Estágio em Farmácia Comunitária

Abreviaturas

FC	Farmácia Comunitária
FDA	Farmácia Dias Amaral
MICF	Mestrado Integrado em Ciências Farmacêuticas
SWOT	*Strengths, Weaknesses, Opportunities, Threats*

1. Introdução

O farmacêutico é um agente de saúde pública, cuja atividade profissional é centrada na pessoa do doente e apresenta como principais finalidades a promoção da saúde e do bem-estar, a informação e o uso racional dos medicamentos.

A fase final do plano de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF) integra a realização de um estágio curricular em Farmácia Comunitária (FC), que representa para muitos estudantes o primeiro contacto com a vida profissional, sob supervisão farmacêutica.

A FC assume um papel de extrema importância na comunidade, uma vez que representa, na maior parte das vezes, o primeiro contato que existe com os cuidados de saúde. É um espaço que a população reconhece pela proximidade, disponibilidade e, principalmente, pela competência profissional, numa relação de confiança contruída ao longo dos tempos [1, 2].

O presente relatório apresenta como principais objetivos proceder à descrição e análise do estágio realizado na Farmácia Dias Amaral (FDA), no período de 11 de janeiro a 30 de abril de 2021. O estágio teve a orientação da Dra. Ana Abrunheiro, apresentando toda a restante equipa técnica um papel fundamental na consolidação dos conhecimentos adquiridos ao longo dos cinco anos de formação e na aprendizagem de novos conteúdos.

O relatório será apresentado de acordo com a análise *Strengths, Weaknesses, Opportunities, Threats* (SWOT). A análise SWOT é efetuada a dois níveis e com a identificação de quatro vertentes, analisando internamente quais os pontos fortes (*Strengths*) e os pontos fracos (*Weaknesses*) e externamente quais as oportunidades (*Opportunities*) e as ameaças (*Threats*) existentes. Deste modo, o âmbito da análise SWOT terá como principal enfoque a unidade Estágio curricular, permitindo encontrar quais as vantagens a preservar e as desvantagens a eliminar.

Posteriormente à análise SWOT serão apresentados alguns casos práticos com os quais tive contacto no decorrer do estágio.

2. Análise SWOT

2.1. Análise Interna

2.1.1. Pontos Fortes

- **<u>Localização da farmácia</u>**

A FDA está situada no centro da freguesia de Arazede, que pertence ao concelho de Montemor-o-Velho, num largo com acesso a diferentes serviços. Assim, apresenta uma localização altamente favorável para o acesso de uma enorme diversidade de utentes à farmácia, tal como a proximidade a umas bombas de combustível, ao banco, à pastelaria e a uma loja de roupa. Para além disso, o facto de todos os meses nos dias 7 e 24 se realizar a feira em Arazede, num recinto também ele próximo, contribui para um afluxo superior de utentes à farmácia, na maior parte das vezes, vindos de outras freguesias. Esta questão permitiu o contacto com um público bastante diversificado e, deste modo, o desenvolvimento da capacidade de comunicação e adaptação à especificidade de cada utente.

- **<u>Utentes fidelizados</u>**

A FDA pela sua localização privilegiada, pela oferta diversificada, mas principalmente pela equipa qualificada, pró-ativa e sempre disposta a informar, esclarecer, ouvir e aconselhar torna-a numa farmácia de destaque no concelho com muitos utentes fidelizados dentro e fora da freguesia. Durante o meu percurso, apercebi-me que a FDA representa para vários utentes muito mais do que "o local que vende os seus medicamentos", mas um local de partilha, de apoio e de bem-estar eleito pelos utentes.

- **<u>Progressão do estágio</u>**

A evolução progressiva do meu estágio permitiu uma adaptação gradual às atividades desempenhadas na FDA, bem como a perceção da realidade da FC.

Deste modo, a primeira parte do estágio foi dedicada às tarefas existentes no *back office*. Primeiro, comecei por fazer a receção de encomendas e, posteriormente, arrumar os medicamentos nos seus locais adequados, tendo sempre em atenção os prazos de validade, os preços e a integridade das embalagens. Esta fase de receção e arrumação permitiu por um lado perceber quais os fornecedores existentes e os horários de entrega e, por outro lado, verificar quais os medicamentos de maior rotatividade, fazer a associação de nomes comerciais aos princípios ativos e, fundamentalmente, ficar a conhecer a grande variedade de produtos existentes na farmácia. Para além disso, também realizei devoluções de produtos ao fornecedor, a regularização de notas de créditos de produtos que eram devolvidos pelos

fornecedores e a transferência de medicamentos e produtos para o posto farmacêutico do Viso. Esta primeira fase do estágio permitiu-me perceber a relevância de todo o trabalho realizado no *back office* e a grande importância que este apresenta para um atendimento ao utente de qualidade.

Passado algum tempo, comecei a realizar pedidos de utentes que eram feitos por telefone, uma vez que a FDA oferece um serviço de entrega ao domicílio, que durante a pandemia adquiriu uma dimensão muito superior. Esta atividade foi uma excelente preparação para a etapa seguinte, o atendimento ao público.

- **Equipa**

A equipa da FDA é bastante diversificada, sendo constituída por farmacêuticos, técnicos e auxiliares de farmácia. É uma equipa qualificada, jovem e pró-ativa que teve um papel fundamental na minha integração nas diversas atividades da farmácia.

Desde o início do estágio foi-me comunicado por parte de todos os elementos da equipa a completa disponibilidade para responder a qualquer dúvida que surgisse e, por outro lado, também me foi concedida autonomia para a realização das tarefas que me eram propostas. Este ambiente foi fundamental para a consolidação e aprendizagem de novos conhecimentos durante o estágio.

- ***Software* 4DigitalCare®**

Na FDA o *software* utilizado é o 4DigitalCare®, ao contrário do que se verifica na maior parte das farmácias que utiliza o Sifarma 2000®. O único contacto que tive com o *software* Sifarma 2000® foi durante o percurso académico, não o tendo experimentado na execução de atividades reais da farmácia e, deste modo, não tenho um meio de comparação. No entanto, considero o 4DigitalCare® um sistema informático bastante intuitivo, simples e com um modo de organização prático, o que permitiu que rapidamente me familiarizasse com a maioria das suas funcionalidades.

A simplicidade e clareza do funcionamento do sistema informático foi, sem dúvida, uma mais-valia para que realizasse um atendimento ao público eficiente e com maior confiança.

- **Serviços oferecidos**

Um fator que sempre considerei muito positivo na FDA foi a diversidade de serviços que esta oferece aos seus utentes. Para além da medição de parâmetros bioquímicos, como a glicémia e o perfil lipídico, a medição da pressão arterial e a administração de injetáveis, a FDA apresenta outros serviços associados ao bem-estar e promoção da saúde. Deste modo, alguns

dos serviços existentes na FDA compreendem a podologia, nutrição e ortopedia, para além de que a FDA também está associada ao espaço "cuida-te", o qual oferece serviços de natureza dermocosmética aos seus utentes.

Devido à pandemia por SARS-CoV-2, surgiram dois novos serviços acessíveis aos utentes, nomeadamente o teste serológico à COVID-19 e o teste rápido de antigénio à COVID-19.

No decorrer do estágio tive a oportunidade de realizar a medição da glicémia, do perfil lipídico e também da pressão arterial num gabinete destinado a estes serviços. Foi uma experiência que permitiu um contato mais próximo com o utente, promovendo a educação para a saúde e o acompanhamento diferenciado de cada utente.

- **Diversidade de oferta**

A FDA apresenta uma oferta bastante diversificada, no sentido de tentar sempre responder às necessidades específicas de cada utente. Este é um dos fatores de distinção da farmácia, em relação à concorrência, e que a torna a primeira escolha de muitos utentes.

A farmácia dispõe de uma ampla variedade de produtos na área da puericultura, nomeadamente de leites, papas, chupetas, biberões, fraldas e produtos de higiene. Outra área de grande desenvolvimento e destaque na farmácia é a dermocosmética, dispondo de variadíssimas marcas, entre as quais Caudalie®, Nuxe®, Vichy®, La Roche-Posay®, Isdin®, Avène® e Klorane®. Durante todo o estágio, esta foi uma área de grande aprendizagem, descobrindo novas gamas de produtos, quais as suas propriedades e indicações e qual o melhor aconselhamento para cada utente, mas tendo a noção que ainda existe muito para conhecer e aprender. Destaco também os inúmeros produtos de ortopedia que me permitiram de igual modo adquirir novos conhecimentos.

Como está inserida num meio rural, a farmácia também apresenta uma grande variedade de produtos e medicamentos de uso veterinário. Apesar de inicialmente ter sido uma das minhas maiores dificuldades a nível do atendimento ao público, sinto também que foi uma das áreas em que mais aprendi durante o estágio.

2.1.2. Pontos Fracos

- **Designação comercial dos medicamentos**

Uma das minhas maiores dificuldades ao longo do estágio foi conseguir fazer a associação entre a designação comercial dos medicamentos e os respetivos princípios ativos. Esta situação dificultava muito a comunicação com os utentes, visto que estes apenas conheciam a sua medicação pelo nome comercial e, na maior parte das vezes, a pronúncia era feita de uma

forma muito própria. Neste aspeto, o *software* 4DigitalCare® foi uma grande ajuda, visto que facilmente me permitia ver o histórico da medicação dos utentes fidelizados.

- ***Stocks* incorretos**

Durante o percurso na FDA, deparei-me várias vezes com erros de *stock* no sistema informático, apresentando tanto um *stock* superior como inferior ao *stock* efetivo. Os erros de stock podem ter várias causas, entre as quais, falhas na receção das encomendas, uma contagem física dos produtos incorreta, a arrumação dos produtos fora dos seus locais e a execução incorreta de transferências para o posto farmacêutico. Esta é uma falha que prejudica a qualidade do atendimento que é prestado aos utentes, uma vez que indica a existência de produtos que na realidade não existem ou, pelo contrário, indica um *stock* nulo ou negativo de um produto que na verdade está presente na farmácia. Consequentemente, estes erros culminam numa perda de tempo durante o atendimento e, maioritariamente, na execução de uma encomenda instantânea ao fornecedor e respetiva reserva.

2.2. Análise Externa

2.2.1. Oportunidades

- **Formações**

Apesar dos cinco anos de MICF me terem proporcionado diferentes conhecimentos, considero que existem algumas lacunas no seu plano de estudos, principalmente no que respeita a algumas situações clínicas de indicação farmacêutica. Neste sentido, considero que seria importante abordar de forma mais aprofundada os problemas de saúde de carácter não grave, visto que é aqui que o farmacêutico vai intervir. Assim, todas as formações a que tive oportunidade de assistir foram bastante enriquecedoras e permitiram que, ao conhecer as características de cada produto, o aconselhamento ao utente fosse de qualidade e que me sentisse mais confiante e confortável ao comunicar com ele.

Infelizmente com a situação pandémica, o número de formações ficou reduzido e estas passaram a ser transmitidas por via remota, no entanto as formações a que tive oportunidade de assistir foram importantes para colmatar algumas dessas lacunas.

- **Dinamização da farmácia**

O estado de emergência devido à COVID-19 levou a que muitas pessoas não se deslocassem com tanta periodicidade à farmácia. Esta diminuição do movimento da farmácia, principalmente, para a aquisição de produtos de beleza, bem-estar e outros que não

medicamentos, obrigou a uma reinvenção da farmácia no sentido de continuar a divulgar os seus produtos e a permitir que todos os seus utentes os pudessem adquirir em segurança. Desta forma, houve uma aposta nas redes sociais que constituíram uma excelente ferramenta para a comunicação e proximidade com os utentes. Assim, todas as publicações que eram feitas tinham o objetivo não só de informar o utente das novidades da farmácia e produtos com desconto como também de contribuir para a literacia em saúde da população. A esta divulgação *online* da oferta da farmácia associou-se um serviço de entrega ao domicílio que permitiu aos utentes receberem os seus medicamentos e produtos em segurança sem saírem de casa e, principalmente, aos que estavam infetados com COVID-19 conseguirem receber a sua medicação.

2.2.2. Ameaças

- **Receitas manuais**

Atualmente, a maioria das receitas prescritas pelos médicos são eletrónicas, no entanto ainda existem algumas exceções que permitem a prescrição de medicamentos através de receitas manuais, nomeadamente, falência do sistema informático, inadaptação fundamentada do prescritor, prescrição ao domicílio e devido a outras situações até um máximo de 40 receitas médicas por mês [3].

Durante o estágio, apesar de maioritariamente contactar com receitas eletrónicas, infelizmente ainda contactei com várias receitas manuais. Sempre que tinha de processar uma receita manual ficava bastante nervosa, visto que não só tinha de proceder à validação da prescrição, verificando vários elementos como também tinha de conseguir perceber o que o médico tinha prescrito [4]. Perante uma situação destas eu tinha de recorrer sempre no *back office* a ajuda da equipa técnica para fazer a verificação da receita e para a decifrar com o intuito de garantir que os medicamentos que entregava eram os corretos e a posologia mencionada ao utente também. Assim, considero que a existência de receitas manuais representa um perigo para o utente, podendo colocar em causa a sua segurança ou a eficácia do tratamento.

- **Medicamentos esgotados**

Ao longo do estágio deparei-me com o facto de existirem vários medicamentos que se encontravam esgotados e outros que existiam apenas em pequenas quantidades disponíveis nos fornecedores. Perante uma situação destas, contactava-se diretamente os nossos fornecedores e também outras farmácias, fazendo tudo o que fosse possível no sentido de

conseguir adquirir os medicamentos necessários, para que o utente não tivesse de interromper a sua medicação.

Do ponto de vista do utente, esta era uma realidade muito difícil de compreender, sendo entendida, muitas das vezes, como uma falha da farmácia, que perdia assim a sua credibilidade. Em algumas situações ainda existia a possibilidade de proceder à substituição do medicamento em questão, recorrendo, por exemplo, a um medicamento genérico. Contudo, na maior parte das vezes, não existia essa possibilidade e a única alternativa consistia em pedir ao utente que contactasse o seu médico de família com o intuito de evitar a interrupção do tratamento.

- **Desinformação**

Hoje em dia, grande parte da população tem acesso a diferentes fontes de informação. No entanto, o facto de não conseguirem rastrear a informação que facilmente encontram através da *internet* provoca uma aquisição desses conhecimentos como verdades indiscutíveis, o que dificulta bastante a comunicação durante o aconselhamento.

Por outro lado, durante o estágio também me apercebi que alguns utentes encaram os medicamentos como produtos vulgares, não tendo a mínima noção do seu perigo caso não sejam utilizados adequadamente. Uma das situações que mais me marcou foi um atendimento de um idoso que ia aviar uma receita para a esposa. Durante a conversa ele referiu que não queria levar o medicamento Palexia® retard 100 mg, uma vez que a sua esposa se sentiu mal após a toma, razão pela qual o médico o retirou. Nesse momento, o utente acabou por me confidenciar que tomou ele o resto dos comprimidos "para não se estragarem". Esta situação deixou-me bastante preocupada ao perceber que ainda há muita desinformação sobre os medicamentos e o perigo que a sua utilização incorreta representa para a saúde.

3. Casos Práticos

Caso Clínico 1

Utente do sexo feminino, com cerca de 45 anos, dirige-se à farmácia solicitando uma solução que permita eliminar uma verruga que lhe apareceu no dedo anelar da mão direita e que a andava a incomodar. Após observação da verruga, a utente referiu já ter passado pela mesma situação há alguns anos.

Face ao que foi exposto, comecei por referir que uma verruga é uma infeção viral, cujo contágio ocorre através do contacto, principalmente em locais públicos onde o ambiente é quente e húmido, tal como em balneários ou ginásios. Assim, para prevenção, seria importante evitar o contacto nestas fontes de contaminação.

Relativamente ao tratamento, aconselhei a utente a utilizar VERRUFILM®, uma solução cutânea cuja substância ativa é o ácido salicílico, que apresenta propriedades queratolíticas. Para uma utilização adequada do produto, recomendei à utente para, antes da aplicação, imergir a zona da verruga em água quente aproximadamente durante 5 minutos, permitindo amolecer aquela área afetada. Posteriormente, de modo a proteger a pele sã circundante de um possível contacto com o produto, aplicar uma camada de creme gordo ou vaselina na zona em redor da verruga. De seguida, proceder à aplicação cuidadosa de uma ou duas gotas de VERRUFILM® apenas na zona a tratar, esperar um pouco até secar e depois tapar com adesivo a área afetada, deixando atuar por 24 horas. Após esse tempo, remover o adesivo, lavar com água quente e, utilizando uma pedra-pomes, raspar um pouco a verruga para remover a pele morta. Referi que teria de repetir este processo todos os dias até conseguir eliminar a verruga, o que poderia demorar cerca de 6 a 12 semanas.

Caso Clínico 2

Utente do sexo feminino, com cerca de 20 anos, dirige-se à farmácia queixando-se que, ultimamente, lhe têm aparecido "umas borbulhas na cara" e solicita uma solução que permita removê-las. Questionada sobre a sua rotina diária de cuidado da pele refere que apenas costuma "passar a cara por água" e que normalmente aplica um creme hidratante.

Analisando a zona T, percebi que se tratava de uma pele oleosa, uma vez que o seu rosto já se encontrava com um ligeiro brilho, por volta das 10 horas, o que foi confirmado pela utente ao referir que a seu pele é "muito gordurosa".

Perante esta situação, expliquei que as bases de uma boa rotina diária de pele assentam na limpeza e hidratação da pele. Referi também que a limpeza da pele não consiste apenas numa lavagem com água, já que esta não consegue eliminar todas as impurezas e oleosidade da nossa pele. Assim, recomendei o gel de limpeza da linha *Normaderm* da Vichy® e a pasta de enxofre S.O.S da mesma linha para aplicar à noite de forma localizada "na borbulha" para a secar, já que a utente referiu que iria utilizar o creme hidratante que já tinha adquirido e que era indicado para pele oleosa. Posteriormente, realcei a importância de aplicar diariamente protetor solar que permite proteger a pele dos danos causados pela radiação ultravioleta e prevenir que fiquem na pele manchas ou marcas das imperfeições. Desta forma, aconselhei como protetor solar o *Anthelios Anti-Imperfections* SPF50 da La Roche-Posay® [5].

Caso Clínico 3

Utente do sexo feminino, com aproximadamente 55 anos, dirige-se à farmácia com queixas de desconforto urinário, referindo que sentia necessidade constante de urinar e ardor.

Perante a situação relatada, questionei a utente relativamente à presença de sangue na urina e à duração dos sintomas. A utente referiu não ter a presença de sangue na urina e que os sintomas tinham começado há poucos dias. Acrescentou ainda que já tinha tido três infeções urinárias e que, portanto, reconhecia estes sintomas. Assim, recomendei a toma de dois comprimidos de Systelle® três vezes ao dia durante 7 dias. Este é um suplemento alimentar que contém extrato seco de uva-ursina, a qual apresenta propriedades antibacterianas e diuréticas devido à presença de arbutina que a nível renal é hidrolisada, libertando o composto ativo hidroquinona. Para além disso, recomendei a ingestão abundante de líquidos para favorecer a diurese e a realização de uma adequada higiene íntima, aconselhando a utilização de Lactacyd® Antissético.

Por último, avisei a utente que no caso de os sintomas persistirem ou se acentuarem deve consultar o seu médico.

Caso Clínico 4

Utente do sexo feminino, com cerca de 50 anos, apresenta-se na farmácia queixando-se que já algum tempo lhe apareceram "umas borbulhas no peito" que lhe causam bastante desconforto e prurido. Refere já ter aplicado Fenivir® que utilizava em caso de herpes labial, mas que não observou qualquer melhora. Questionada sobre quando verificou o aparecimento daquela lesão cutânea, a utente referiu que esta teve início nos "dias de maior calor". Acrescentou também que, como tem os seios grandes, transpira muito e que, por isso, nos dias de mais calor é normal aparecer-lhe uma vermelhidão, contudo desta vez havia ficado muito mais exacerbado, causando bastante prurido.

Perante os sintomas relatados pela utente e o facto de se tratar de um ambiente quente e húmido, concluí que seria uma infeção de origem fúngica, razão pela qual não houve resposta ao Fenivir®, uma vez que não era de natureza viral. Desta forma, aconselhei a utilização de um spray secante da A-Derma® e de uma pomada com antifúngico (miconazol), a Nutraisdin® AF Pomada Reparadora. Recomendei a aplicação do *spray* secante seguido da pomada com antifúngico até à regeneração da pele e, posteriormente, continuar com a aplicação do spray secante de modo a evitar a criação de um ambiente favorável ao crescimento de fungos e, assim, prevenir o desenvolvimento de outra infeção fúngica.

Caso Clínico 5

Utente do sexo feminino, com cerca de 70 anos, dirige-se à farmácia para pedir ajuda, uma vez que teve uma ninhada de leitões, mas que a "porca acabou por morrer com uma infeção". Desesperada, questiona o que poderá dar aos leitões como substituto das mamadas. Face ao

exposto, aconselhei a utente a dar o Zoomilk® e como referiu que os leitões estavam bastante fracos, tendo já morrido três, recomendei também o Anima Strath® para fortalecer o seu sistema imunitário e aumentar o apetite, ajudando na digestão e absorção dos nutrientes.

4. Considerações Finais

O estágio curricular na FDA constituiu uma experiência bastante enriquecedora quer a nível profissional quer a nível pessoal. Por outro lado, permitiu-me conhecer a realidade do dia-a-dia de um farmacêutico comunitário e, para além disso, verificar a importância que a FC representa para os seus utentes, tanto para questões de saúde como de bem-estar.

O facto de ter experienciado diferentes situações foi fundamental para a aquisição de novos conhecimentos, para além de ter permitido consolidar e aplicar vários conhecimentos teóricos adquiridos ao longo do MICF. Importante foi também verificar que um farmacêutico comunitário é um profissional multifacetado, cujas atividades desempenhadas exigem uma atualização constante dos seus conhecimentos, tal como a capacidade de estabelecer uma comunicação efetiva com os seus utentes, ao saber gerir as emoções e criar empatia e conquistar a confiança dos utentes.

Com esta experiência, verifiquei a enorme responsabilidade da atividade profissional do farmacêutico na sociedade, incentivando o uso racional dos medicamentos, garantindo a segurança e efetividade da sua terapêutica e contribuindo para uma maior literacia em saúde dos utentes.

Para finalizar, quero agradecer sinceramente à Dra. Paula Amaral e ao Dr. Pedro Andrade pela possibilidade de realização do estágio na FDA, à minha orientadora de estágio, a Dra. Ana Abrunheiro, tal como a toda a restante equipa técnica pelo acolhimento, pelo apoio e por todos os ensinamentos transmitidos ao longo deste percurso.

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Parte II

Relatório de Estágio em Farmácia Hospitalar

Abreviaturas

CHUC	Centro Hospitalar e Universitário de Coimbra
HP	Hospital Pediátrico
HUC	Hospitais da Universidade de Coimbra
MICF	Mestrado Integrado em Ciências Farmacêuticas
SWOT	*Strengths, Weaknesses, Opportunities, Threats*
UMIV	Unidade de misturas intravenosas

1. Introdução

Como profissional de saúde, o farmacêutico pode exercer a sua atividade em diversas áreas, nas quais se incluem, entre outras, Farmácia Comunitária, Farmácia Hospitalar e Indústria Farmacêutica.

O Mestrado Integrado em Ciências Farmacêuticas (MICF) proporciona aos seus estudantes a oportunidade de, no 5º ano do plano de estudos, poderem realizar um estágio curricular numa área do seu interesse e que desperte a sua curiosidade, para além do estágio obrigatório em Farmácia Comunitária. De acordo com o meu interesse em conhecer a realidade da Farmácia Hospitalar e qual o papel que o farmacêutico desempenha para a prestação de cuidados de saúde eficazes, seguros e de qualidade numa estrutura tão complexa como o meio hospitalar, decidi realizar estágio no Centro Hospitalar e Universitário de Coimbra, EPE (CHUC).

Segundo o Decreto-Lei n.º 44 204, de 2 de fevereiro de 1962 [1], que regula as atividades de Farmácia Hospitalar, estas atividades são exercidas por meio dos serviços farmacêuticos, os quais constituem atualmente departamentos com autonomia técnica e científica. No meio hospitalar, os serviços farmacêuticos integram as equipas de prestação de cuidados de saúde e têm como missão garantir o acesso aos medicamentos a todos os doentes, assegurando a sua qualidade, eficácia e segurança, bem como promover ações de ensino e de investigação científica [2].

O CHUC é um centro hospitalar constituído por várias unidades, Hospitais da Universidade de Coimbra (HUC), Hospital Geral, Hospital Pediátrico de Coimbra (HP), Hospital Sobral Cid, Maternidade Bissaya Barreto e Maternidade Daniel de Matos [3, 4]. Os Serviços de Farmácia Hospitalar fazem parte da estrutura organizacional do CHUC, são dirigidos pelo Dr. José Feio e encontram-se centralizados nos HUC. Englobam diversos setores, alguns dos quais tive a oportunidade de contactar, nomeadamente os setores da distribuição, farmacotecnia, ensaios clínicos e gestão e aprovisionamento.

O presente relatório apresenta como principais objetivos proceder à descrição e análise do estágio realizado no CHUC, no período de 3 de maio a 29 de junho de 2021, sob orientação da Doutora Marília João Rocha, o qual será apresentado de acordo com a análise *Strengths, Weaknesses, Opportunities, Threats* (SWOT). A análise SWOT é efetuada a dois níveis e com a identificação de quatro vertentes, analisando internamente quais os pontos fortes (*Strengths*) e os pontos fracos (*Weaknesses*) e externamente quais as oportunidades (*Opportunities*) e as ameaças (*Threats*) verificadas durante o estágio curricular em Farmácia Hospitalar.

2. Análise SWOT

2.1. Análise Interna

2.1.1. Pontos Fortes

- **<u>Plano de estágio</u>**

Os primeiros dias de estágio foram fundamentais para a minha integração e orientação nos Serviços de Farmácia Hospitalar do CHUC. O facto de ter havido uma breve apresentação pelos coordenadores de cada setor permitiu ficar logo com uma noção de qual o objetivo desses setores e algumas das atividades desempenhadas. Posteriormente, tive a possibilidade de fazer uma visita às instalações de cada setor que constitui os Serviços de Farmácia Hospitalar dos HUC bem como ao edifício de São Jerónimo, proporcionando uma noção real da dinâmica e organização do serviço. Para além disso, também me foi entregue um planeamento de todo o estágio, no qual eu e os restantes cinco estagiários fomos organizadamente distribuídos por cada setor de modo a que houvesse apenas um estagiário em cada local e que fosse possível contactar com múltiplos setores. Do mesmo modo, foi disponibilizado o "Caderno do Estagiário" que apresentava quais os conhecimentos a adquirir e as atividades a realizar em cada setor. A organização e estruturação do estágio bem como a visita aos setores constituiu uma mais-valia para o decorrer do estágio, uma vez que já conhecia as instalações e os objetivos de cada setor.

- **<u>Contacto com vários setores dos Serviços de Farmácia Hospitalar</u>**

Durante o estágio tive a possibilidade de contactar com quatro setores dos Serviços de Farmácia Hospitalar do CHUC e de perceber o seu funcionamento.

No setor da distribuição, assisti à cedência e controlo de *stocks* de estupefacientes, psicotrópicos e hemoderivados, para além da dispensa de medicamentos em regime de ambulatório aos doentes. O ambulatório no edifício de São Jerónimo constituiu para mim uma experiência bastante enriquecedora, uma vez que me permitiu verificar todo o aconselhamento que é feito ao doente aquando da dispensa da medicação e a forma como este aconselhamento é feito, pois normalmente estamos perante um doente debilitado, bem como o alerta para possíveis efeitos adversos que possam decorrer da toma dos medicamentos. Para além disso, também presenciei a preparação dos medicamentos no ambulatório dos HUC para o programa de entrega de medicamentos em proximidade (PEMProx), que evita que os doentes se desloquem ao hospital apenas para levantar os seus medicamentos.

No setor da farmacotecnia tive a possibilidade de contactar com várias áreas, incluindo a unidade de misturas intravenosas (UMIV), unidade de preparação de medicamentos não estéreis, radiofarmácia e unidade de preparação de citotóxicos. Na UMIV assisti à preparação de alguns manipulados, nomeadamente da enzima elosulfase, da pomada oftálmica de tacrolímus e de monodoses de soro autólogo. Do mesmo modo, na unidade de preparação de medicamentos não estéreis tive a oportunidade de observar a preparação de vários manipulados bem como de colaborar na preparação de alguns, como por exemplo, de bisnagas de nitroglicerina a 0,25% e cinchocaína a 0,25% e de papéis de fortificante do leite materno.

No setor dos ensaios clínicos presenciei uma visita de qualificação, na qual o monitor de um ensaio clínico de fase II verificou se existiam as condições necessárias para a realização do estudo no hospital, para além de ter participado na cedência dos medicamentos experimentais.

Por fim, no setor da gestão e aprovisionamento passei pelo armazém onde observei a receção e confirmação das encomendas, bem como a dispensa de medicamentos armazenados no Kardex® de frio para a satisfação dos pedidos feitos pelos serviços.

A passagem por todos estes setores foi relevante para perceber o funcionamento dos Serviços de Farmácia Hospitalar de uma forma integrada.

- **Contacto com medicamentos sujeitos a legislação específica**

Durante o estágio, observei a dispensa de medicamentos sujeitos a legislação específica, nomeadamente de hemoderivados, estupefacientes e de medicamentos experimentais [5], tendo a possibilidade de perceber os circuitos específicos deste tipo de medicamentos. Relativamente aos estupefacientes ainda assisti a algumas requisições realizadas segundo o anexo X, modelo n.º 1509, apesar da maioria ser feita através do sistema informático. Da mesmo forma, contactei pela primeira vez com algumas requisições de hemoderivados, tais como da albumina humana, de sistemas adesivos de fibrina e de fibrinogénio humano, o que constituiu uma experiência relevante no meu estágio.

2.1.2. Pontos Fracos

- **Impossibilidade de passar por todas as unidades do CHUC**

No decorrer do estágio curricular, apenas tive a possibilidade de contactar com duas unidades do CHUC, designadamente os HUC e o HP. Deste modo, considero que seria bastante relevante para a minha experiência e para a aprendizagem de novos conceitos ter passado pela Maternidade Bissaya Barreto ou pela Maternidade Daniel de Matos, de forma a ter uma noção de qual o papel que o farmacêutico desempenha para um bom funcionamento

de uma maternidade e quais as especificidades de trabalhar nessas unidades. Contudo, considero que um período de estágio de apenas dois meses não seja o mais adequado para nos permitir passar por todas as unidades do CHUC nem para conseguir adquirir conhecimentos mais detalhados.

- **Instalações**

Um fator que considero ser um ponto negativo no meu estágio foi o desajuste da maior parte das instalações para as atividades que eram realizadas e o facto de não estarem preparadas para a receção de estagiários. Relativamente aos HUC, onde passei praticamente todo o meu estágio, entendo que as instalações demasiado pequenas e com poucas condições de trabalho dificultaram o acompanhamento das atividades dos farmacêuticos, principalmente a sala de validação da unidade de preparação de citotóxicos, localizada no edifício de São Jerónimo, cujas dimensões são bastante reduzidas, sem a existência de janelas e com fraca refrigeração do local, razão pela qual apenas foi possível passar lá um dia.

2.2. Análise Externa

2.2.1. Oportunidades

- **Aprendizagem de novos conceitos**

Com o estágio curricular no CHUC tive a oportunidade de adquirir novos conhecimentos que praticamente não foram abordados no plano de estudos do MICF. Uma das áreas que considero ser bastante promissora é radiofarmácia, apesar de ser uma área um pouco desconhecida por mim. Na Medicina Nuclear dos HUC observei a preparação de radiofármacos em condições específicas, incluindo *hotte* blindada, seringas com proteção de chumbo, bem como a utilização de dosímetros, em forma de anel, que permitem medir a radiação a que o manipulador está exposto. Estas preparações radiofarmacêuticas podem ser utilizadas para terapêutica ou para o diagnóstico de várias patologias. Uma das preparações que tive a oportunidade de presenciar foi a marcação de um bifosfonato, ácido oxidrónico (Osteocis®), com o radionuclídeo tecnécio-99 metaestável, um dos mais usados em Medicina Nuclear, com o objetivo de verificar as áreas de maior atividade óssea, o que é utilizado para deteção de tumores ósseos por meio de uma cintigrafia óssea.

No edifício de São Jerónimo, tanto no ambulatório como na unidade de preparação de citotóxicos foi feita uma abordagem geral aos protocolos de quimioterapia, no entanto o tempo reduzido que lá estive não permitiu adquirir conhecimentos mais detalhados. Do

mesmo modo, na UMIV tive a oportunidade de presenciar a preparação de bolsas para nutrição parentérica, verificando a importância da adição ordenada de cada elemento.

A oportunidade de, neste estágio curricular, ter tido uma abordagem geral sobre estes temas constituiu uma mais-valia, pois demonstrou outras vertentes da Farmácia Hospitalar para as quais não estava desperta.

- **Realização de um trabalho e apresentação**

Durante o primeiro mês de estágio foi proposta a realização de um trabalho relacionado com o consumo de desinfetantes e antissséticos a nível do CHUC e a comparação desse consumo com a quantidade que está predefinida no *stock* para cada serviço (Anexo I). A elaboração deste trabalho permitiu-me ficar com uma noção da enorme quantidade de serviços que existem no CHUC, bem como da quantidade e tipo de desinfetantes e antissséticos que são utilizados e algumas das indicações para os quais são aplicados. Posteriormente, houve a possibilidade de estar presente numa reunião com diferentes profissionais, nomeadamente farmacêuticos, médicos e enfermeiros, e proceder à apresentação do trabalho, analisando mais pormenorizadamente o consumo de desinfetantes e antissséticos por cada serviço do hospital. No final da apresentação, existiu um debate com troca de ideias e opiniões entre os diferentes profissionais, ao qual tive a oportunidade de assistir em que se tentou perceber quais os pontos em que se pode melhorar de modo a haver uma utilização mais eficaz e segura dos desinfetantes e antissséticos no hospital, evitando a troca de produtos ou o seu uso desadequado. O facto de ter estado presente nesta reunião foi bastante enriquecedor para o meu estágio, ao perceber alguns problemas reais do hospital, contactar com outros profissionais de saúde e perceber toda a gestão que é necessária para um funcionamento eficiente do hospital.

2.2.2. Ameaças

- **Reduzida interação com o doente**

Durante o período em que estive no CHUC verifiquei que a interação entre o farmacêutico e o doente internado é praticamente inexistente e, naturalmente, também devido à situação pandémica por SARS-CoV-2, este contacto com o doente foi muito afetado. Contudo, considero ser fundamental que exista interação com o doente, o que possibilita perceber se, por exemplo, há alguma patologia que ainda não esteja a ser tratada, se existe algum efeito adverso provocado pelos medicamentos, se é possível adaptar ainda mais o plano terapêutico de acordo com o contexto clínico do doente, tal como adequação da via de

administração ou ajuste de dose. Ou seja, o contacto com o doente, mas também com o médico que o segue, permite que se obtenha um plano terapêutico mais eficiente bem como uma melhoria da qualidade de vida do doente.

- **Reduzida oferta de emprego em Farmácia Hospitalar**

Apesar de ser conhecida a falta de recursos humanos em Farmácia Hospitalar, a oferta de emprego para constituir carreira como farmacêutico hospitalar é reduzida e, além disso, mais inacessível para recém-farmacêuticos que ainda não têm experiência profissional em Farmácia Hospitalar. De acordo com isso, em alguns setores era visível a sobrecarga de trabalho dos farmacêuticos, o que dificultava por vezes o acompanhamento e orientação dos estagiários da forma que eles próprios consideram ser a mais adequada.

3. Considerações Finais

O estágio curricular em Farmácia Hospitalar no CHUC representou uma experiência muito importante no meu percurso académico, permitindo-me adquirir, principalmente, novas competências e uma grande variedade de novos conhecimentos. Além disso, possibilitou o contacto com a realidade hospitalar, ficando com uma perceção de qual o circuito do medicamento num hospital de grandes dimensões e com uma grande multiplicidade de serviços, da forma como os Serviços de Farmácia Hospitalar estão organizados e quais as atividades desempenhadas pelos farmacêuticos em cada setor. A abordagem geral de temas não muito associados à atividade farmacêutica, permitiu-me conhecer outras vertentes de Farmácia Hospitalar, das quais destaco radiofarmácia e ensaios clínicos, que considero serem áreas promissoras com um grande potencial.

Assim, quero agradecer a todos os farmacêuticos e também técnicos superiores de diagnóstico e terapêutica que me acompanharam por toda a atenção e ensinamentos e por me mostrarem a realidade e a organização da Farmácia Hospitalar.

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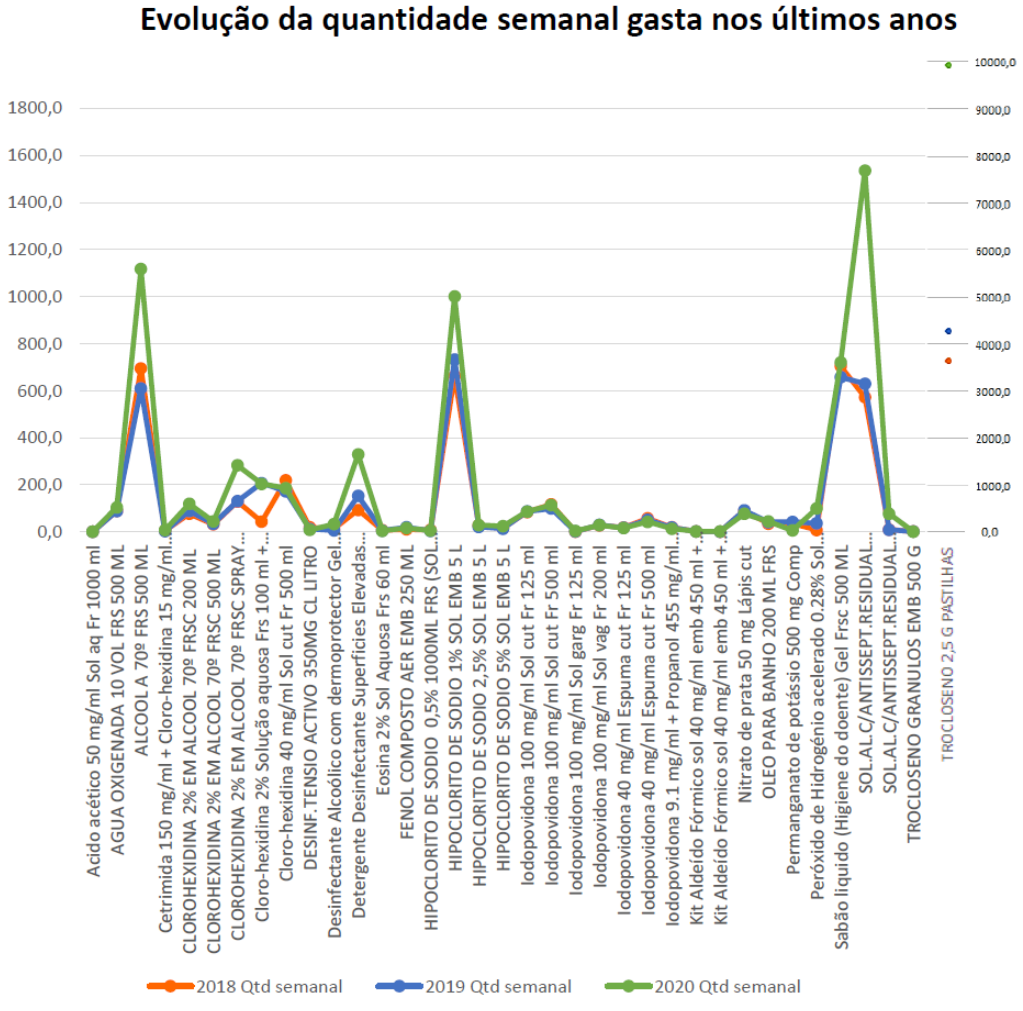
Anexo I – Parte do trabalho realizado durante o estágio curricular. Apresentação de alguns dos serviços existentes no CHUC e representação gráfica da quantidade semanal de desinfetantes e antisséticos gastos nos anos de 2018, 2019 e 2020 no CHUC.

- Alimentação
- Anatomia Patológica
- Anestesiologia
- Antisepticos e Desinfetantes
- Bloco operatório central
- Bloco Operatório
- Cardiologia
- Casa Mortuária
- Cinesiterapia Respiratória
- Cirurgia A e B
- Cirurgia cardiotorácica
- Cirurgia de ambulatório
- Cirurgia geral
- Cirurgia Maxilo-Facial
- Cirurgia Pediátrica e Queimados
- Cirurgia plástica
- Cirurgia Vascular
- Consultas externas
- COVID-19
- Dermatologia
- Diálise e Diálise Peritoneal
- Endocrinologia
- Endoscopias e Endoscopia Respiratória
- Estomatologia
- Fisiopatologia Respiratória
- Gastrenterologia
- Gastroenterologia
- Ginecologia
- Hematologia
- Hemodinâmica
- Hospital dia
- Hospital Geral
- Hospital Pediátrico
- Imagiologia
- Imunoalergologia
- Infeciologia
- Laboratório de Citogenética
- Maternidade Bissaya Barreto
- Maternidade Daniel de Matos
- Medicina Física e Reabilitação
- Medicina intensiva
- Medicina interna
- Nefrologia
- Neontatologia
- Neurocirurgia
- Neurologia
- Obstetrícia
- Oftalmologia
- Oncologia e Oncologia Pediátrica
- Ortopedia
- Otorrinolaringologia
- Pneumologia
- Psiquiatria
- Queimados
- Radiologia
- Radioterapia
- Reumatologia
- S. Reprodução Humana
- Sala de Partos
- Serv. de Sangue
- Serv. Farmacêuticos
- Serviço de Instalações e Equipamentos
- Transplantação Hepática
- Transplante Renal
- Transporte de doentes
- Urgência
- Urologia
- VMER



Parte III

"Stem Cell Membrane-Coated Nanosystems for Biomedical Applications"

Resumo

A nanotecnologia tem sido largamente explorada no sentido de desenvolver novos nanossistemas de diagnóstico e terapêutica. Apesar dos avanços inovadores, algumas limitações podem ser atribuídas aos nanossistemas, nomeadamente a rápida eliminação pelo sistema imunitário, a falta de direcionamento para as células alvo e a insuficiente biocompatibilidade. Deste modo, novas estratégias têm recebido especial atenção para melhorar o perfil farmacocinético e de segurança dos nanossistemas, baseando-se na abordagem biomimética. O revestimento biomimético de núcleos de nanopartículas com membranas celulares derivadas de células de diferentes origens surge, assim, como uma estratégia promissora. As células estaminais têm sido um foco de investigação para o desenvolvimento de sistemas de entrega direcionada de fármacos, devido à sua capacidade de direcionamento, particularmente para os locais de tumor e de inflamação. Esta capacidade de direcionamento é conferida pelas proteínas presentes na membrana das células estaminais, que não só permitem aumentar a eficácia terapêutica, como também diminuir os efeitos adversos, devido à maior acumulação de fármaco nos locais alvo. Para além disso, o revestimento das nanopartículas com membrana celular funciona como uma camuflagem ao impedir a sua rápida eliminação, e assim aumentar o seu tempo de semi-vida. Neste artigo de revisão serão descritos os progressos da utilização de nanossistemas revestidos por membrana das células estaminais no âmbito das aplicações biomédicas.

Palavras-chave: célula estaminal; membrana da célula estaminal; revestimento por membrana celular; nanopartícula; sistema de entrega direcionada de fármaco.

Abstract

Nanotechnology has been extensively explored for developing new diagnostic and therapeutic nanosystems. Despite the groundbreaking advances, some limitations can be attributed to nanosystems, namely the rapid elimination by the immune system, lack of targeting to specific cells and insufficient biocompatibility. Therefore, novel strategies have been receiving special attention to improve the pharmacokinetic and safety profile of nanosystems, building on a biomimetic approach. An exciting strategy consists in the biomimetic coating of nanoparticle cores with cell membranes derived from distinct source cells. Stem cells have been a focus of research for the development of targeted drug delivery systems due to their ability to target, particularly to tumor and inflammation sites. This targeting ability is conferred by proteins present in the stem cell membrane that not only increase the therapeutic efficacy but also decrease the adverse effects, due to greater accumulation of drug in the target sites. Furthermore, the coating of the nanoparticles by the cell membrane acts as a camouflage by preventing their rapid elimination and thus increasing their circulation time. This review will describe the advances in the use of stem cell membrane-coated nanosystems for biomedical applications.

Keywords: stem cell; stem cell membrane; cell membrane coating; nanoparticle; targeted drug delivery system.

Abbreviations

ADSC	Adipocyte-derived stem cell
ALCAM	Activated leukocyte cell adhesion molecule
ASC	Adult stem cell
bFGFR	Basic fibroblast growth factor receptor
cRA@STCM	Stem cell membrane-coated isotretinoin nanoparticles
CUR-LIPO@STCM	Functionalized curcumin-loaded liposomes with stem cell membrane
DLS	Dynamic light scattering
DOX	Doxorubicin
DOX-PLGA@STCM	Stem cell membrane-coated doxorubicin-loaded poly(lactic-co-glycolic acid) nanoparticles
DOX-SPIO@STCM	Stem cell membrane-coated doxorubicin-loaded superparamagnetic iron oxide nanoparticles
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeation retention
ESC	Embryonic stem cell
Gly-PLGA@STCM-CXCR4	Stem cell membrane-coated glyburide-loaded poly(lactic-co-glycolic acid) nanoparticles overexpressing CXCR4
GNG@STCM	Stem cell membrane-coated gelatin nanogels
HIF-1	Hypoxia-inducible factor 1
HSC	Hematopoietic stem cell
ICAM-1	Intercellular adhesion molecule-1
ICAM-2	Intercellular adhesion molecule-2
IFN-ɣ	Interferon-gamma
IFN-ɣR	Interferon-gamma receptor
IL-6	Interleukin-6

IL-6R	Interleukin receptor 6
KGN-Fe_3O_4@STCM	Stem cell membrane-coated kartogenin-loaded iron oxide nanoparticles
M-LP-VTP-PFC/O_2	Stem cell membrane functionalized liposome composed by sonosensitizer verteporfin and oxygen-loading perfluorocarbon
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cell
NIR	Near infrared
NP	Nanoparticle
NSC	Neural stem cell
OSCC	Oral squamous cell carcinoma
PDA-Au-Ag@STCM	Stem cell membrane-coated polydopamine-coated Au-Ag nanoparticles
PDA-Fe_3O_4@STCM	Stem cell membrane-coated polydopamine-coated iron oxide nanoparticles
PDCD4	Programmed cell death 4
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
pDNA	Plasmid complementary DNA
PEG	Poly(ethylene glycol)
PEX	Hemopexin-like domain
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
Plk1	Polo-like kinase 1
PTEN	Phosphate and tension homology deleted on chromosome ten
PTX-PLGA@STCM	Stem cell membrane-coated paclitaxel-loaded poly(lactic-co-glycolic acid) nanoparticles

RA	Retinoic acid
RARE	Retinoic acid-response element
ROS	Reactive oxygen species
SDF1	Stromal cell derived factor 1
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
siRNA	Small interfering RNA
SPIO	Superparamagnetic iron oxide
SPIO@STCM	Stem cell membrane-coated superparamagnetic iron oxide nanoparticles
sTRAIL	Soluble form of tumor necrosis factor-related apoptosis-inducing ligand
TEM	Transmission electron microscopy
TNFR	Tumor necrosis factor receptor
TNF-α	Tumor necrosis factor α
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

1. Introduction

The therapeutic activity of drugs is determined by their concentration in the target tissues. Therefore, the lack of drug targeting and their short half-life not only compromises therapeutic success but can also induce toxicity in non-targeted tissues [1]. To overcome these disadvantages, researchers have turned to nanotechnology for the development of new drug delivery systems, using nanoparticles (NPs) that can have different sizes within the nanometric range and also different morphologies [2]. Some of the most commonly used NPs in biomedical applications are polymeric NPs, iron oxide NPs, liposomes, silica NPs and gold NPs [3]. The use of NPs as drug delivery systems has several advantages, including the protection of the cargo from biodegradation or inactivation, increasing their half-life, and the capacity of controlled drug release [4, 5]. The loading capacity varies according to the type of NPs, as an example, inorganic NPs, namely silica mesoporous NPs, have a very high loading capacity [6]. However, NPs can be captured by the mononuclear phagocytic system according to the size of the NP, leading to a short half-life and low accumulation of the NPs at the target site [7]. The surface functionalization of NPs with hydrophilic polymers has been explored to improve circulation profile of NPs. For example, addition of synthetic hydrophilic polymer poly(ethylene glycol) (PEG) onto the surface of NPs improves their immune escape properties; however, some studies have shown that successive administration of PEGylated NPs could generate an immune response, leading to a more accelerated clearance [8-10].

Considering the particular case of tumor drug delivery, two common strategies can be applied for targeted NP delivery to diseased tissues. Passive targeting occurs through the enhanced permeation retention (EPR) effect, whereby increasing tumor size leads to the formation of new blood vessels, which have a much higher permeability compared to the endothelium of normal tissues. Additionally, tumor tissues do not have an adequate lymphatic drainage, causing NPs to be retained inside [11, 12]. However, the EPR effect is only present in tumors that are well vascularized, whereas in most tumors there are areas under hypoxic conditions, leading to lower expression of the EPR effect [13]. On the other hand, the active targeting promotes the uptake of NPs by functionalizing the surface of the NPs with targeting ligands directed to membrane proteins that are overexpressed in the target cells. This strategy enables both targeting to tumor cells and intracellular delivery of drugs, which minimizes toxicity in normal cells and has been described to improve therapeutic efficacy [14]. Even then, NPs can exhibit heterogeneous intratumoral distribution because of their entrapment in the dense extracellular matrix of the tumor microenvironment. Furthermore, NPs may not have the ability to target infiltrative areas in an effective way [15]. Thus, new platforms for drug delivery have been explored, namely biomimetic NPs that have the ability to combine the

advantages of biological materials and synthetic nanomaterials [16]. Biological materials widely used in the constitution of biomimetic NPs include, among others, proteins, cells, exosomes and cell membranes [17].

Cell membrane-coated NPs consist of a synthetic NP core camouflaged by a natural cell membrane [18]. While surface modification of an NP is complicated to obtain and fabricate [8], this approach makes it possible to obtain a coating that retains all the compounds that make up the source cell membrane. The presence of the large number of surface recognition molecules is essential for the homotypic targeting of the nanosystem, which relies on the specific interaction with cells presenting the same membrane surface composition, and enabling cellular recognition [19]. When compared to conventional NPs, cell membrane-coated NPs exhibit improved immune system escape, prolonged circulation time as well as an improved specific targeting capability [20]. Among the cells investigated as a source of cell membrane are red blood cells [21], platelets [22], leukocytes [23], macrophages [24], tumor cells [25], stem cells [26] and bacteria [27].

Stem cells are a peculiar cell type characterized by their ability to self-renew and differentiate into specialized cell types [28]. Furthermore, stem cells demonstrated an easy proliferation *in vitro* and a very important ability of targeting not only to the tumor but also inflammatory sites [1]. This is the basis for their use as targeted delivery systems for biomedical applications, particularly in inflammatory diseases and cancer. While stem cells *per se* have already been used as a vehicle for various therapeutic and diagnostic systems, including a vehicle for drugs [29], nucleic acids [30], NPs [31] and drug-loaded NPs [32], without losing their targeting properties [33, 34], some concerns have emerged, including the influence of the transported drugs on the "stemness" properties of stem cells. Stem cell membrane-coated nanosystems retain all the surface compounds present on the source stem cell membrane, thus maintaining its targeting ability, and the ability to protect the cargo from being captured by the immune system. These benefits allow not only to increase the therapeutic efficacy of the drugs, but also to improve their safety profile [35, 36].

This review covers the development and variety of biomedical applications of stem cell membrane-coated NPs as next-generation biomimetic drug delivery nanosystems. The most common production methods for producing stem cell membrane-coated NPs will be highlighted, as well as the recent advances in their application in various biomedical areas, such as cancer and inflammatory diseases, and the future prospects of this technology.

2. An introduction to stem cells

The life of an organism begins in a cell, the fertilized egg, which constitutes the primordial stem cell. From a single cell, through multiplication and differentiation, all the cells that make up an organism are formed, presenting this stem cell a potential of totipotency. As multiplication occurs, the resulting cells present a smaller potential of totipotency, acquiring differentiated functions. This process is called determination, which means that the cells formed have a reduced ability to form different types of cells and increasingly acquire specialized functions [37].

Stem cells are cells that have unique capacities that distinguish them from other existing cells, namely the multiplication and differentiation properties which enclose the ability to self-renew and to originate specialized cells, respectively [28]. Two major types of stem cells can be considered: embryonic stem cells (ESCs) and adult stem cells (ASCs), the latter also called non-embryonic stem cells [38].

Although ESCs display an enormous degree of plasticity, which allows them to originate practically any type of cell, thus showing great potential at the therapeutic level, their use raises many ethical issues particularly relating to the use of human embryos [39]. In this sense, ASCs have shown a growing interest, since they can overcome some of these ethical problems. ASCs have the very important function of tissue self-renewal and the ability to differentiate into some specialized cell types, awakening the interest of its use to repair tissue injury and its usefulness for treatment of cancer [40, 41].

Most ASCs are multipotent and can be found in various adult tissues and organs, namely in the bone marrow, where a large amount of stem cells can be found. There are also ASCs in fetal structures, namely in umbilical cord blood and placenta. Among the most investigated ASCs are neural stem cells (NSCs), mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and adipocyte-derived stem cells (ADSCs) [42] (Figure 1).

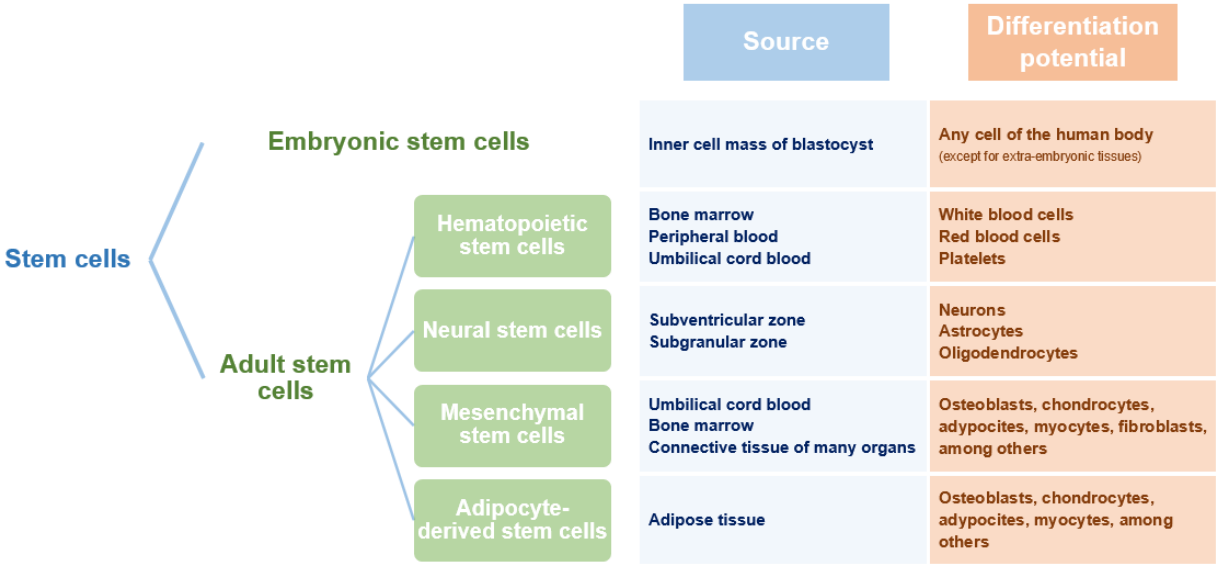


Figure 1. Summary of the source and the distinct differentiation potentials of the two major types of stem cells - embryonic stem cells [37] and the main adult stem cells, namely hematopoietic stem cells [28], neural stem cells [42, 43], mesenchymal stem cells [38, 44] and adipocyte-derived stem cells [45].

In the process of differentiation of an ASC into a specialized cell an intermediate cell - called a progenitor cell - can be identified. While an ASC always gives rise to at least one more stem cell when it divides, a progenitor cell can only form two specialized cells after division [38].

2.1. Surface repertoire and physiology

Physiologically, ASCs are present in various tissues in the form of small cellular niches, which play a determining role in stem cell homeostasis [46]. The fact that ASCs are organized in small populations makes their isolation difficult, since they can exist in quite small proportions compared to the amount of differentiated cells around them. For example, in one hundred thousand differentiated cells there may be one stem cell, in the case of HSCs [28].

Morphologically, ASCs are very similar to other cells in the tissues where they are present, which makes microscopic isolation impossible. In order to overcome this problem, cytological markers are used to identify and isolate stem cells. The fact that each type of stem cells has a specific set of markers allows only the identification of the target stem cells. Most often cell surface proteins that are characteristic for a specific type of ASCs are used as markers. In this way, it is possible to use antibodies that specifically bind to the surface proteins of a specific type of ASCs, allowing their identification [38].

The division of ASCs can occur in two distinct forms, i.e., by symmetric cell division or by asymmetric cell division (Figure 2). Regarding symmetric cell division, one stem cell originates two daughter cells identical to the mother cell, maintaining its stem properties [47]. This type of division is responsible for the capacity of stem cells to self-renewal and maintain their cellular repository [38]. When ASCs receive the signal for cell differentiation from the stem cell niche, symmetric cell division gives way to asymmetric cell division. In asymmetrical cell division, stem cells originate a stem cell, which remains within the niche, and a differentiating cell, which is usually placed outside the niche [48].

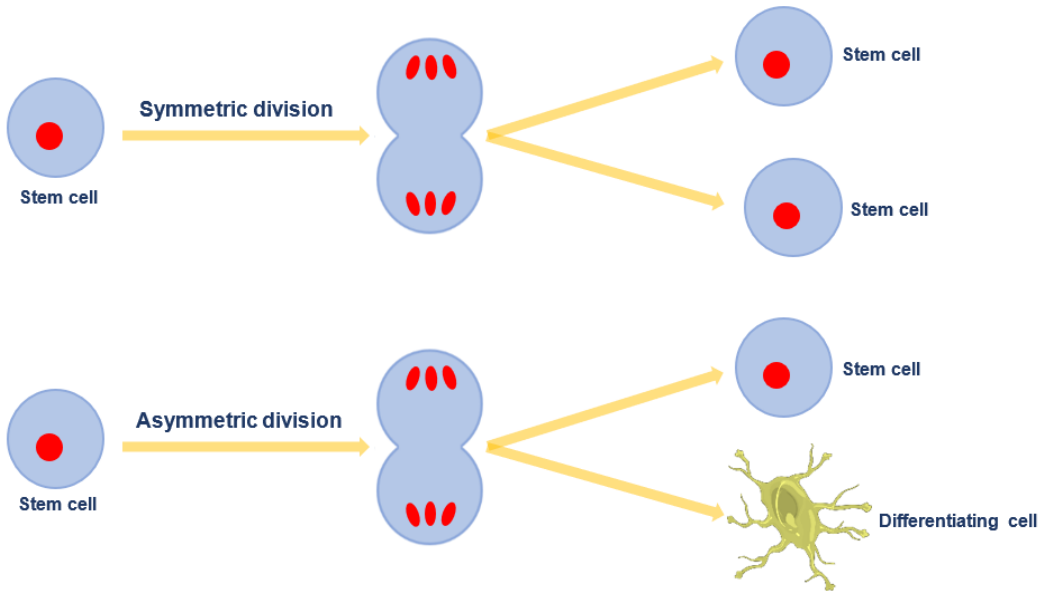


Figure 2. Schematic representation of symmetric stem cell division and asymmetric stem cell division. In symmetrical division, a stem cell divides to form two daughter cells identical to the mother cell. Through asymmetric division, stem cells originate a daughter cell similar to the mother cell and another daughter cell more specialized.

Stem cell surface markers that allow for their identification and isolation have been investigated, however no marker is known that is only expressed on these types of ASCs. The CD44 marker is a surface molecule that is present in several ASCs, particularly in HSCs, MSCs and ADSCs [49]. For HSCs, the first surface marker discovered and most relevant was CD34. Regarding NSCs, one known marker is nestin, an intermediate filament protein [50]. In the case of MSCs and according to the definition that the International Society for Cell & Gene Therapy proposed, MSCs express the surface markers CD73, CD90 also known as thymocyte differentiation antigen-1 and CD105, a transmembrane protein also known as endoglin, not expressing the endothelial and hematopoietic surface markers CD34, CD19, CD14, CD45 and HLA-DR, a major histocompatibility complex II receptor [51]. In addition to these, MSCs can express CD13 (alanine aminopeptidase) and CD29, also referred to as integrin beta-1 [52].

Another relevant biomarker also present in MSCs is CD47 that allows protecting these cells from elimination by the immune system, as its presence on the surface of MSCs is like a signal to macrophages and other immune cells that the cell is not to be eliminated [53, 54]. In the membrane of MSCs there are several chemokine receptors, such as CXCR4, CXCR5, CXCR6, and CX3CR1, which are critical for the migration of MSCs to tumor and inflammatory tissues [55], and multiple cytokine receptors, including interleukin receptor 6 (IL-6R), interferon-gamma receptor (IFN-γR), and tumor necrosis factor receptor (TNFR), which allow the targeting of MSCs to damaged tissues [56]. Several adhesion molecules present a role also relevant for MSCs homing and migration, such as intercellular adhesion molecule-1 (ICAM-1), intercellular adhesion molecule-2 (ICAM-2), activated leukocyte cell adhesion molecule (ALCAM), vascular cell adhesion molecule-1 (VCAM-1) as well as integrins α1, α2, α4, β1 and β3 among others [57]. The surface of MSCs also presents multiple growth factor receptors that are important for the differentiation and self-renewal properties of these cells, such as platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and basic fibroblast growth factor receptor (bFGFR) [58] (Figure 3).

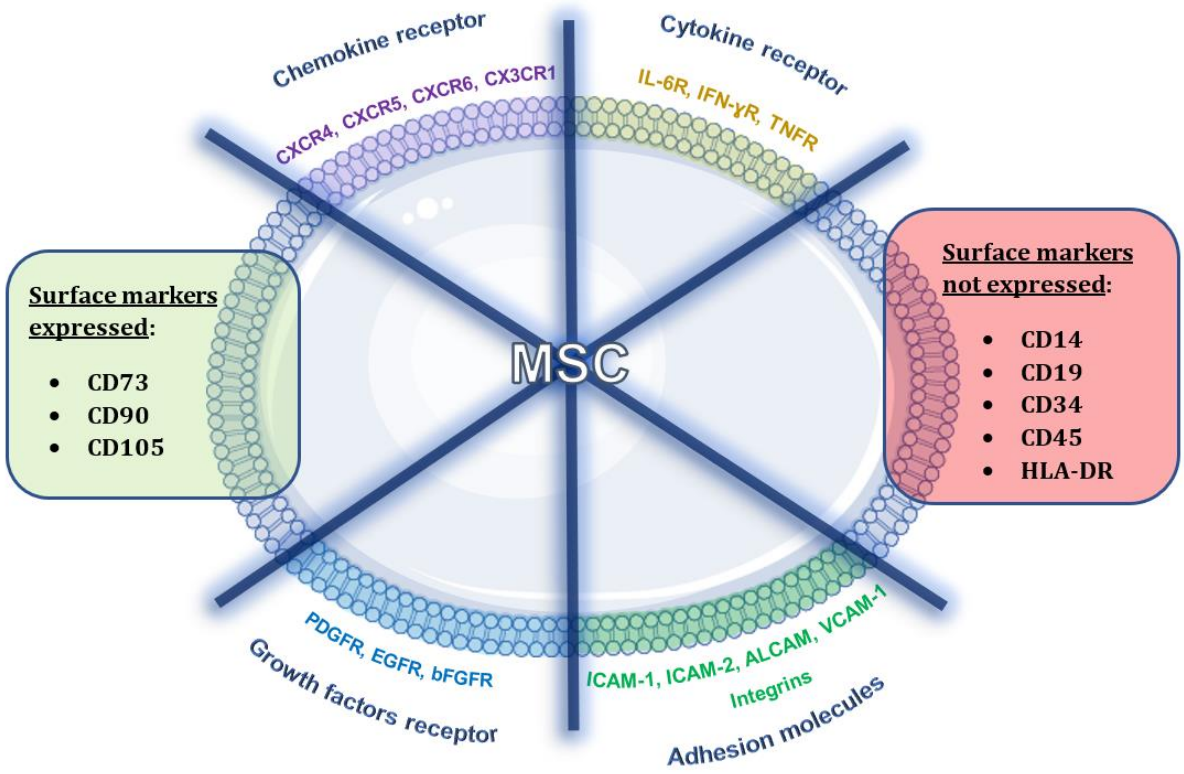


Figure 3. Schematic representation of the surface markers that are expressed on the MSC membrane and those that are not expressed, as defined by the International Society for Cell & Gene Therapy. Illustration of some examples of the multiple receptors present on the surface of MSCs, namely some chemokine receptors, cytokine receptors, growth factor receptors and adhesion molecules.

2.2. Cell-cell communication in physiological and pathophysiological processes

Stem cell behavior is controlled and influenced by the local microenvironment through direct interactions with the other cells that make up the stem cell niche or through indirect interactions using molecules that are secreted by these cells [42].

Several signaling pathways are known to regulate endogenous stem cell activity namely Wnt, Notch and retinoic acid (RA) signaling pathways. Wnt signaling controls both differentiation and renewal of stem cells through short-range intercellular signals. When Wnt signaling is not present, phosphorylated β-catenin existing in the destruction complex leads this complex to the ubiquitination and subsequent degradation in the proteasome [42]. On the other hand, in the presence of Wnt proteins, originating from the stem cell niche, their binding to receptors present on stem cells inhibits the destruction complex, preventing β-catenin degradation. Thus, β-catenin accumulates at the nuclear level, leading to up-regulation of target genes, which causes cell proliferation, and in some tissues induces asymmetric cell division [59, 60].

In contrast to Wnt signaling, Notch signaling needs cell-cell contact to be present for the signaling pathway to be activated. Thus, the binding between the ligand and the receptor, both transmembrane proteins, leads to proteolytic cleavage of the Notch intracellular domain, which migrates to the nucleus, regulating gene transcription [42]. This results in the homeostasis of the stem cell niche, as observed in Wnt signaling, controlling stem cell differentiation and self-renewal [61].

The RA signaling pathway also plays an important role in stem cell regulation. The RA signaling factor by interacting with receptors on the nuclear membrane leads to the binding of these receptors with the DNA region designated retinoic acid-response element (RARE) and, consequently, to the transcription of genes that are involved in differentiation, proliferation and apoptosis [62, 63].

The microenvironment surrounding stem cells, through the supply of molecular factors, is responsible for regulating self-renewal, differentiation and, ultimately, the number of stem cells. In this way, the stem cell niche can also cause dysregulation of ASCs function. Uncontrolled self-renewal of stem cells would lead to an abnormal increase in the number of cells and a cancer stem cells could arise [60].

In the specific case of cancer, the mechanism responsible for the tumor-tropic properties of stem cells is quite complex, based not only on the interaction between the chemokines produced by tumor cells and tumor stromal cells with the receptors present on the surface of the stem cells, as well as adhesion to endothelial cells [64]. One of the possible explanations for the tumor tropism capacity of stem cells is the SDF1/CXCR4 axis (Figure 4), since tumor cells express stromal cell derived factor 1 (SDF1), and its receptor, CXCR4, is present on the surface of stem cells. Thus, stem cells migrate towards the tumor according to the increase in SDF1 concentration, that is, according to its concentration gradient [42, 65].

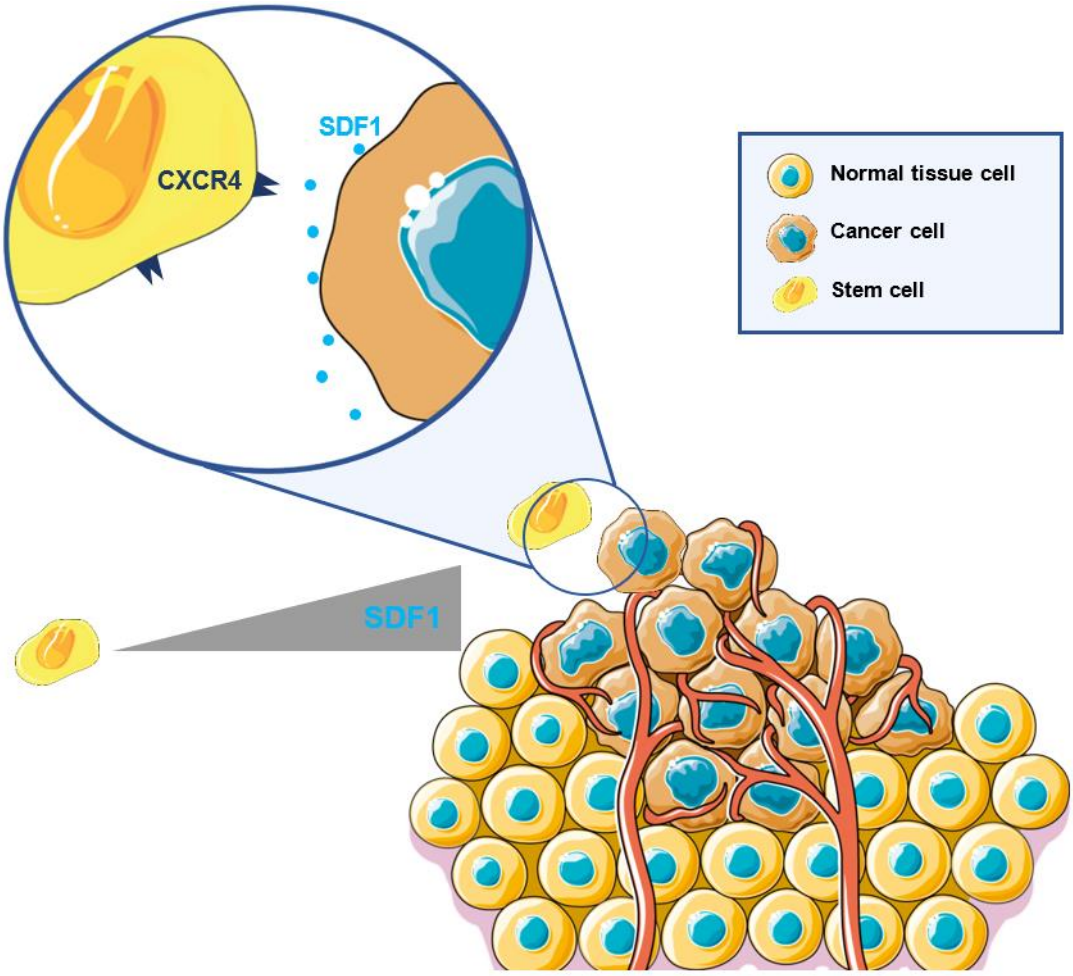


Figure 4. Schematic illustration of stem cells targeting to the tumor *via* the SDF1/CXCR4 axis. Stem cells express the receptor CXCR4 on their surface and, its ligand, the stromal cell derived factor 1 (SDF1), is secreted by tumor cells, leading to the migration of stem cells to the tumor, according to the SDF1 concentration gradient.

In addition to the chemokine SDF1, there are other inflammatory signals secreted by non-cancer cells that constitute the tumor and inflammatory microenvironment, such as by macrophages, neutrophils, and myeloid-derived suppressor cells. MSCs also travel toward the tumor in response to these signals, particularly in response to inflammatory cytokines such as

interferon-gamma (IFN-ɣ), tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6); and growth factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [13, 66], due to the presence of the receptors of these cytokines and growth factors on the MSCs membrane.

In order for circulating MSCs to target the tumor tissue in response to these inflammatory cytokines, first the MSCs have to adhere to the vascular endothelium and subsequently pass the endothelial layer of the tumor vessels. In tumor tissues there is a high concentration of TNF-α and it was verified that the expression of VCAM-1 in MSCs is induced by TNF-α through the NF-kB signaling pathway. Therefore, the concentration of TNF-α in tumor tissues is also responsible for the accumulation of MSCs [67].

Similarly, in the tumor microenvironment there are high concentrations of IL-6 that contribute to the agglomeration of MSCs in the tumor, as MSCs express IL-6R. Parallelly, the presence of large amounts of growth factors in the tumor tissue also leads to the accumulation of MSCs due to the expression of the receptors for these growth factors on the surface of the MSCs. Additionally, hypoxia-inducible factor 1 (HIF-1) is relevant in the targeting of MSCs, as normally tumor environments present hypoxic conditions. In breast cancer, low oxygen partial pressures were found to induce the expression of the chemokine receptor, CXCR3, which led to the recruitment of MSCs to these tumor sites, as MSCs express high concentrations of its ligand, CXCL10 [68].

Thus, it is verified that stem cell targeting to tumors and inflammatory sites results from the various surface receptors expressed on their membranes.

2.3. Stem cells as nanosystems for delivery of diagnostic and therapeutic compounds

The particularity of stem cells for exhibiting specific tropism towards tumor and inflammatory tissues has led to the development of diagnostic and therapeutic systems based on stem cells as living cell-based delivery systems. Stem cells have been used as delivery systems of, among others, paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) NPs [13, 69], doxorubicin (DOX)-loaded PLGA NPs [32], DNA-complexed polymers [30], paclitaxel-loaded superparamagnetic iron oxide (SPIO) NPs [70], chlorine e6-loaded manganese dioxide NPs [33], capsules containing gold nanorods, silica and vincristine [34] and chlorine e6-conjugated polydopamine NPs [71].

Some examples of studies using stem cells as delivery systems for cancer treatment include glioblastoma, leukemia, lung cancer and to a much lesser extent for diagnostics. Drug-loaded

NPs can be transported by stem cells using mainly two strategies, according to the studies presented below, the loading of stem cells with drug-loaded NPs and the attaching of drug-loaded NPs to the surface of stem cells (Figure 5).

Glioblastoma is the most common primary malignant brain tumor of the central nervous system. This tumor is quite lethal, as it leads to aggressive invasion of brain tissue and diffuse infiltration of brain tumor cells [5, 72]. NSCs and MSCs exhibit a particular tropism for glioblastoma and ability to be loaded with NPs, making them excellent candidates for therapeutic cellular vectors [72]. MSCs loaded with poly(lactic acid) (PLA) NPs and lipid nanocapsules have been investigated as a therapeutic strategy for glioblastoma. According to the study, it was found that MSCs loaded with NPs exhibited the same migration properties around the tumor as unloaded MSCs and that both PLA-NPs and lipid nanocapsules remained retained inside the stem cells for at least 7 days [5].

Now addressing the application of stem cells as delivery systems in the treatment of leukemia, a cancer of the stem cells that give rise to the myeloid lineage. One of the therapeutic solutions used in the treatment of leukemia is the use of a compound with the ability to induce differentiation of leukemic cells. For this purpose, RA has been successfully used, however is known a serious complication associated with its use, differentiation syndrome. In order to develop safer RA formulations, the transport of RA-containing NPs by leukemic cells has been tested in which by light action the polymeric NPs disassemble and release RA. Leukemic cells exhibit the ability to migrate to the leukemic stem cell niche and upon activation by blue laser there is a release of RA that promotes stem cell differentiation [73].

For the treatment of lung cancer, a nanosystem was developed with the goal of attaching NPs to the surface of stem cells instead of internalizing them into the cell, in order to try to decrease the toxicity of the load to the carrier cells. Thus, a biotinylated polymeric NP was developed to carry a drug (curcumin) and biotin was also inserted into the MSC membrane. Consequently, it was possible to promote the binding of NP to the surface of a MSC by means of avidin, which presents the biotin binding sites present in these structures. The biotin-avidin bond has high affinity, which allows the NPs to be delivered to the tumor site, not occurring their release during the time that MSCs need to reach the tumor tissue. The study demonstrated the high antitumor efficacy of this delivery system in pulmonary melanoma metastasis [74].

The use of stem cells as living cell-based delivery systems not only has therapeutic but also diagnostic applicability, but to a much more limited extent. Of the few reported approaches to using these nanosystems for diagnosis, magnetic NPs internalized in MSCs have been developed in order to perform cancer diagnosis by detecting them by magnetic resonance imaging (MRI) [31]. Experiments have been performed with MSCs loaded with magnetic NPs verifying specific displacement of these nanosystems to the tumor, in particular verified in orthotopic prostate tumor model [75]. However, there are still few studies for its use in diagnostics.

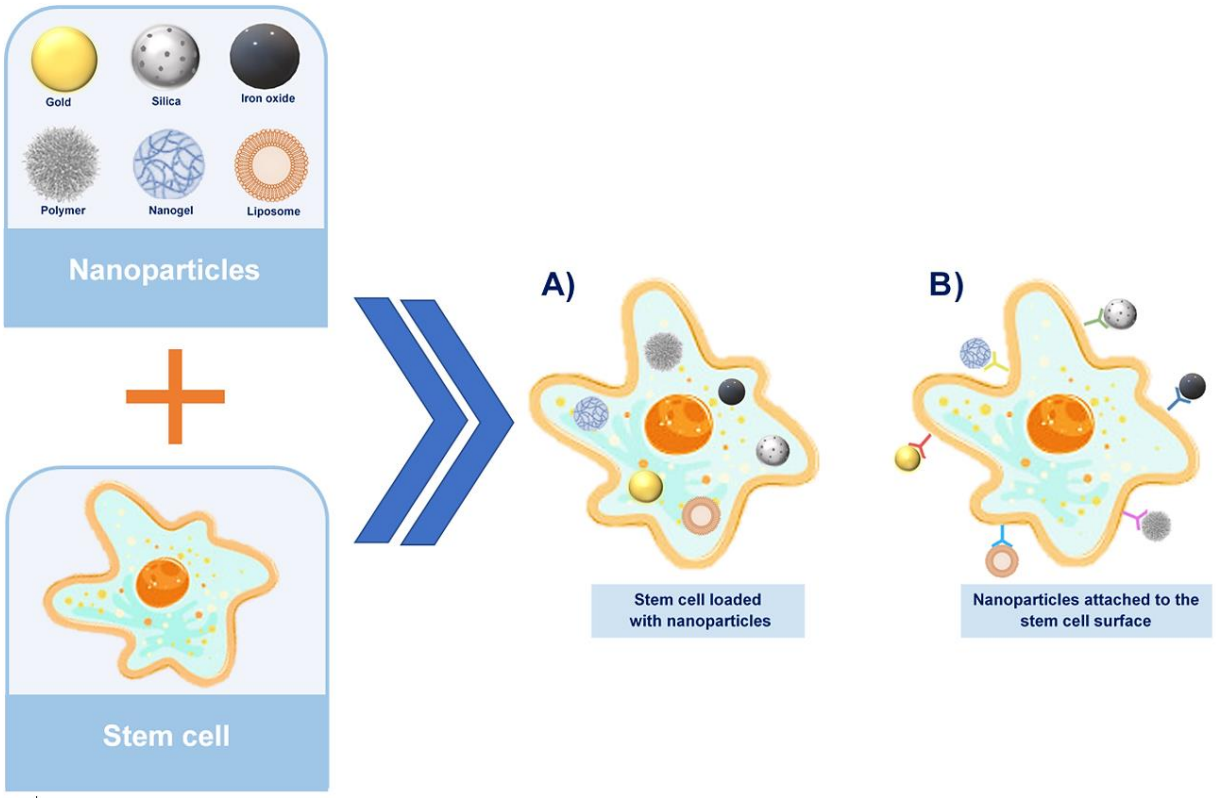


Figure 5. Schematic representation of stem cells as living cell-based delivery systems for therapeutics and diagnostics. Drug-loaded nanoparticles can be transported by stem cells using two strategies. One of the strategies used is loading stem cells with drug-loaded nanoparticles (A) and the other way is attaching the drug-loaded nanoparticles to the stem cells surface (B).

3. Stem cell membrane coatings

The evolution in the development of targeted drug delivery systems has allowed the transition from the use of drug-loaded NPs to carrier cells of these NPs, improving the retention and targeting ability of the therapeutic system. However, some concerns have arisen, namely the influence of the transported cargo on "stemness" properties of stem cells, as well as the difficulty in controlling drug release from the carrier cell. In order to obtain targeted drug delivery systems with higher efficiency, scientists explored biomimetic coatings for NPs based on cell membranes [8]. Accordingly, a core consisting of a synthetic NP is coated with a

cell membrane, giving it several properties specific of the source cells, including specific targeting and prolonged circulation. As these NPs have a natural "cover" that preserves all the ligands and receptors of the original cell membranes, they are able to mimic a great deal of their essential functionalities for an effective biointerface [7].

The interaction of cells with the environment that surrounds them is indispensable for their survival and proliferation, being supported mainly by the cell membrane. The cell membrane consists of lipids, which are responsible for its structure and fluidity, proteins, important in signaling and adhesion, and carbohydrates that provide the interface functions of the membrane. Surface protein markers still make it possible to determine very important properties for each cell type according to their expression profile such as their location, the influence they exert on surrounding cells, and how the cells respond to signals from the environment [76]. On the other hand, these proteins allow interaction with cells presenting the same membrane, designated homotypic interaction, and interaction with distinct cells, known as heterotypic interaction. Therefore, by using cell membranes to coat foreign particles to the body, it is possible to avoid elimination by the immune system and have highly efficient targeting systems [3].

The first cell membranes used as coating material for NPs were the membranes of red blood cells in 2011 [21]. Since then, many other cells have been used as sources of cell membranes, including platelets, leukocytes, stem cells, cancer cells, and even bacterial membranes. Also the NPs present in the core can be of different origins and have different applications, including drug delivery, imaging therapy, photothermal therapy, detoxification or immune modulation [8].

Stem cell membrane-coated NPs constitute a potential effective and biocompatible system for targeted drug delivery. The use of only the cell membrane, as the nucleus and remaining cytoplasmic contents are removed, prevents potential risks associated with using live stem cells, such as promoting tumor progression, representing a safer structure for drug delivery [77-80]. One of the relevant properties of this nanosystem is the ability to target not only the tumor tissue but also the inflammatory tissue, allowing a remarkable accumulation of the drug at the target site [1]. Surface receptors expressed on the stem cell membrane are crucial for nanosystem migration, such as the CXCR4 receptor, enabling their recruitment to the target tissue [81]. Another relevant advantage of stem cell membrane coating is the ability to evade detection by the immune system, increasing blood circulation half-life [82]. Furthermore, a nanosystem that allows not only stable carrier of drugs, but also of genetic material, is formed and able to preserve the high loading efficiency conferred by the NP. [26, 83] (Figure 6). In this

section, the methods used for the production of stem cell membrane-coated NPs and the subsequent characterization of the obtained nanosystem will be presented.

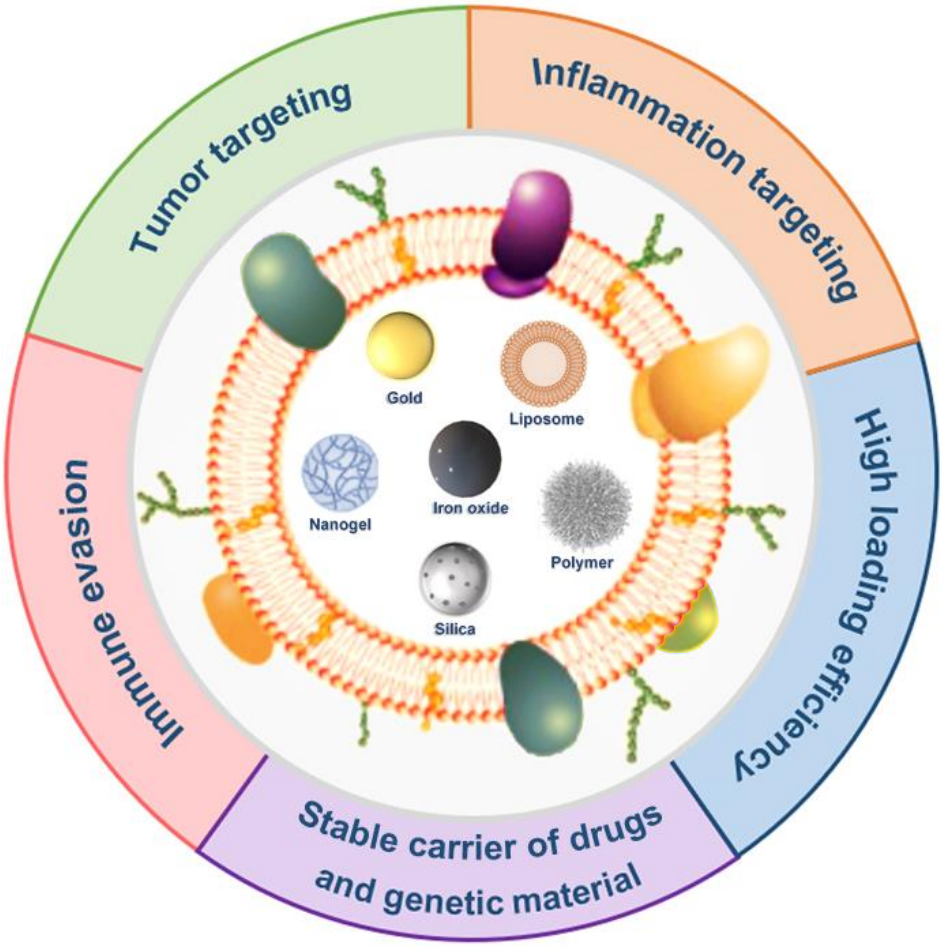


Figure 6. Potentialities of stem cell membrane-coated nanoparticles as delivery systems of diagnostic and therapeutic compounds. The coating of nanoparticles with the stem cell membrane provides the nanosystem with the ability to target both tumor and inflammatory tissues, evade detection by the immune system, form a stable drug and genetic material carrier, and preserve the high loading efficiency of nanoparticles.

3.1. Synthesis methods

The synthesis of stem cell membrane-coated NPs is mainly divided into two steps, starting with the isolation of cell membrane-derived nanovesicles followed by their fusion with NP cores.

3.1.1. Isolation of stem cell membrane-derived nanovesicles

Stem cells are more complex cells compared to red blood cells or platelets, as the latter cells are devoid of a nucleus, which makes the process of isolating the cell membrane-derived nanovesicles of stem cells also more complex.

Initially, isolated and purified stem cells need to be obtained. ASCs present greater advantage of not having the limitations of use when compared to ESCs and induced pluripotent stem cells, which can be associated with ethical issues and also the possibility of teratoma formation. In particular, MSCs can be easily isolated. Although initially isolated from bone marrow, currently MSCs can also be obtained from umbilical cord and adipose tissue, representing less invasive and higher yielding processes [32, 53].

After the purification of the stem cells a complex sequence of procedures follows, namely hypotonic lysis using a hypotonic buffer, followed by ultrasonication, disintegrating the membrane, and to completely remove the intracellular contents a discontinuous sucrose density centrifugation is performed. This is the most convenient and the most used procedure [3]. However, it is possible to apply other methods instead of hypotonic lysis. Another possible procedure is successive freeze-thaw cycles, which lead to the formation of ice crystals, leading to membrane rupture [84]. The electroporation technique can also be applied, allowing the formation of pores in the membrane as a consequence of its exposure to electric fields, leading to its semipermeability [85]. Physical homogenization is also another method that allows the cell membrane to be obtained [3]. After the cell membranes are obtained, they are extruded by passing through a porous polycarbonate membrane, forming the membrane nanovesicles of the desired size (Figure 7).

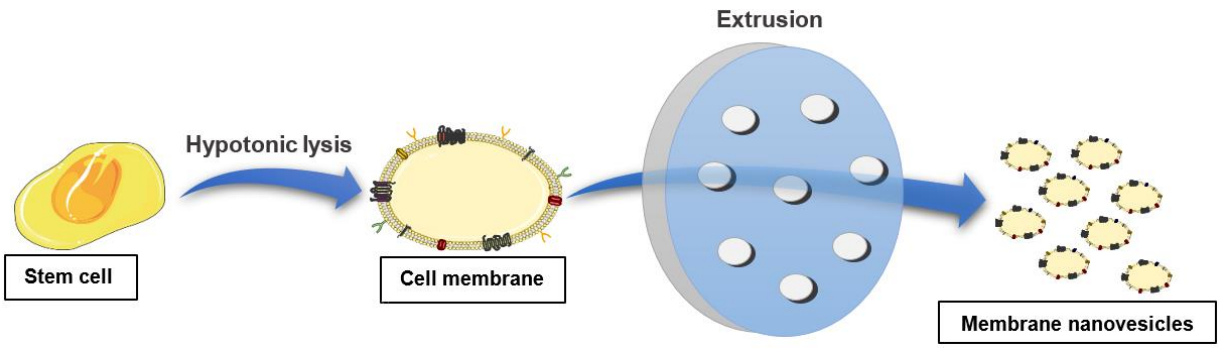


Figure 7. Representation of the hypotonic lysis method. Obtaining the stem cell membrane-derived nanovesicles from the source cells using a hypotonic buffer and then downsizing through a porous polycarbonate membrane.

3.1.2. Coating of nanoparticle cores by membrane nanovesicles

Different methods can be applied to coat the NP cores by the obtained membrane nanovesicles. In this process, it is essential that the natural membrane disposition is successfully reproduced in order to expose the surface proteins to the outside. For this, it is necessary that this conformation is the most energetically favored. It is also important that during this process the fluidity of the lipid membrane is maintained [3].

The first and easiest membrane coating method used is physical co-extrusion, which was based on the conventional method of nanoliposome formation [86]. Here, membrane nanovesicles and NPs are co-extruded by-passing multiples times through a porous polycarbonate membrane and then sonicated, allowing the formation of stable cell membrane-coated NPs. The mechanical force applied to pass through the porous membrane allows the rupture of the membrane structure, which is recovered involving the NPs [53] (Figure 8).

The coating of NP cores can also occur through sonication [87]. This more recent method consists in the use of ultrasonication energy by applying a specific frequency, amplitude and sonication time to obtain an effective coating. The mixture of membrane nanovesicles and NP cores easily form a core-shell structure by the action of this disruptive force [3]. Sonication process is suitable for NPs obtained by extrusion and also allows less material loss [53].

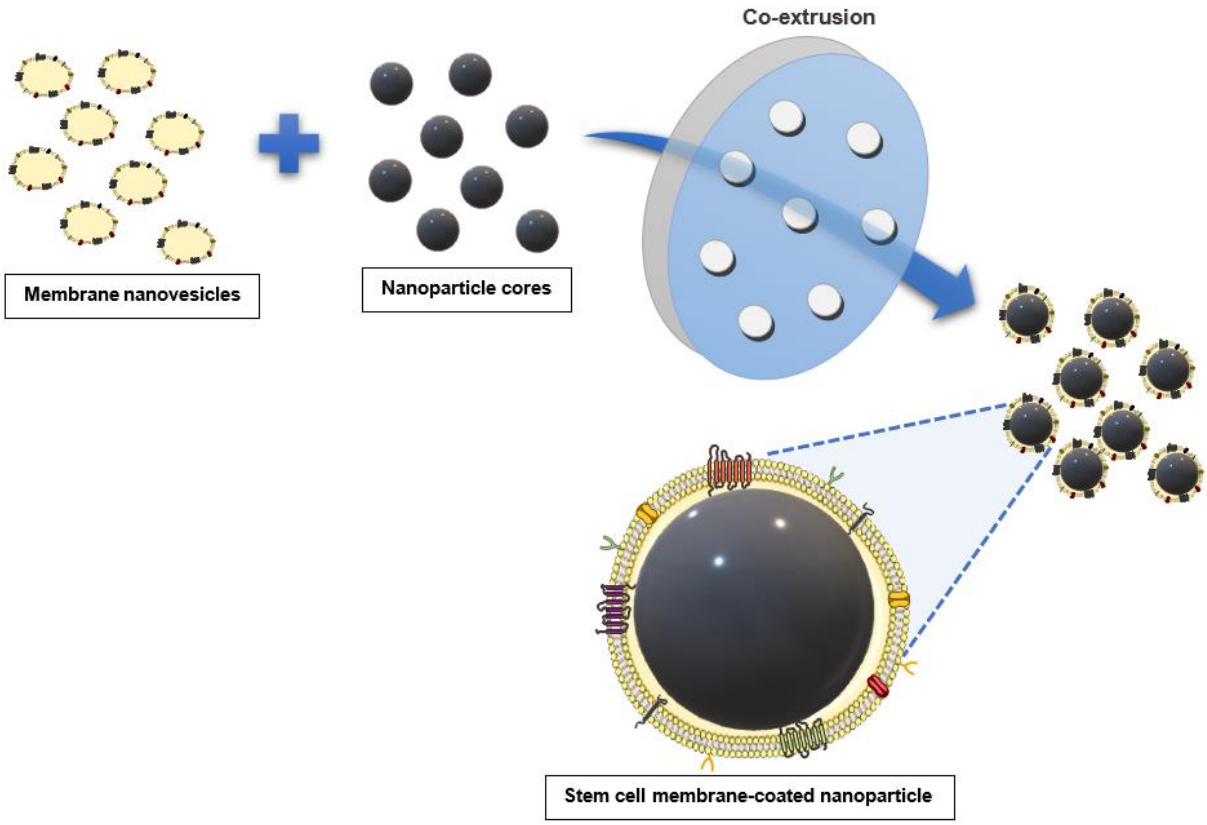


Figure 8. Representation of nanoparticle cores coated by membrane nanovesicles through the co-extrusion method using a porous polycarbonate membrane.

3.2. Characterization of stem cell membrane-coated nanoparticles

Characterization of stem cell membrane-coated NPs obtained from the synthesis process involves several techniques in order to verify the successful coating of the NPs with cell membranes and with the purpose of analyzing their therapeutic capability.

Transmission electron microscopy (TEM) is widely used to evaluate the morphology of NPs, showing that cell membrane-coated NPs exhibit a halo around the NPs, unlike uncoated NPs [3]. Size is a feature that can be determined using dynamic light scattering (DLS) and allows us to confirm the successful coating of the NPs by the cell membrane [26]. In this case, the difference between the diameter of the coated NPs and the diameter of the uncoated NPs should correspond to the thickness of the cell membrane. Also the surface charge is an important indicator, as the zeta potential of the produced cell membrane-coated NPs should be identical to the zeta potential of the membrane nanovesicles, as these are the ones that are coating the surface of the NPs [3]. Thus, the zeta potential of bare PLGA NPs is -50.8 ± 2.1 mV, while the zeta potential of stem cell membrane-coated PLGA NPs is -30.3 ± 2.6 mV, similar to that of membrane nanovesicles [54]. The fact that the zeta potential of the coated NPs is negative ensures the stability of these nanosystems in the bloodstream by preventing non-specific absorption of proteins, as well as favoring their cellular uptake [88]. Similarly, a negative surface charge of the NP cores has been shown to be an advantage for better coating by the cell membrane, as a positive charge leads to strong interactions with the membranes, giving rise to a cross-linked structure [53, 76].

In addition, it is possible to evaluate the protein content of the membrane that coat the NPs allowing to verify if the coating was performed correctly and if the membrane retains the composition of its original membrane, comparing with the membrane of natural stem cells. The technique of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used for this analysis [26].

In order to determine the stability of the coating of the NPs, ultraviolet-visible spectroscopy and confocal laser scanning microscopy can also be used. Other techniques can be applied to characterize the obtained nanosystems, namely flow cytometry, fluorescence microscopy and western blotting [89].

4. Biomedical applications of stem cell membrane-coated nanoparticles

This section will cover the studies that have explored stem cell membrane-coated NPs for cancer applications (Table I in appendix I) and for other different biomedical applications, particularly acne, ischemic stroke, severe hindlimb ischemia, myocardial infarction, articular cartilage damage and, in the additional application of biodistribution assessment of NPs (Table 2 in appendix II).

4.1. Cancer

Cancer is still a highly prevalent disease and one of the leading causes of death in the world. Thus, oncology is one of the areas that have experienced the development of new drugs [14]. The current standard cancer therapy consists mainly of surgery followed by chemotherapy and radiotherapy. Currently, most of the existing chemotherapeutic drugs have reduced efficacy, on account of poor targeted drug delivery and inefficient blood circulation half-life [90]. Furthermore, chemotherapeutic drugs have the ability to act not only on tumor cells, but also on normal cells, which causes serious adverse effects due to its cytotoxicity [14]. One of the major difficulties of drugs is to be able to cross barriers, such as the blood-brain barrier in the case of brain cancer, as well as the enormous difficulty in penetrating solid tumors, due to their dense extracellular matrix [91].

Stem cell membrane-coated NPs have attracted the attention of researchers for being a highly efficient biocompatible drug delivery platform that allows specific targeting of drugs and evade detection by the immune system, prolonging blood circulation half-life [92]. The major advantage of these nanosystems is their ability to retain the large number of surface recognition molecules that exist on stem cells in the membrane nanovesicles formed [76]. Table 1 in appendix I briefly describes the studies that have used stem cell membranes as a coating for cancer applications. Of these studies some have application to cancer in general, while the rest were developed to fight a specific type of cancer.

For cancer treatment, Gao *et al.* developed a DOX delivery platform consisting of gelatin nanogels camouflaged by bone marrow derived MSC membrane (GNG@STCM) obtained by co-extrusion [26] (Appendix III A). The observation through TEM demonstrated the spherical core-shell structure, which reflects the gelatin core coated by a thin layer (Appendix III B). Considering that the thickness of the cell membrane is about 5 to 10 nm, it was confirmed that the gelatin nanogels coating was achieved, as it was found by DLS that the hydrodynamic diameters increased the corresponding to this thickness. Also, the change of zeta potential allowed to confirm the success of the coating of gelatin nanogels, approaching the zeta potential of the stem cell membrane nanovesicles (Appendix III C). Using SDS-PAGE it was found that the protein composition of the membranes was maintained during the synthesis of the nanovesicles (Appendix III D) and the long-term stability of GNG@STCM was confirmed. The researchers were also able to demonstrate that GNG@STCMs exhibited negligible cytotoxicity and did not cause morphological modifications in the blood, which is critical for an *in vivo* drug delivery system. Subsequently, the researchers encapsulated DOX in the gelatin nanogels that formed the core of the nanosystem. In this way, they found that GNG@STCM-DOX had the ability to release the drug depending on pH, since there was a much higher

release of DOX at acidic pH, which normally corresponds to the pH of tumor tissues [93], on account of the fact that tumor cells produce large amounts of lactic acid as a result of hypoxia (Appendix III E). GNG@STCM have been shown to have a high DOX loading capacity and superior uptake by cancer cells relative to free DOX and bare gelatin nanogels-DOX. The intravenous administration of GNG@STCM-DOX in mice grafted with cancer cells showed a great capacity for accumulation at the tumor site. The coating with stem cell membrane conveyed not only improved circulation time but also enhanced the antitumor efficiency of the nanosystem, showed by a significant decrease in tumor volume, when compared to mice treated with free DOX or with bare gelatin nanogels-DOX (Appendix III F, G). Although free DOX is known for its severe cardiotoxicity [94], the fact that the drug is encapsulated in GNG@STCM allowed to avoid this side effect of DOX owing to a targeted delivery, thus exhibiting superior safety [26].

Stem cell membrane coating has also shown applicability in photodynamic therapy for cancer treatment. In this way, stem cell membrane coated upconversion NPs were first developed by Gao and coworkers [95]. This research aimed, on the one hand, to overcome the shallow depth of penetration of visible light and, on the other hand, to increase the circulation time in the body of the upconversion NPs and improve its ability to target the tumor. Upconversion NPs exhibit the ability to absorb near infrared (NIR) light and convert it into higher energy photon emission [96]. Thus, using NIR light, greater deep penetration of light into tissues was achieved in relation to visible light, which is necessary to obtain effective therapy against an internal tumor [97]. In this way, the therapy developed consisted of upconversion NPs loaded with photosensitizers, ZnPC and MC540, which were coated by stem cell membrane by a co-extrusion method. This means that the upconversion NPs converted the NIR light with which they were irradiated into visible light, which acted on the photosensitizers. Excited photosensitizers had the ability to transfer energy to surrounding oxygen, which led to the production of reactive oxygen species (ROS), causing tumor cell death [98]. This production of cytotoxic oxygen compounds by photosensitizers is the fundamental element of photodynamic therapy. The role triggered by stem cell membrane was not only to decrease the toxicity of the therapy but also to increase their antitumor efficacy by enhancing the active targeting to the tumor, which was proven by the higher accumulation in the tumor of the coated NPs. Furthermore, by camouflaging this foreign material to the body, the upconversion NPs, with a natural cell membrane, enabled to prevent its elimination by the reticuloendothelial system. These were the results obtained in studies performed on mice grafted with tumor cells showing superior photodynamic antitumor efficacy of stem cell

membrane-coated upconversion NPs compared to uncoated upconversion NPs, with a tumor growth inhibition efficacy of about 23% [95].

4.1.1. Breast cancer

Breast cancer is one of the most prevalent cancers in women. The most common therapeutic options used for breast cancer include surgery, radiotherapy and chemotherapy, whereby a combination of these therapies is often used. However, these treatment options have several adverse effects and the possibility of recurrence or metastasis [99].

For advanced breast cancer therapy, a widely used chemotherapeutic agent is paclitaxel, although the adverse effects it causes limit its use in the clinic [100, 101]. In an attempt to circumvent these limitations, Tian and coworkers fabricated bone marrow-derived MSC membrane-coated paclitaxel-loaded PLGA NPs (PTX-PLGA@STCM) with the aim of obtaining a more effective and safer therapy for the treatment of breast cancer [101] (Appendix IV A). The morphology analysis of PTX-PLGA@STCM NPs evidenced the core-shell structure (Appendix IV B), the hydrodynamic diameter of the NPs increased, coinciding the difference in size with the thickness of the stem cell membrane nanovesicle and the value of the zeta potential approached that of the membrane nanovesicles (Appendix IV C). All these analyzed features indicated the successful coating of the membrane nanovesicles to the surface of paclitaxel-loaded PLGA NPs. The paclitaxel release behavior was also verified, concluding that the presence of the cell membrane coating PLGA NPs allowed a more controlled paclitaxel release (Appendix IV D). In this way, there was a much lower release during circulation compared to paclitaxel-loaded PLGA NPs, which enabled more efficient on-target drug delivery and lower off-target toxicity, since the presence of stem cell membrane also allowed for increased targeting ability to the tumor. In order to verify the antitumor efficacy *in vivo*, orthotopic breast tumor-bearing mice were subjected to the sample formulation and the controls, verifying different tumor inhibition rates. Free paclitaxel showed the lowest antitumor activity, with tumor inhibition rates of 32.0 ± 6.7%. Then, paclitaxel-loaded PLGA NPs showed superior activity with tumor inhibition rates of 53.6 ± 8.3% due to a passive targeting mechanism and to a paclitaxel controlled release pattern. However, PTX-PLGA@STCM NPs showed the highest antitumor efficacy with tumor inhibition rates of 78.4 ± 10.6%, which may be a consequence of their excellent tumor targeting ability, higher stability and long-time circulation (Appendix IV E-G). The study also indicated the biocompatibility of PTX-PLGA@STCM NPs and its ability to minimize the side effects caused by free paclitaxel. Thus, it was possible to conclude that the use of stem cell membrane as a coating for NPs was an effective option for the treatment of breast cancer [101].

4.1.2. Liver cancer

Despite advances in its therapy, primary liver cancer, especially hepatocellular carcinoma, remains one of the cancers whose cure is most difficult to achieve, so research is ongoing to develop new treatments [102].

The use of human umbilical cord as a source of MSCs allowed to overcome possible injury caused by isolation of MSCs from bone marrow, providing a resource for easy obtaining and expansion of MSCs with maintaining stemness [103]. Accordingly, Yang *et al.* developed umbilical cord-derived MSC membrane-coated DOX-loaded PLGA NPs (DOX-PLGA@STCM) for treatment of liver cancer [54] (Appendix V A). TEM analysis demonstrated that MSCs membrane-coated NPs exhibited a spherical core-shell structure and on the surface a lipidic bilayer. In contrast, bare PLGA NPs showed a uniform and spherical structure (Appendix V C). In addition, they analyzed the hydrodynamic size and surface charge using DLS, which also suggested the success of coating the PLGA NPs with MSC membrane (Appendix V B). Furthermore, they verified that the protein profile of MSCs membrane-coated NPs was identical to that of MSCs membrane nanovesicles by western blot assays and SDS-PAGE, which suggested that during the coating process no protein loss or degradation occurred. For this purpose, the expression level of the chemokine receptor CXCR4 and the transmembrane protein Na^+/K^+ ATPase was analyzed. Then, the researchers constructed DOX-PLGA@STCM NPs that exhibited very similar characteristics compared to MSCs membrane-coated PLGA NPs. Furthermore, this nanosystem demonstrated a significantly higher cellular uptake efficiency than bare PLGA NPs, which suggested that coating with MSCs membrane favored uptake by target cells. Observation by confocal microscopy also revealed that upon uptake by the target cells, DOX-PLGA@STCM NPs were directed to the acidic organelles, which caused effective DOX release and consequently enhanced tumor cell killing (Appendix V E). Moreover, it was verified that the functionalization of NPs with MSCs membrane was crucial to improve the targeting to the tumor due to the presence of receptors, such as PDGF receptor and CXCR4, since the corresponding chemokines are present in tumor tissues [104-106]. In addition to enhanced tumor accumulation, the expression of CD47 on the MSC membrane allowed to avoid elimination by the macrophages, specifically by Kupffer cells [107, 108]. CD47 is a membrane protein, present on all human cells, that binds to the inhibitory receptor signal regulatory protein alpha (SIRPα) that is abundantly found on macrophages and also on some non-phagocytic cells. From this binding occurs the activation of a phosphatase, SHP-1, which inhibits phagocytosis of opsonized cells and particles [107]. Thus, CD47 has a very important function, allowing to distinguish what is proper from what is foreign to the organism [108]. The antitumor efficacy of this nanosystem was tested in liver tumor-bearing mice

demonstrating an extraordinary inhibition of tumor growth and consistent apoptosis status within tumor (Appendix V D). The results obtained demonstrated that umbilical cord-derived MSC membrane-coated PLGA NPs allow a successful tumor-targeted drug delivery [54].

MSCs have a limited drug loading capacity, which leads to certain cytotoxic drugs not being able to reach the therapeutic quantities in the tumor tissue, possibly compromising the antitumor efficacy of the treatment [53]. On the other hand, there are some safety issues associated with the use of MSCs, since it has been found that malignant transformations could occur [109, 110]. Thus, using only the membrane of MSCs allows avoiding these safety issues and, by maintaining the same protein composition as the source MSCs membrane, also retains its ability to target the tumor, penetrate the tumor and reduces clearance by monocytes, mainly due to the presence of the marker CD47. The combination of MSC membrane with a NP core further allows to associate the high loading capacity and controlled drug release capability of the NPs. Accordingly, Li *et al.* constructed MSC membrane-coated DOX-loaded mesoporous silica NPs through sonication [111] (Appendix VI A, B). After coating with MSC membrane it was verified by western blot that the MSC-specific markers CD44, CD90, and CD105 as well as the membrane marker CD47 remained intact (Appendix VI C). The researchers found that higher DOX release occurred in acidic environment, as the ionization of the drug weakened its interaction with mesoporous silica NPs. In addition, the MSC membrane-coated NPs demonstrated an improved ability to penetrate the tumor compared to mesoporous silica NPs. To verify the antitumor activity, MSC membrane-coated DOX-loaded mesoporous silica NPs were administered to mice grafted with HepG2 cells, a hepatocellular carcinoma model, which were shown to cause a greater inhibition of tumor growth compared to free DOX and DOX-loaded mesoporous silica NPs (Appendix VI D). The administration of mesoporous silica NPs has caused the appearance of inflammation in the lungs, however their coating with MSC membrane allows preventing the interaction of the NPs with monocytes, thus preventing the occurrence of inflammation [112]. Therefore, the coating with MSC membrane did not affect the loading capacity of mesoporous silica NPs nor the controlled drug release and still allowed to increase not only the targeting, accumulation and penetration of the nanosystem into the tumor but also its uptake by HepG2 cells, improving the antitumor activity [111] (Appendix VI E, F).

4.1.3. Oral cancer

Oral squamous cell carcinoma (OSCC) represents the most common oral cancer [113]. Conventional treatments such as chemotherapy, radiotherapy and surgery have many limitations, as they exhibit a low effectiveness, various systemic adverse effects and can cause

oral dysfunction [114, 115]. In this regard, Sun and coworkers have developed sonodynamic therapy for the treatment of OSCC, which consisted of MSC membrane functionalized liposome composed by sonosensitizer verteporfin and oxygen-loading perfluorocarbon (M-LP-VTP-PFC/O_2) [36] (Appendix VII A). DLS and TEM analysis of M-LP-VTP-PFC/O_2 demonstrated uniform spherical morphology (Appendix VII B). Other characteristics were analyzed, among which the protein profile through SDS-PAGE (Appendix VII D) and Western blot verifying the high expression of integrin α4β1 in the membrane of MSCs that contributes to the targeting to the tumor, since it is recognized by cancel cell VCAM-1. On the other hand, the optimal stability of the system was also demonstrated, as in the absence of ultrasound, only a minimal amount of the cargo was detected, indicating the lowest risk of adverse effects (Appendix VII C). In order to verify the antitumor effect of M-LP-VTP-PFC/O_2 *in vivo*, they were administered in mice grafted with orthotopic OSCC. The presence of MSC membrane allowed targeting to the tumor, minimizing adverse effects and potentiating the anti-tumor effects. Oxygen-loading perfluorocarbon allowed to relieve the hypoxia characteristic of the tumor microenvironment, allowing the ultrasound-activated verteporfin and in the presence of oxygen to produce a high amount of ROS, leading to apoptosis of the tumor cells [36] (Appendix VII E, F).

4.1.4. Osteosarcoma

Osteosarcoma is a malignant bone tumor that mainly affects children and adolescents, constituting the most frequent bone tumor [116, 117]. For the treatment of osteosarcoma, Zhang and coworkers developed a chemo-photothermal therapy [82]. The researchers constructed MSC membrane-coated polydopamine NPs encapsulating 7-ethyl-10-hydroxycamptothecin (SN38), a hydrophobic anticancer drug. The camouflaging of the NPs with a cellular membrane allowed to decrease their elimination by macrophages and thus increase their lifetime in circulation, in addition to decreasing TNF-α secretion, compared to uncoated NPs, suggesting their higher biocompatibility. The release of SN38 by NPs was shown to be higher in the presence of more acidic pH, characteristic of the tumor microenvironment, and in the presence of laser radiation. *In vivo* studies performed in mice grafted with MG63 osteosarcoma cells showed that coating SN38-loaded NPs with MSC membrane led to a higher accumulation of NPs at the tumor site and higher uptake efficiency of SN38 by tumor cells, which resulted in an effective inhibition of osteosarcoma growth. Thus, by combining the antitumor action of SN38 with photothermal conversion, the researchers constructed a promising nanosystem for the treatment of osteosarcoma [82].

4.1.5. Colon cancer

Colon cancer is the third cause of death from cancer in the worldwide. Chemotherapy is the main therapeutic strategy in its treatment, however the conventional drugs used have the great drawback of toxicity and lack of targeting to the tumor [118]. Thus, in order to develop a targeted drug delivery platform for colon cancer, Liu and coworkers constructed MSC membrane-coated DOX-loaded SPIO NPs (DOX-SPIO@STCM) [35] (Appendix VIII A). TEM analysis demonstrated spherical core-shell structure with an outer membrane (Appendix VIII B). Among other characteristics, the researchers found that DOX-SPIO@STCM NPs exhibited pH-response release, which is important for tumor-targeted action and the non-occurrence of adverse effects (Appendix VIII C). The coating with MSC membrane prevented macrophage uptake and decreased complement activation, resulting in less release of C5a, a protein relevant in the recruitment and activation of immune system cells [119]. The anti-tumor efficacy of DOX-SPIO@STCM NPs was evaluated in mice grafted with MC38 colon cancer cells, in which MSC membrane coating was found to decrease systemic toxicity, increase cellular uptake efficiency, and thus have enhanced antitumor activity [35] (Appendix VIII D, E).

4.1.6. Prostate cancer

Prostate cancer is the second most frequent type of cancer in men [120]. Although it mostly presents as a localized and curable disease, it can evolve in some cases to advanced stages and metastasis [121]. Here, the cure is much more difficult to achieve and new therapeutic strategies are being explored, including gene therapy.

Stem cell membrane-coated NPs are also useful for the delivery of nucleic acids in gene therapy, as they allow for gene stability. Hence, a platform for the delivery of small interfering RNA (siRNA) was developed by Mu and coworkers consisting of MSCs membrane-coated polydopamine-coated iron oxide NPs (PDA-Fe_3O_4@STCM) [122] (Appendix IX A). In this way, they were able to overcome the difficulty of siRNA to enter cells given its polyanionic charge and also instability [123]. Due to the use of iron oxide NPs they were able to obtain two functionalities, the photothermal therapeutic agent and a way to track their distribution through their MRI capabilities. Thus, in a single system they managed to combine gene silencing, photothermal, and MRI capabilities. The use of photothermal therapy as a non-invasive cancer treatment consists in the use of photothermal agents that by accumulating at the tumor site and absorbing near-infrared light have the ability to convert light energy into heat energy to kill the cancer cells [124]. In addition, the researchers used polydopamine to coat the hydrophobic iron oxide NPs, still allowing the binding of various biomolecules, such as RNA, through the various functional groups that polydopamine has on its surface, like amine and

catechol [125]. This nanosystem allowed siRNA delivery against polo-like kinase 1 (Plk1) gene, highly expressed in tumor cells, which led to enhanced cell apoptosis, suppressing tumor growth [126]. Regarding the morphology of PDA-Fe_3O_4@STCM NPs, they observed the core-shell structure in TEM image (Appendix IX B). The hydrodynamic size was determined by DLS and showed the increase in size of the NPs after coating with MSC membrane, which suggested the success of the coating since this increase in size is consistent with the thickness of the lipid bilayer. Similarly, it was found that the surface charge approached the characteristic value of MSC membrane nanovesicles (Appendix IX C) and that PDA-Fe_3O_4@STCM NPs remain structurally intact even after their internalization by cells. Intravenous administration of PDA-Fe_3O_4@STCM NPs in mice grafted with DU145 cells, a human prostate cancer model, allowed to verify that thanks to the presence of MSC membrane there was an increase in the cellular uptake efficiency, a much higher accumulation of the nanosystem in the tumor site, indicating its high targeting ability to the tumor, which also allowed to decrease its toxicity. They also confirmed that the coating did not affect the MRI capability of the NPs and that the photothermal property of the iron oxide NPs is identical to that seen after coating with MSC membrane (Appendix IX D). All these properties associated with gene therapy allowed PDA-Fe_3O_4@STCM NPs to show improved anti-tumor efficacy [122] (Appendix IX E).

Long circulation capability is essential for SPIO NPs to exhibit higher efficacy as diagnostic and therapeutic tools, due to their capabilities for MRI and magnetic hyperthermia properties, respectively [127]. For this, surface modifications are required, namely coating the SPIO NPs with stem cell membrane (SPIO@STCM), a platform developed by Lai *et al.* [128]. The characterization of SPIO@STCM NPs demonstrated the retention of the characteristic protein profile of the stem cell membrane, namely the surface marker CD44, the stability of the NPs, the identical magnetic property of SPIO NPs after coating by the cell membrane and their very important role in reducing the uptake of SPIO@STCM NPs by macrophages. The incubation of SPIO@STCM NPs in mouse prostate cancer cells allowed to demonstrate the enhanced anti-tumor efficacy by increasing the temperature through the action of an external magnetic field and also its good biocompatibility [128].

The potential of using stem cell membrane as a targeted drug delivery platform was investigated in the two studies that will be presented below, for prostate cancer therapy, in which the developed systems did not use an inner NP core.

In cancer therapy, it is critical to have nanosystems that can selectively deliver drugs to cancer cells, mainly because of its toxicity [129]. In the study of Furman *et al.* they constructed MSC-derived membrane nanovesicles, which they called nano-ghosts, that were loaded with the soluble form of tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) [90]

(Appendix X A). Subsequently, these nanosystems were characterized by demonstrating in particular its unilamellar morphology (Appendix X B). The use of stem cell membrane allowed to overcome the hepatoxicity of free sTRAIL and its short half-life. The anti-tumor efficacy of this drug delivery platform was tested in mice grafted with human prostate cancer, in which it was found that sTRAIL-encapsulating membrane nanovesicles led to greater apoptosis of tumor cells (89%) compared to mice treated with free sTRAIL (53%), thus leading to greater inhibition of prostate cancer growth (Appendix X C - E). Furthermore, the researchers detected a much higher accumulation of MSC-derived membrane nanovesicles at the tumor site compared to smooth muscle cell-derived membrane nanovesicles, reflecting the targeting ability that MSC membrane confers to the nanosystem [90].

The discovery that MSC-derived membrane nanovesicles could reach not only the cytoplasm, but also the nucleus of target cancer cells led to the development of a non-viral platform for gene delivery [130]. Hence, Kaneti *et al.* developed MSC-derived membrane nanovesicles loaded with plasmid complementary DNA (pDNA) encoding for hemopexin-like domain (PEX), which corresponds to a fragment of human matrix metalloprotease-2 [130]. Although the negative surface charge of membrane nanovesicles makes them safer vectors, this characteristic makes them difficult to load with also negatively charged pDNA, which led to the use of the electroporation method to load the membrane nanovesicles with pPEX [131]. MSC-derived membrane nanovesicles loaded with pPEX was administered to mice grafted with subcutaneous prostate cancer showing significant inhibition of tumor growth with an increase in tumor apoptosis. This gene delivery platform was also administered in mice grafted with orthotopic metastatic pulmonary non-small cell lung carcinoma, in which its anti-tumor efficacy was also verified. Thus, it was found that MSC-derived membrane nanovesicles loaded with a cancer-toxic gene showed efficient targeting and transfection to tumor cells without leading to adverse effects, allowing a large increase in the survival of tumor-bearing animals [130].

With these examples it is possible to verify that the coating with stem cell membrane proves to be a promising new class of drug delivery with a great applicability in various types of cancers.

4.2. Acne

The discovery of the ability of stem cells to target the sites of inflammation was the key to the development of several studies in which the stem cell membrane was used as a vehicle for targeted drug delivery to the sites of inflammation.

A very common inflammatory disease in adolescents is acne, which occurs at the level of the sebaceous glands present in the hair follicles [132]. In order to try to obtain an improved treatment for acne, Meng *et al.* developed stem cell membrane-coated polydopamine-coated Au-Ag NPs (PDA-Au-Ag@STCM) [133]. To verify the photothermal effect of PDA-Au-Ag@STCM NPs on sebaceous glands, golden hamsters were injected with *Propionibacterium acnes*. Thus, it was found that PDA-Au-Ag@STCM NPs, after irradiation, showed efficacy to inhibit the proliferation of sebaceous gland cells. Although polydopamine improves the stability and the photothermal conversion efficiency, the coating with MSC membrane not only enhanced the above features but also reduced the toxicity by enhancing targeting to specific cells and improved the uptake of the NPs by these target cells [133].

Isotretinoin is a drug widely used in the treatment of acne, as it is the only one that can act on the four factors that lead to the development of acne, namely colonization of the follicle by *Propionibacterium acnes*, altered keratinization, excessive production of sebum and inflammation [132]. However, the topical application of isotretinoin has several adverse effects, such as xerosis and dermatitis, which are the most common. For this purpose, Wang and coworkers constructed MSC membrane-coated isotretinoin NPs (cRA@STCM) [134]. The characterization of cRA@STCM showed a high encapsulation efficiency and a superior stability of isotretinoin. Furthermore, cRA@STCM demonstrated a controlled release of the drug, increased transdermal ability and lower skin irritation [134].

4.3. Ischemic stroke

The use of stem cell membrane as a drug delivery system to ischemic regions has been investigated. The tropism exhibited by stem cells to these regions comes from the interaction between chemokine factors present on the stem cell surface and their ligands abundantly located in the ischemic regions, mostly the SDF-1/CXCR4 axis interaction [135]. Glyburide is a hypoglycemic drug used in the treatment of type 2 diabetes, as it inhibits ATP-sensitive potassium channels present in pancreatic beta cells. However, these potassium channels are also found in other tissues, particularly in the nervous system [136]. Thus, glyburide may constitute a potential anti-shock agent, however its major hurdle is to overcome the blood brain barrier in a therapeutic amount [137]. Ma *et al.* developed NSC membrane-coated glyburide-loaded PLGA NPs for stroke treatment [138]. The researchers found that overexpression of CXCR4 in NSCs membrane, through lentiviral transduction, significantly increased its tropism for ischemic regions (Appendix XI D, E) and accordingly synthesized NSC membrane-coated glyburide-loaded PLGA NPs overexpressing CXCR4 (Gly-PLGA@STCM-CXCR4) (Appendix XI A, B). Gly-PLGA@STCM-CXCR4 were administered to mice

submitted to middle cerebral artery occlusion surgery, in which a significant increase in the therapeutic effect of glyburide in stroke treatment was observed compared to the free drug [138] (Appendix XI C).

Another drug delivery system was developed for the treatment of ischemic stroke. Wu and coworkers functionalized curcumin-loaded liposomes with MSC membranes (CUR-LIPO@STCM) using the sonication method which allowed to increase the production yield compared to the co-extrusion method [139]. The liposomes were loaded with curcumin, as curcumin presents an anti-inflammatory potential that, in an earlier stage of ischemic stroke, allows decreasing the loss of neurons and increasing the survival rate. Western-blot analysis of CUR-LIPO@STCM NPs demonstrated the retention of MSC membrane specific markers, namely CD44, CD90 and CD105 indicating the successful formation of the nanosystem, since the liposomes did not present any protein in their constitution. Furthermore, the retention also of CD47 protein in CUR-LIPO@STCM NPs plays a relevant role by preventing detection by the immune system, prolonging their circulation time. The functionalization of the liposomes with MSC membrane enabled that, in a situation of inflammation, the CUR-LIPO@STCM NPs show an increased permeability of the blood brain barrier due to the expression of adhesion molecules, such as VCAM-1. This demonstrates that the presence in CUR-LIPO@STCM NPs of ligands expressed on the MSCs membrane provides them with the ability to target and migrate to inflammatory tissues [140]. To verify *in vivo* efficacy, CUR-LIPO@STCM NPs were administered in middle cerebral artery occlusion mice showing an effective targeting to the damaged brain regions, allowing a recovery of neuronal functions far superior to curcumin-loaded liposomes or free curcumin [139].

4.4. Severe hindlimb ischemia

Peripheral arterial disease is characterized by obstruction of blood flow in the arteries, most commonly in the lower limbs and critical limb ischemia is the most severe form of the disease [141]. In the study of Bose *et al*. VEGF-loaded PLGA NPs coated with human ADSCs membrane were developed for the treatment of severe hindlimb ischemia (Appendix XII A) [142]. One of the objectives of the study was to compare VEGF-loaded PLGA NPs coated with non-engineered ADSCs membranes and with engineered ADSCs membranes that overexpressed CXCR4, in order to increase targeting to injured tissues that secrete SDF-1. To produce ADSCs that overexpressed CXCR4, it was used a retroviral vector that encoded for CXCR4, and subsequently the correct orientation of the receptor on the surface of the ADSCs membranes was confirmed. The activity of these formulations was evaluated in mice with hindlimb ischemia by retro-orbital injection. This research demonstrated that the coating

with ADSC membrane led to decreased uptake by macrophages, significantly increased penetration of the nanosystem across the endothelial barrier, and that the presence of the cell membrane allowed more efficient control of VEGF release kinetics. However, engineered ADSCs overexpressing CXCR4 showed increased targeting and migration to ischemic tissues, leading to a large accumulation in these tissues, allowing faster restoration of blood reperfusion and thus greater limb salvage compared to non-engineered ADSCs [142] (Appendix XII B - E).

4.5. Myocardial infarction

Myocardial infarction is an ischemic event caused by the sudden total occlusion of a coronary artery producing irreversible damage to the myocardial muscle [143]. New therapeutic options have been explored for their treatment. Yao and coworkers designed a nanostructure for microRNA delivery consisting of MSC membrane-coated mesoporous silica NPs, which prevented microRNA degradation [83]. The researchers loaded mesoporous silica NPs with microRNA-21 with the aim of inhibiting cardiomyocyte apoptosis as it silences the expression of the genes programmed cell death 4 (PDCD4) and phosphate and tension homology deleted on chromosome ten (PTEN) [144]. The MSC membrane coating allowed not only to prevent the elimination of NPs by the immune system, increasing their circulation time, but also to ensure tropism for ischemic cardiomyocytes, allowing the delivery of NPs to the target cells and thus decreasing adverse effects, such as myocardial fibrosis and remodeling. Additionally, it also enables efficient delivery of the miRNA to the target cells by avoiding its intracellular degradation by the liposomes. This targeting can be explained, for example, by the interaction between integrin β2 present in the stem cell membrane and ICAM-1 which is overexpressed in the ischemic myocardium [145]. On the other hand, research has shown that stem cell membrane also favors the multiplication of cardiomyocytes. Administered to mice with myocardial infarction, MSC membrane-coated microRNA-loaded mesoporous silica NPs have been shown to have high therapeutic efficiency, decreasing cardiomyocyte apoptosis and improving cardiac function [83].

4.6. Articular cartilage damage

Cartilage damage is very common and can lead to severe pain and osteoarthritis. The fact that the cartilage has a very limited capacity for self-renewal, since cartilage is an acellular and avascular tissue, makes damaged cartilage still a problem in the clinic without an effective solution [146]. Thus, Zhang *et al.* [147] developed a therapeutic strategy for cartilage regeneration that consisted of MSC membrane-coated kartogenin-loaded iron oxide NPs (KGN-

Fe_3O_4@STCM), where kartogenin is a molecule that after entering chondrocytes exhibits a chondroprotective effect [148]. The fact that it only used stem cell nanovesicles made the therapeutic system more simple to manage and safer, since there is no cell nucleus that could lead to an exaggerated immune response and even malignant transformation. After production of KGN-Fe3O4@STCM NPs it was verified by western blot that the protein profile remained identical to that of MSC, namely CXCR4, CD29 and CD90. This is very relevant for the formed nanosystem to have the ability to target and migrate to the sites of damage and inflammation, in which the CXRC4 receptor plays a key role. Furthermore, this study demonstrated that the presence of the stem cell membrane allowed to recruit MSCs that aided in the repair of damaged cartilage. KGN-Fe_3O_4@STCM NPs were intra-articularly injected into rats with osteochondral autograft transplantation (Appendix XIII) demonstrating an excellent biosafety profile, efficient cellular uptake and, in terms of effectiveness, led to earlier cartilage regeneration than pure KGN and with an excellent organized structure [147].

The use of stem cell-derived membrane nanovesicles as a delivery system of antisense oligonucleotide was evaluated for application in the field of regenerative medicine, especially for cartilage repair. Oieni *et al.* [149] developed MSC-derived membrane nanovesicles loaded with a microRNA inhibitor (anti-microRNA-221) to verify the loading capacity of the nanosystem as well as the targeting to endogenous MSCs and the delivery of anti-microRNA into the cytoplasm of the cell in order to silence microRNA-221. In previous studies, it was demonstrated that silencing microRNA-221, an antichondrogenic regulator, stimulated cartilage repair [150]. For the loading of MSC-derived membrane nanovesicles with anti-microRNA-221 was performed electroporation, which was found to be an effective method for encapsulating anti-microRNA. The membrane nanovesicles produced retained the membrane constitution of the source MSCs which attributed to them the ability to target endogenous MSCs, cells important in regenerative medicine, and also the ability to avoid lysosomal degradation. Subsequently, to verify the effectiveness of the delivery system *in vivo*, they proceeded to subcutaneous implantation in mice of a calf osteochondral biopsy model. Using this model, they demonstrated that MSC-derived membrane nanovesicles were safe and efficient anti-microRNA delivery systems, since after 7 days it was found that at the intracellular level in endogenous MSCs there was 97% of membrane nanovesicles and 95% of anti-microRNA, which proves the ability of the nanosystem to transfect endogenous cells [149].

4.7. Additional application: biodistribution assessment of nanoparticles

Although there are many studies of nanosystems constituted by stem cell membrane, it was still a difficulty to measure their biodistribution with good sensitivity. One of the most

used techniques was fluorescent labeling, but this technique has some limitations, namely being exclusively semi-quantitative and limited to small animals. In this way, Khait and coworkers developed radiolabeled stem cell membrane-based nanovesicles with ^{14}C-linoleic acid, a labeling technique that allowed to overcome these limitations of fluorescent labeling [151]. In this study, MSCs were incubated with ^{14}C-linoleic acid, an essential fatty acid that is part of the constitution of cell membranes, thus occurring its incorporation into the MSCs membrane. The presence of ^{14}C-linoleic acid had practically no impact on the characteristics of nanovesicles, just as it also had no impact on the MSC membrane targeting ability. The use of cell membranes instead of living cells allows to constitute therapies with more stability and safety, as there is no response to external stimuli. Radiolabeling, unlike fluorescence techniques, allowed to determine how much NPs were captured by the cells and also how much NPs each cell captured. Therefore, it is found that radiolabeling nanovesicles constitutes a more advantageous alternative to fluorescence techniques with the ability to provide a large amount of additional information [151].

5. Future prospects

Despite the great advances in both research and production of stem cell membrane-coated NPs, this nanotechnology is very recent, still presenting several questions not completely clarified and some limitations.

As mentioned in several studies, the production technology of stem cell membrane-coated NPs needs to be improved and new techniques need to be discovered that make it possible to obtain an excellent production yield, unlike the most used traditional method, the co-extrusion. In this sense, it is necessary to discover new coating techniques that enable to obtain good production yields associated with a good quality of the obtained product.

As it is a relatively new nanotechnology there are still many improvements that can be made in order to maximize its potential. Few studies have explored the modification of stem cell membrane, leading to the overexpression of certain surface proteins that can enhance not only targeting but also internalization into the target cells by interacting with overexpressed membrane proteins. The studies of Ma *et al.* [138] and Bose *et al.* [142] explored the potential of overexpression of the CXCR4 receptor on the membrane of stem cells, achieving considerable improvements in the properties of NPs. Thus, stem cell membrane modification is a very attractive area that can be further explored. The modification of the stem cell membrane with ^{14}C-linoleic acid in the study of Khait *et al.* [151] allowed to obtain a tool for theranostic applications, being an example of how through cell membrane modifications new applications can be obtained. Thus, investigating stem cell membrane modifications with

imaging agents, such as fluorescent labeling and radiolabeling, will allow to obtain potential successful diagnostic applications.

The use of stem cell membrane for coating of NPs presents several advantages, already mentioned above, due to the retention of the properties of the source cell. However, it has been recently explored the use of two types of cell membranes to coat NPs, called hybrid membrane, combining the different functionalities of the source cells. Bu and coworkers developed NPs coated with cancer stem cell-platelet hybrid membrane, constituting an improved treatment for cancer [152]. This is a recent area of research that could offer very promising new drug delivery platforms.

In the future, a major advance would be to apply this nanotechnology in large-scale clinical trials, however for these further studies are still needed to rigorously assess whether the long-term administration of stem cell membrane-coated NPs can cause some kind of adverse effects in living organisms. Therefore, more studies are needed to conclude about the biosafety of this new nanotechnology that could be a promising therapy for various diseases.

6. Conclusion

This review covers targeted drug delivery systems consisting of a NP core coated by a stem cell membrane, which allowed to combine the advantages of both synthetic NPs and biological membranes. On one hand, a NP core allows for loading of a therapeutic agent, protecting it from degradation and enables its controlled release; on another hand, a biomimetic cell membrane-based coating retain the main surface composition of the source cell membrane and is able to conserve its native biofunctionality. The main benefits conferred by stem cell membrane coating, as mentioned in most of the studies presented, is the ability to target both tumor and inflammatory tissues, increased tissue penetration, due to the retention of all ligands and receptors expressed on the stem cell membrane. This allows for greater accumulation at the target site, which not only enhances the efficacy of therapy but also decreases its adverse effects. Furthermore, the stem cell membrane coating allows for prolonging the circulation time of the nanosystem by preventing its detection by the immune system and constitutes a stable nanodelivery system for a variety of therapeutic compounds including drugs and nucleic acids. Hence, stem cell membrane-coated NPs constitute an innovative, multifunctional and versatile biomimetic approach towards improved diagnostics and therapeutics for a plethora of biomedical applications, with maximized efficacy and safety.

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Appendix I

Table 1. Summary of studies involving stem cell membrane as a coating of a nanosystem for cancer applications.

Cancer type	Characteristics of stem cell membrane-coated NPs							Animal model	Outcomes	Ref.
	Inner core	Coating membrane	Drug	Method of production	Particle size (nm)	Zeta potential (mV)	Other properties			
Cancer	Gelatin nanogels	Bone marrow-derived mesenchymal stem cell membrane	Doxorubicin	• Hypotonic lysis • Co-extrusion	146.40	-33.05	• Protein profile • Stability • Integrity • Cytotoxicity • Load capacity • Release profile	Nude mice grafted with human cancer HeLa cells	• ↑ accumulation in tumor sites • ↑ antitumor efficiency • ↑ safety profile	[26]
Cancer	Mesoporous silica-coated upconversion NP	Stem cell membrane	Photosensitizers ZnPC and MC540	• Hypotonic lysis • Co-extrusion	152.00	-30.34	• Protein profile • Stability • Cytotoxicity • Cancer cell binding ability	Female BALB/c nude mice grafted with HeLa cells	• ↑ biocompatibility • ↑ tumor-targeting ability • ↑ accumulation in tumor sites • ↑ photodynamic effect • ↑ antitumor activity	[95]
Breast cancer	PLGA NP	Bone marrow-derived mesenchymal stem cell membrane	Paclitaxel	• Lysis • Co-extrusion	92.50	-36.22	• Stability • Release profile • Cytotoxicity • Biodistribution	Female BALB/c mice bearing orthotopic 4T1 breast cancer	• ↑ drug concentration in tumor sites • ↑ antitumor activity • ↓ toxicity of drug	[101]
Liver cancer	PLGA NP	Umbilical cord-derived mesenchymal stem cell membrane	Doxorubicin	--------- • Sonication	105.00	-27.20	• Protein profile • Stability • Release profile	BALB/c nude mice grafted with MHCC97H liver tumor	• ↑ cellular uptake efficiency • ↑ accumulation in tumor sites • ↑ antitumor efficiency	[54]

Table 1. (Continued)

Cancer type	Characteristics of stem cell membrane-coated NPs							Animal model	Outcomes	Ref.
	Inner core	Coating membrane	Drug	Method of production	Particle size (nm)	Zeta potential (mV)	Other properties			
Liver cancer	Mesoporous silica NP	Mesenchymal stem cell membrane	Doxorubicin	• Freeze-thaw cycles • Sonication	180.00	-25.00	• Protein profile • Stability • Integrity • Cytotoxicity • Load capacity • Release profile • Biodistribution	Nude mice grafted with HepG2 tumor	• ↓ uptake by macrophages • ↑ tumor-targeting ability • ↑ tumor penetration ability • ↑ cellular uptake efficiency • ↑ safety profile • ↑ antitumor activity	[111]
Oral cancer	Liposome	Mesenchymal stem cell membrane	Verteporfin PFC/O_2	•Homogenization buffer • Co-extrusion	153.50	-23.20	• Protein profile • Stability • Cytotoxicity • Load capacity	Mice grafted with orthotopic OSCC	• ↑ accumulation in tumor sites • ↑ safety profile • ↑ antitumor activity	[36]
Osteosarcoma	Polydopamine NP	Mesenchymal stem cell membrane	SN38	• Hypotonic lysis • Sonication	191.00	-28.40	• Protein profile • Stability • Cytotoxicity • Load capacity • Release profile	Female BALB/c nude mice grafted with MG63 osteosarcoma cells	• ↑ biocompatibility • Retain photothermal effect • ↓ uptake by macrophages • ↑ accumulation in tumor sites • ↑ cellular uptake efficiency	[82]
Colon cancer	SPIO	Mesenchymal stem cell membrane	Doxorubicin	• Freeze-thaw cycles and sonication • Co-extrusion	125.00	---------	• Protein profile • Stability • Integrity • Cytotoxicity • Release profile	C57BL/6 female mice grafted with MC38 colon cancer cells	• ↑ cellular uptake efficiency • ↑ antitumor activity • ↑ safety profile	[35]

Table 1. (Continued)

Cancer type	Characteristics of stem cell membrane-coated NPs							Animal model	Outcomes	Ref.
	Inner core	Coating membrane	Drug	Method of production	Particle size (nm)	Zeta potential (mV)	Other properties			
Prostate cancer	Polydopamine-coated iron oxide NP	Mesenchymal stem cell membrane	siRNA siPlk1	• Hypotonic lysis • Co-extrusion	109.00	-30.28	• Protein profile • Stability • Photothermal capability • Integrity • Cytotoxicity • MRI capability	BALB/c nude mice grafted with DU145 tumor	• ↑ biocompatibility • Retain photothermal ablation ability • ↑ cellular uptake efficiency • ↑ tumor-targeting ability • ↑ antitumor activity	[122]
Prostate cancer	SPIO	Adipose-derived mesenchymal stem cell membrane	---------	• Hypotonic lysis • Sonication	---------	---------	• Protein profile • Stability	Mouse prostate cancer cells	• ↓ uptake by macrophages • ↑ cellular uptake efficiency • Identical magnetic induced hyperthermia effect	[128]
Prostate cancer	---------	Mesenchymal stem cell membrane	sTRAIL	• Hypotonic lysis	180.00	-12.00	• Protein profile • Cytotoxicity • Load capacity • Release profile	Nude mice grafted with prostate cancer	• ↑ antitumor activity • ↑ tumor-targeting ability	[90]
Prostate cancer	---------	Mesenchymal stem cell membrane	Plasmid DNA encoding for the PEX	• Electroporation	204.00	-17.00	• Stability • Cytotoxicity • Load capacity	Mice grafted with subcutaneous prostate cancer	• ↑ accumulation in tumor sites • ↑ antitumor activity • ↑ safety profile • ↑ expression of PEX in the entire tumor bulk	[130]

Abbreviations: MRI – magnetic resonance imaging; NP – nanoparticle; OSCC – oral squamous cell carcinoma; PEX – hemopexin-like domain; PFC/O2 – oxygen-loading perfluorocarbon; PLGA – poly(lactic-co-glycolic acid); Plk1 – polo-like kinase 1; siRNA – small interfering RNA; SN38 – 7-ethyl-10-hydroxycamptothecin; SPIO – superparamagnetic iron oxide NPs; sTRAIL – soluble form of tumor necrosis factor-related apoptosis-inducing ligand

Appendix II

Table 2. Summary of studies involving stem cell membrane as a coating of a nanosystem for different biomedical applications, particularly acne, ischemic stroke, severe hindlimb ischemia, myocardial infarction, articular cartilage damage and, in the additional application of biodistribution assessment of nanoparticles.

Biomedical application	Characteristics of stem cell membrane-coated NPs							Animal model	Outcomes	Ref.
	Inner core	Coating membrane	Drug	Method of production	Particle size (nm)	Zeta potential (mV)	Others properties			
Acne	Polydopamine-coated Au-Ag NP	Mesenchymal stem cell membrane	---------	• Hypotonic lysis • Sonication	---------	---------	• Cytotoxicity • Photothermal capability	Male golden hamsters injected with *Propionibacterium acnes*	• ↑ photothermal conversion efficiency • ↑ cellular uptake efficiency • ↑ antiproliferative effect • ↑ safety profile	[133]
Acne	---------	Umbilical cord-derived mesenchymal stem cell membrane	Isotretinoin	• Hypotonic lysis • Sonication	56.90	-34.90	• Stability • Load capacity • Release profile	Rabbit model of hyperkeratinization	• ↑ transdermal ability • ↓ skin irritation • Significant therapeutic activity	[134]
Ischemic stroke	PLGA NP	CXCR4-overexpressing neural stem cell membrane	Glyburide	• Freeze-thaw cycles and sonication • Co-extrusion	---------	---------	• Protein profile • Cytotoxicity • Release profile	Middle cerebral artery occlusion mice	• ↑ therapeutic effect of glyburide	[138]
Ischemic stroke	Liposome	Mesenchymal stem cell membrane	Curcumin	• Freeze-thaw cycles • Sonication	70.00 to 80.00	-17.20	• Protein profile • Stability • Cytotoxicity • Load capacity • Release profile	Middle cerebral artery occlusion mice	• ↑ survival rate • Prevents weight loss tendency	[139]
Severe hindlimb ischemia	PLGA NP	CXCR4-overexpressing adipose-derived stem cell membrane	VEGF	• Hypotonic lysis • Sonication	127.00	-15.00	• Protein profile • Stability • Cytotoxicity • Release profile	Female C57BL/6 mice with hindlimb ischemia	• ↓ uptake by macrophages • ↑ targeting to ischemic tissues • ↑ limb salvage	[142]

Table 2. (Continued)

Biomedical application	Characteristics of stem cell membrane-coated NPs							Animal model	Outcomes	Ref.
	Inner core	Coating membrane	Drug	Method of production	Particle size (nm)	Zeta potential (mV)	Others properties			
Myocardial infarction	Mesoporous silica NP	Mesenchymal stem cell membrane	microRNA-21	--------- • Sonication	110.00	---------	• Protein profile • Stability • Load capacity • Release profile	Mice with myocardial infarction	• ↑ targeting to infarcted myocardium • ↑ inhibition of cardiomyocyte apoptosis • ↓ myocardial fibrosis and remodeling	[83]
Articular cartilage damage	Iron oxide NP	Mesenchymal stem cell membrane	Kartogenin	• Hypotonic lysis • Sonication	266.33	-27.20	• Protein profile • Stability • Cytotoxicity • Load capacity • Release profile	Rats with osteochondral autograft transplantation	• ↑ cartilage regeneration activity • ↑ biosafe profile	[147]
Articular cartilage damage	---------	Mesenchymal stem cell membrane	MicroRNA inhibitor (anti-microRNA-221)	• Hypotonic lysis • Sonication	---------	---------	• Protein profile • Stability • Cytotoxicity • Load capacity • Release profile	Subcutaneous implantation in mice of a calf osteochondral biopsy model	• Efficient cellular uptake • Delivery of load to the cytoplasm avoiding lysosomal degradation • Efficient delivery system of anti-microRNA	[149]
Biodistribution assessment of NPs	---------	Membrane of mesenchymal stem cell cultured with ^{14}C-Linoleic acid	---------	• Hypotonic lysis • Sonication	---------	-7.80	• Stability • Cytotoxicity • Release profile	Nude mice	• ↑ quantification of cellular uptake • Allows determining how many NPs were uptaken by cells	[151]

Abbreviations: NP – nanoparticle; PLGA – poly(lactic-co-glycolic acid); VEGF – vascular endothelial growth factor.

Appendix III

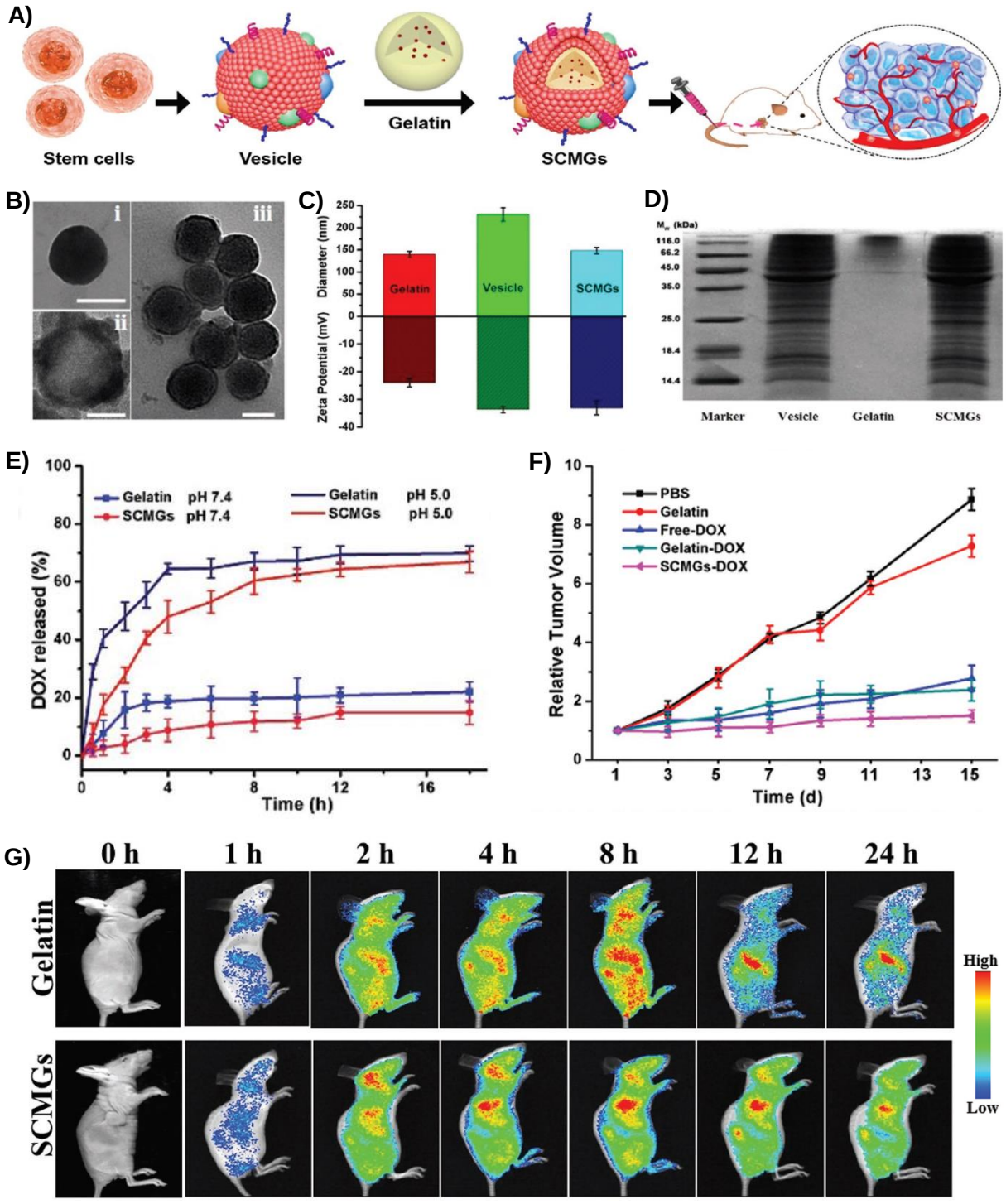


Figure 9. A) Schematic representation of the production of SCMGs and their tumor targeting ability *in vivo*. B) Transmission electron microscopy of gelatin nanogel (i), membrane vesicle (ii) and SCMGs (iii). Scale bar = 100 µm. C) Hydrodynamic diameter and zeta potential of gelatin nanogels, membrane vesicles and SCMGs. D) Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of the protein composition of membrane vesicles, gelatin nanogels and SCMGs. E) DOX release profile of gelatin nanogels and SCMGs at pH 7.4 and pH 5.0. F) Evolution of tumor volume in mice submitted to different treatments. G) *In vivo* distribution of gelatin nanogels and SCMGs, both dyed with Cyanine 7, administered in mice. Reproduced with permission from reference [26].

Abbreviations: DOX – doxorubicin; PBS – phosphate buffered saline; SCMGs – mesenchymal stem cell membrane-coated gelatin nanogels, referred to in the text as GNG@STCM.

Appendix IV

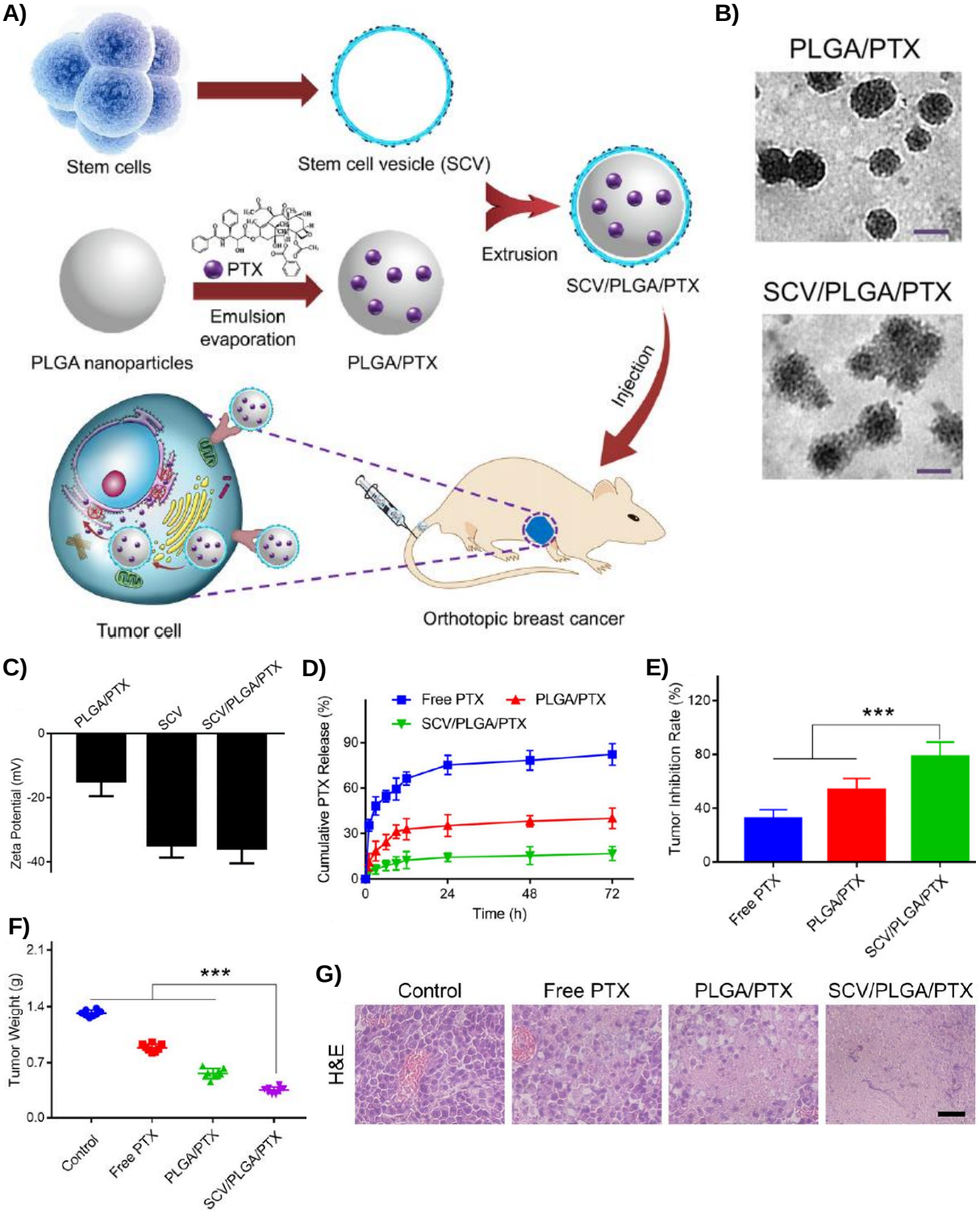


Figure 10. A) Schematic representation of the production of SCV/PLGA/PTX and their tumor targeting ability in orthotopic breast tumor-bearing mice. B) Transmission electron microscopy images of PLGA/PTX NPs and SCV/PLGA/PTX NPs. Scale bar = 100 nm. C) Zeta potential of PLGA/PTX NPs, stem cell vesicles and SCV/PLGA/PTX NPs. D) PTX release profile of free PTX, PLGA/PTX NPs and SCV/PLGA/PTX NPs at pH 7.4 at 37 °C *in vitro*. E) *In vivo* tumor inhibition rates of free PTX, PLGA/PTX NPs and SCV/PLGA/PTX NPs. F) *In vivo* tumor weights of free PTX, PLGA/PTX NPs and SCV/PLGA/PTX NPs. G) H&E-stained tumor tissue images treated with free PTX, PLGA/PTX NPs and SCV/PLGA/PTX NPs. Scale bar = 50 μm. Reproduced with permission from reference [101].

Abbreviations: H&E – hematoxylin and eosin; PLGA/PTX – poly(lactic-co-glycolic acid) nanoparticles loaded with paclitaxel; PTX – paclitaxel; SCV/PLGA/PTX – mesenchymal stem cell membrane-coated paclitaxel-loaded poly(lactic-co-glycolic acid) nanoparticles, referred to in the text as PTX-PLGA@STCM.

Appendix V

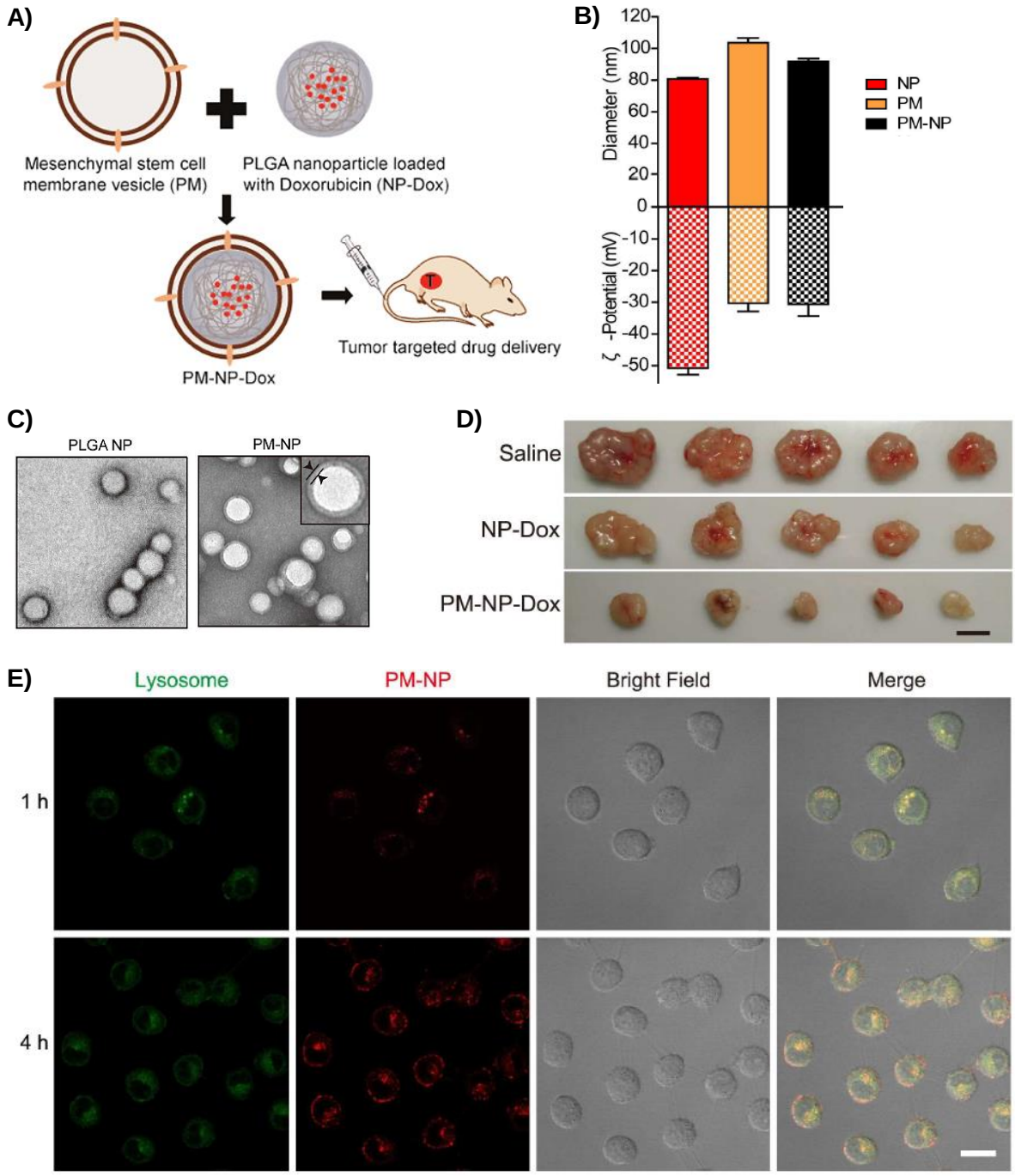
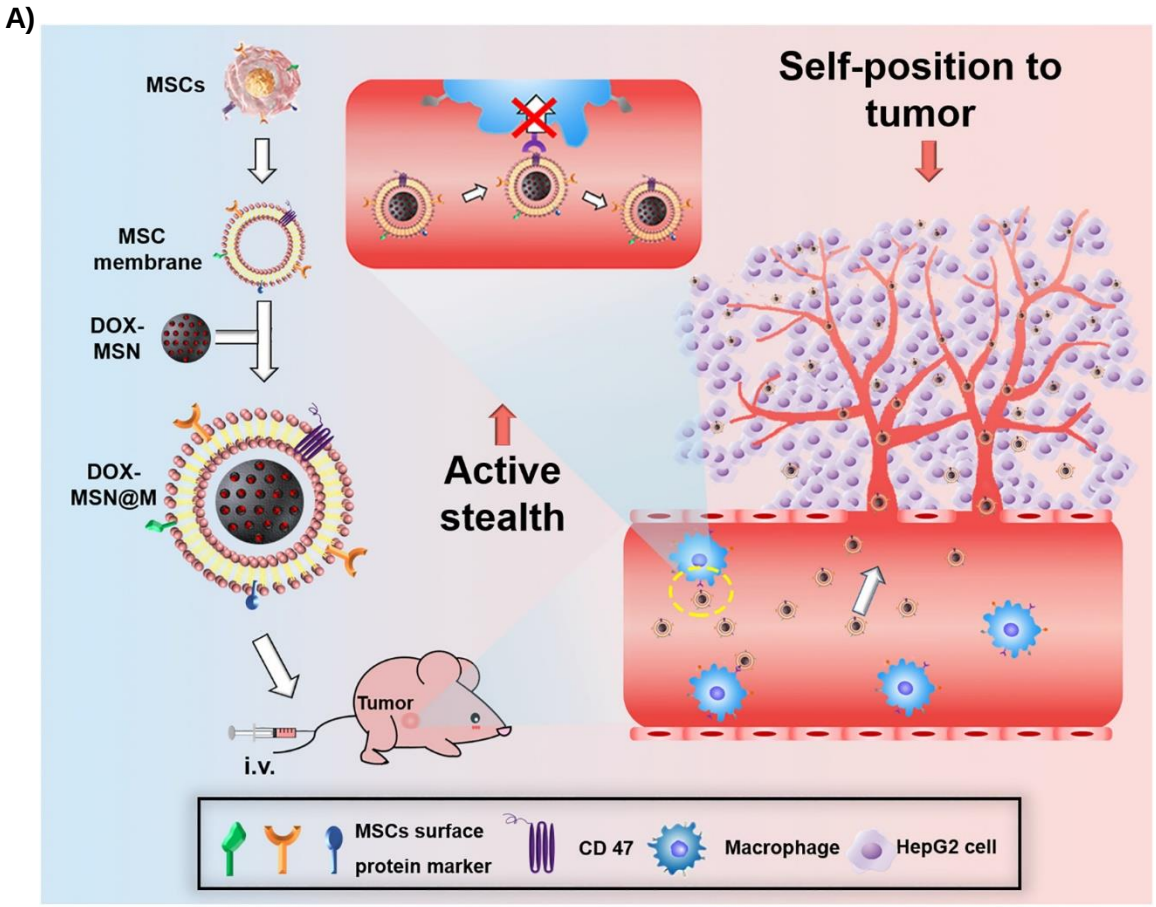
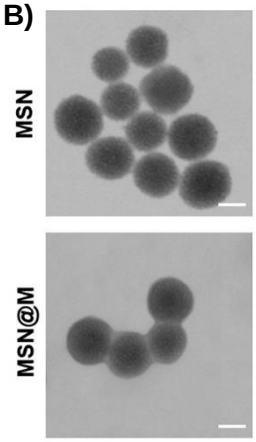


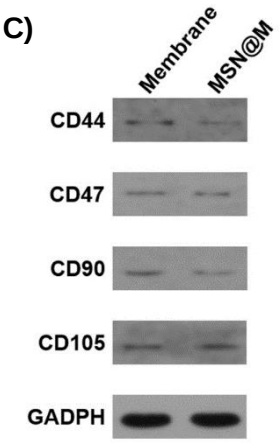
Figure 11. A) Schematic representation of the production of PM-NP-Dox and their tumor targeting ability in liver tumor-bearing mice. B) Zeta potential and hydrodynamic diameter of PLGA NP, membrane vesicles and PM-NP. C) Transmission electron microscopy images of PLGA NP and PM-NP. Scale bar = 100 nm. D) Tumor imagens from tumor-bearing mice submitted to different treatments for 15 days. E) Confocal microscopy images of lysosomes stained with FITC-lysosensor and PM-NP stained with Rhodamine B, allowing to verify the co-localization of both in the cell. Scale bar = 20 μm. Reproduced with permission from reference [54].

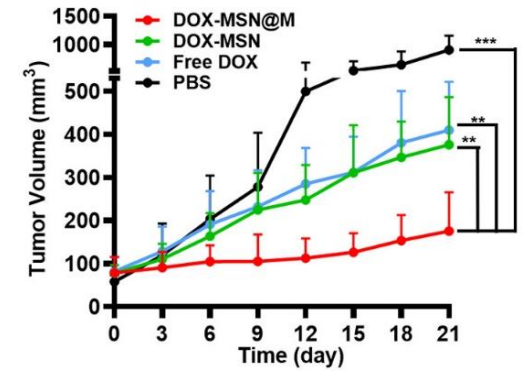
Abbreviations: PLGA NP – poly(lactic-co-glycolic acid) nanoparticle; PM-NP – mesenchymal stem cell membrane-coated poly(lactic-co-glycolic acid) nanoparticle; PM-NP-Dox – mesenchymal stem cell membrane-coated doxorubicin-loaded poly(lactic-co-glycolic acid) nanoparticle, referred to in the text as DOX-PLGA@STCM.

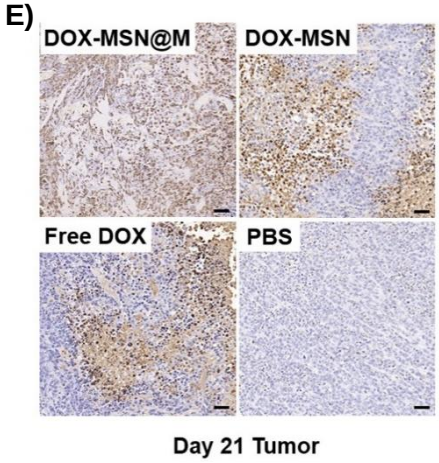
Appendix VI


A)
MSCs
MSC membrane
DOX-MSN
DOX-MSN@M
Self-position to tumor
Active stealth
Tumor
i.v.
MSCs surface protein marker
CD 47
Macrophage
HepG2 cell


B)
MSN
MSN@M


C)
Membrane
MSN@M
CD44
CD47
CD90
CD105
GADPH


D)
DOX-MSN@M
DOX-MSN
Free DOX
PBS
Tumor Volume (mm3)
Time (day)
0
100
200
300
400
500
1000
1500
0 3 6 9 12 15 18 21
***
**
**


E)
DOX-MSN@M
DOX-MSN
Free DOX
PBS
Day 21 Tumor

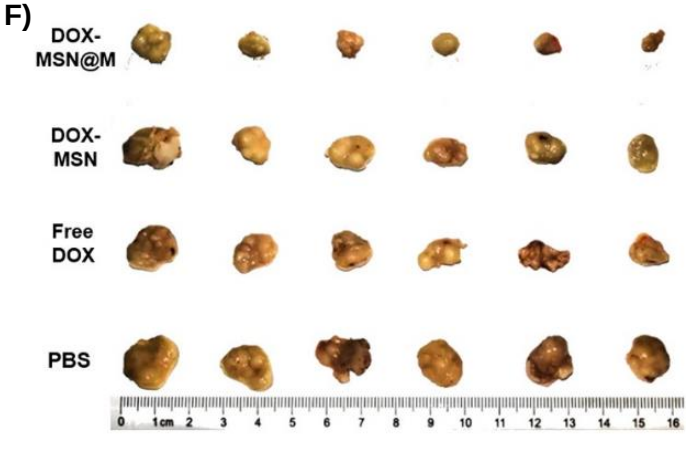

F)
DOX-MSN@M
DOX-MSN
Free DOX
PBS

Figure 12. A) Schematic representation of the production of DOX-MSN@M. Ability of DOX-MSN@M to target the tumor and prevent clearance by macrophages *via* the marker CD47. B) Images from scanning transmission electron microscopy of MSN and MSN@M. Scale bar = 50 μm. C) Western blot analysis of membrane markers in MSC membrane and MSN@M. D) Evolution of tumor volume in mice submitted to different treatments. E) TUNEL-stained tumor tissue images treated with DOX-MSN@M, DOX-MSN, free DOX and PBS. Scale bar = 50 μm. F) Tumor imagens from tumor-bearing mice submitted to different treatments for 21 days. Reproduced with permission from reference [111]. Abbreviations: DOX – doxorubicin; DOX-MSN – mesoporous silica nanoparticles loaded with doxorubicin; DOX-MSN@M – mesenchymal stem cell membrane-coated doxorubicin-loaded mesoporous silica nanoparticles; MSC – mesenchymal stem cell; MSN – mesoporous silica nanoparticle; PBS - phosphate-buffered saline.

Appendix VII

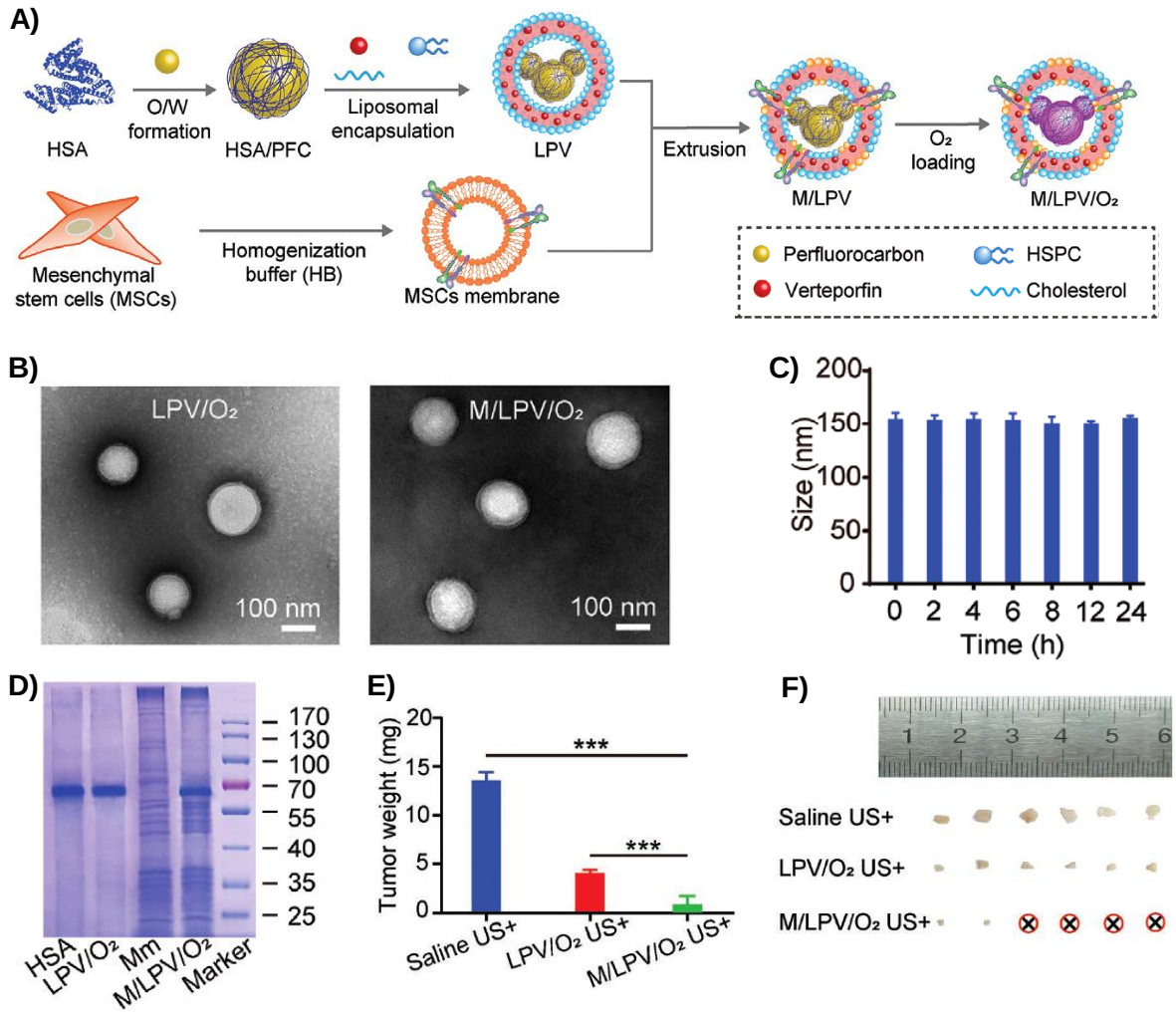


Figure 13. A) Schematic representation of the production of M/LPV/O_2. B) Transmission electron microscopy images of LPV/O_2 and M/LPV/O_2. Scale bar = 100 nm. C) Stability of M/LPV/O_2 in 50 % fetal bovine serum in absence of ultrasound at 37 °C for 24h. D) Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of the protein composition of LPV/O_2, membrane vesicles and M/LPV/O_2. E) Representation of tumor weight in mice submitted to different treatments for 28 days in the presence of ultrasound. F) Tumor imagens from orthotopic oral tumor-bearing mice submitted to different treatments for 28 days in the presence of ultrasound. Reproduced with permission from reference [36].

Abbreviations: HSA – human serum albumin; LPV – liposome constituted by sonosensitizer verteporfin and perfluorocarbon without oxygen; LPV/O_2 – liposome constituted by sonosensitizer verteporfin and oxygen-loading perfluorocarbon; M/LPV – mesenchymal stem cell membrane functionalized liposome composed by sonosensitizer verteporfin and perfluorocarbon; M/LPV/O_2 – mesenchymal stem cell membrane functionalized liposome composed by sonosensitizer verteporfin and oxygen-loading perfluorocarbon, referred to in the text as M-LP-VTP-PFC/O_2; Mm – mesenchymal stem cell membrane.

Appendix VIII

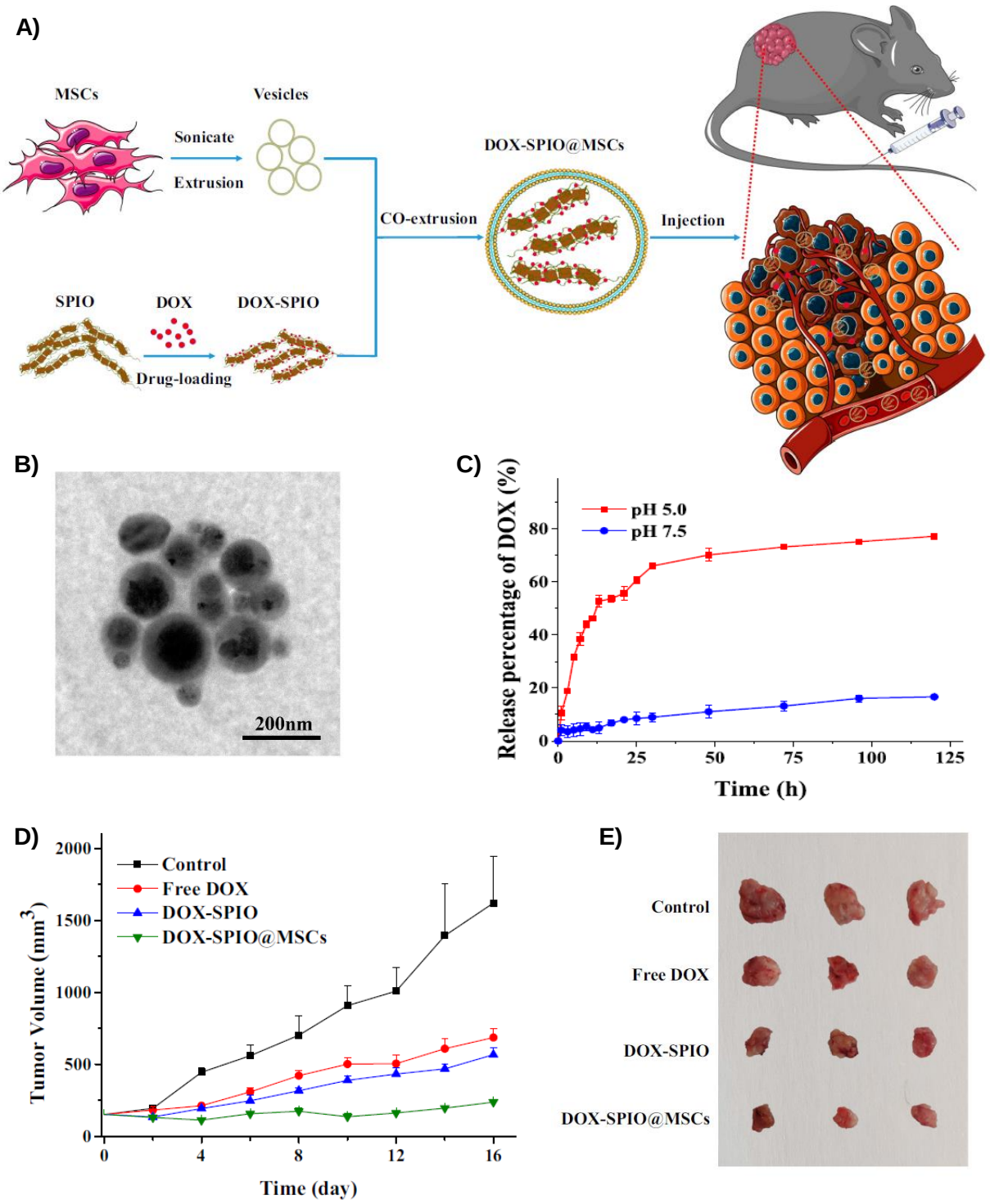


Figure 14. A) Schematic representation of the production of DOX-SPIO@MSCs and their tumor targeting ability in mice grafted with colon cancer cells. B) Transmission electron microscopy image of DOX-SPIO@MSCs NPs. Scale bar = 200 nm. C) DOX release profile of DOX-SPIO@MSCs NPs at pH 7.5 and pH 5.0. D) Evolution of tumor volume in tumor-bearing mice submitted to different treatments. E) Tumor imagens from tumor-bearing mice submitted to different treatments. Reproduced with permission from reference [35].

Abbreviations: DOX – doxorubicin; DOX-SPIO – superparamagnetic iron oxide nanoparticle loaded with doxorubicin; DOX-SPIO@MSC – mesenchymal stem cell membrane-coated doxorubicin-loaded superparamagnetic iron oxide nanoparticle, referred to in the text as DOX-SPIO@STCM; MSC – mesenchymal stem cell; SPIO – superparamagnetic iron oxide.

Appendix IX

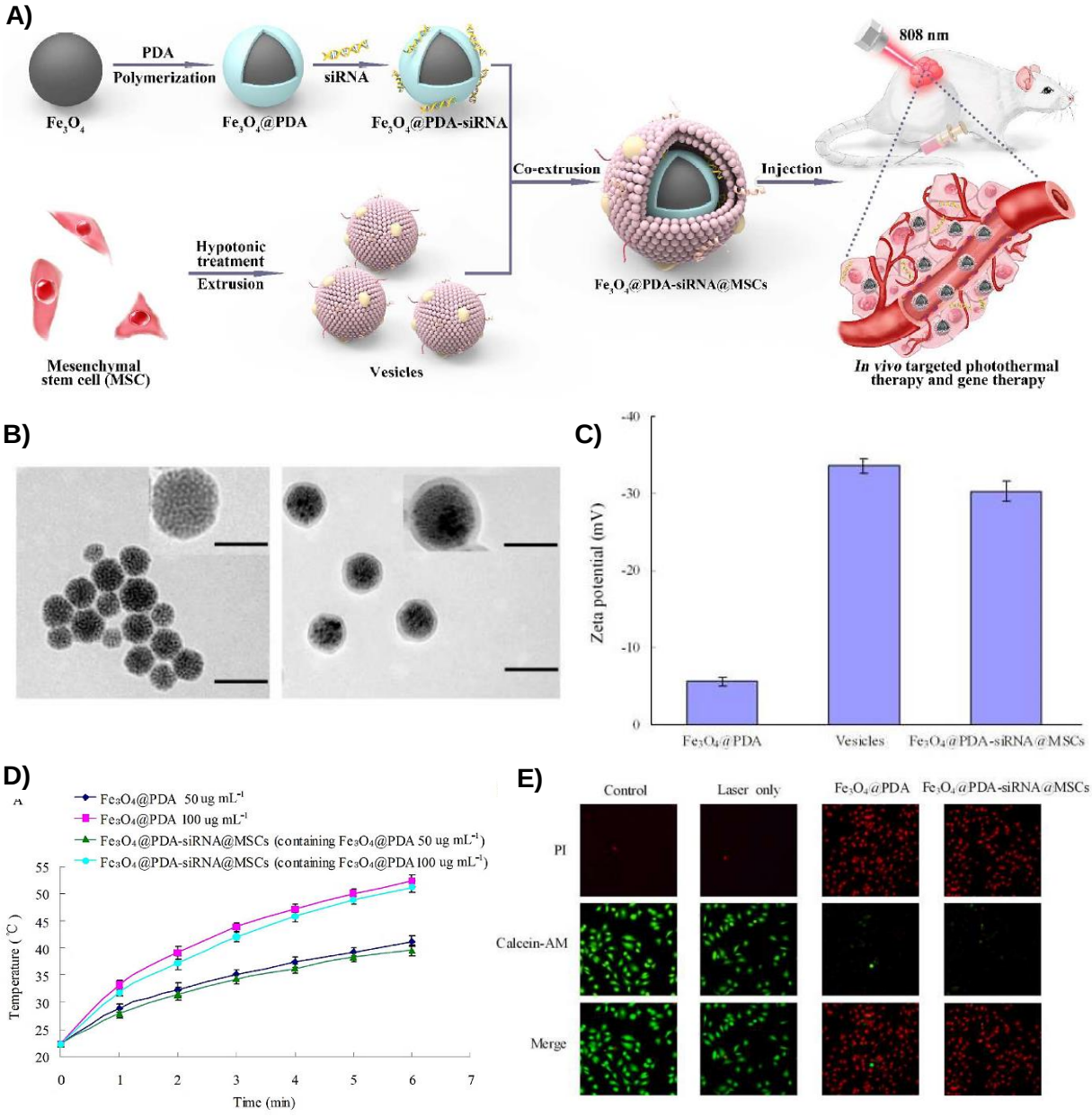
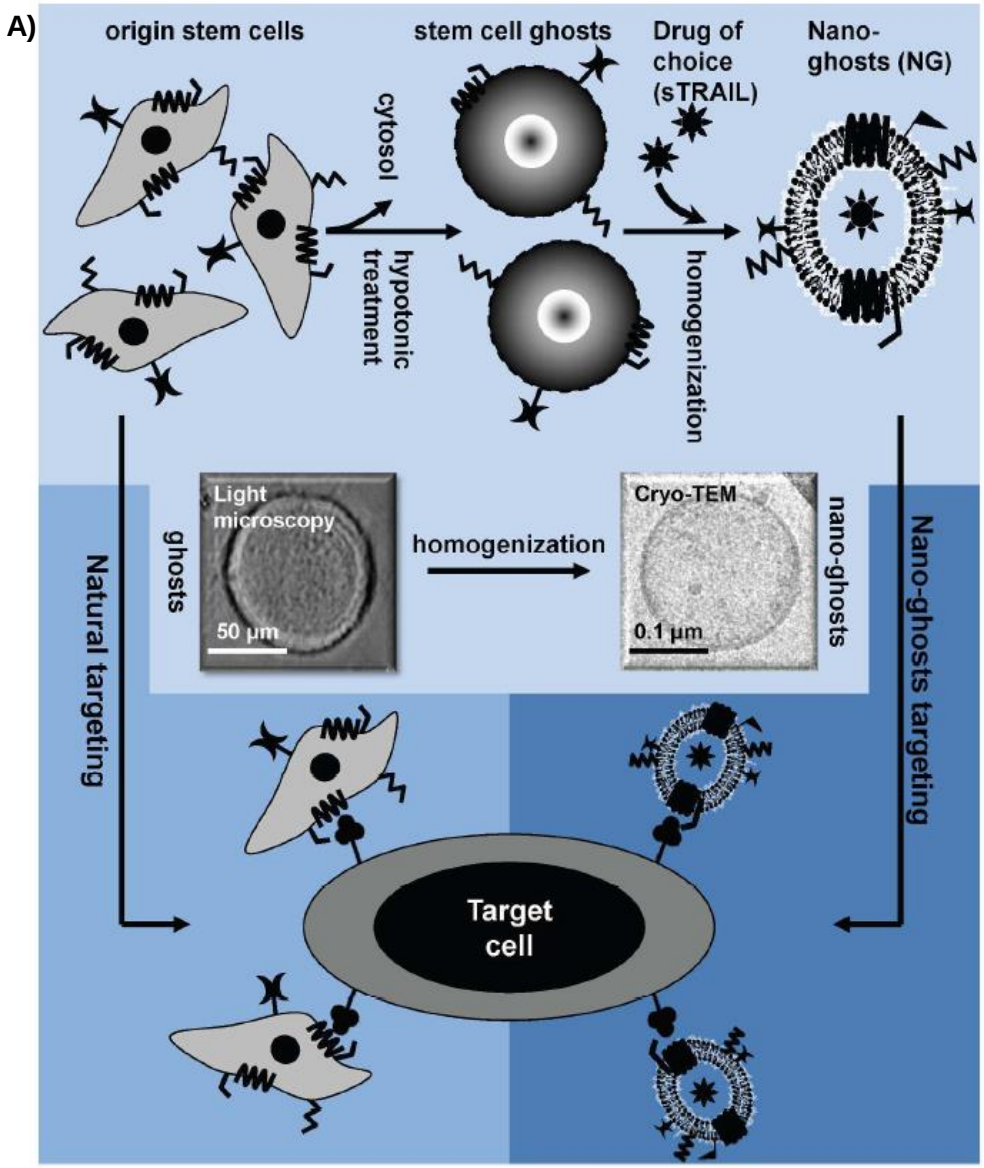
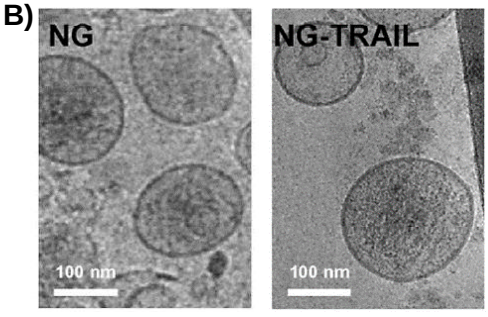
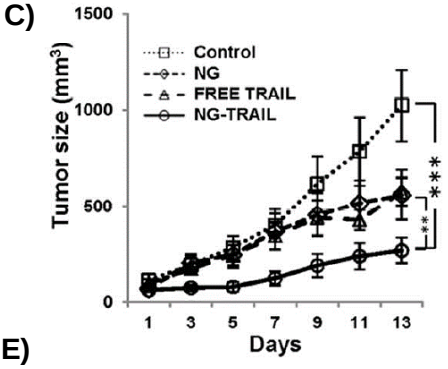


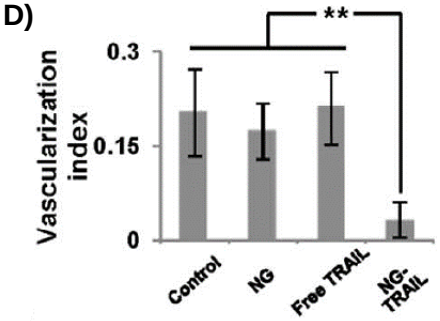
Figure 15. A) Schematic representation of the production of Fe_3O_4@PDA-siRNA@MSCs and their tumor targeting ability *in vivo*. B) Transmission electron microscopy images of Fe_3O_4@PDA NPs (left) and Fe_3O_4@PDA-siRNA@MSCs NPs (right). Scale bar = 100 nm. In the insets are transmission electron microscopy images of Fe_3O_4@PDA NPs (left) and Fe_3O_4@PDA-siRNA@MSCs (right). Scale bar = 50 nm. C) Zeta potential of Fe_3O_4@PDA NPs, membrane vesicles and Fe_3O_4@PDA-siRNA@MSCs NPs. D) Representation of photothermal heating curves of Fe_3O_4@PDA NPs and Fe_3O_4@PDA-siRNA@MSCs NPs in aqueous medium following an 808 nm laser irradiation at different concentrations of Fe_3O_4@PDA. E) Confocal laser scanning microscopy images of green cells stained with Calcein-AM, indicating live cells, red cells stained with PI, indicating apoptotic cells and co-stained cells irradiated with an 808 nm laser. Reproduced with permission from reference [122].
Abbreviations: Fe_3O_4@PDA – polydopamine-coated iron oxide nanoparticles; Fe_3O_4@PDA-siRNA – polydopamine-coated iron oxide nanoparticles loaded with small interfering RNA; Fe_3O_4@PDA-siRNA@MSCs – mesenchymal stem cell membrane-coated polydopamine-coated iron oxide-small interfering RNA nanoparticles, referred to in the text as PDA-Fe_3O_4@STCM; PDA – polydopamine; PI – propidium iodide; siRNA – small interfering RNA.

Appendix X


A)
origin stem cells
stem cell ghosts
Drug of choice (sTRAIL)
Nano-ghosts (NG)
cytosol
hypotonic treatment
homogenization
Light microscopy
50 µm
ghosts
homogenization
Cryo-TEM
0.1 µm
nano-ghosts
Natural targeting
Nano-ghosts targeting
Target cell


B)
NG
NG-TRAIL
100 nm
100 nm


C)
Tumor size (mm³)
1500
1000
500
0
Control
NG
FREE TRAIL
NG-TRAIL
1
3
5
7
9
11
13
Days
***
***


D)
Vascularization index
0.3
0.15
0
**
Control
NG
Free TRAIL
NG-TRAIL

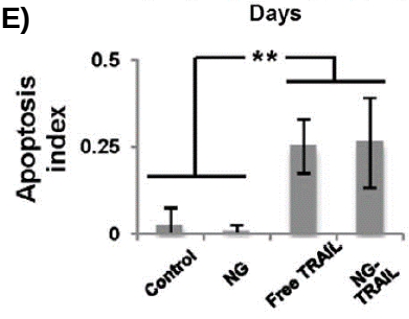

E)
Apoptosis index
0.5
0.25
0
**
Control
NG
Free TRAIL
NG-TRAIL

Figure 16. A) Schematic representation of the production of NG-TRAIL and their tumor targeting ability. B) Transmission electron microscopy images of NG and NG-TRAIL. Scale bar = 100 nm. C) Evolution of tumor size in tumor-bearing mice submitted to different treatments. D) Vascularization index and E) apoptosis index of tumor in tumor-bearing mice compared with untreated mice. Reproduced with permission from reference [90].
Abbreviations: NG – mesenchymal stem cell-derived membrane nanovesicles; NG-TRAIL – mesenchymal stem cell membrane loaded with soluble form of tumor necrosis factor-related apoptosis-inducing ligand; sTRAIL – soluble form of tumor necrosis factor-related apoptosis-inducing ligand.

Appendix XI

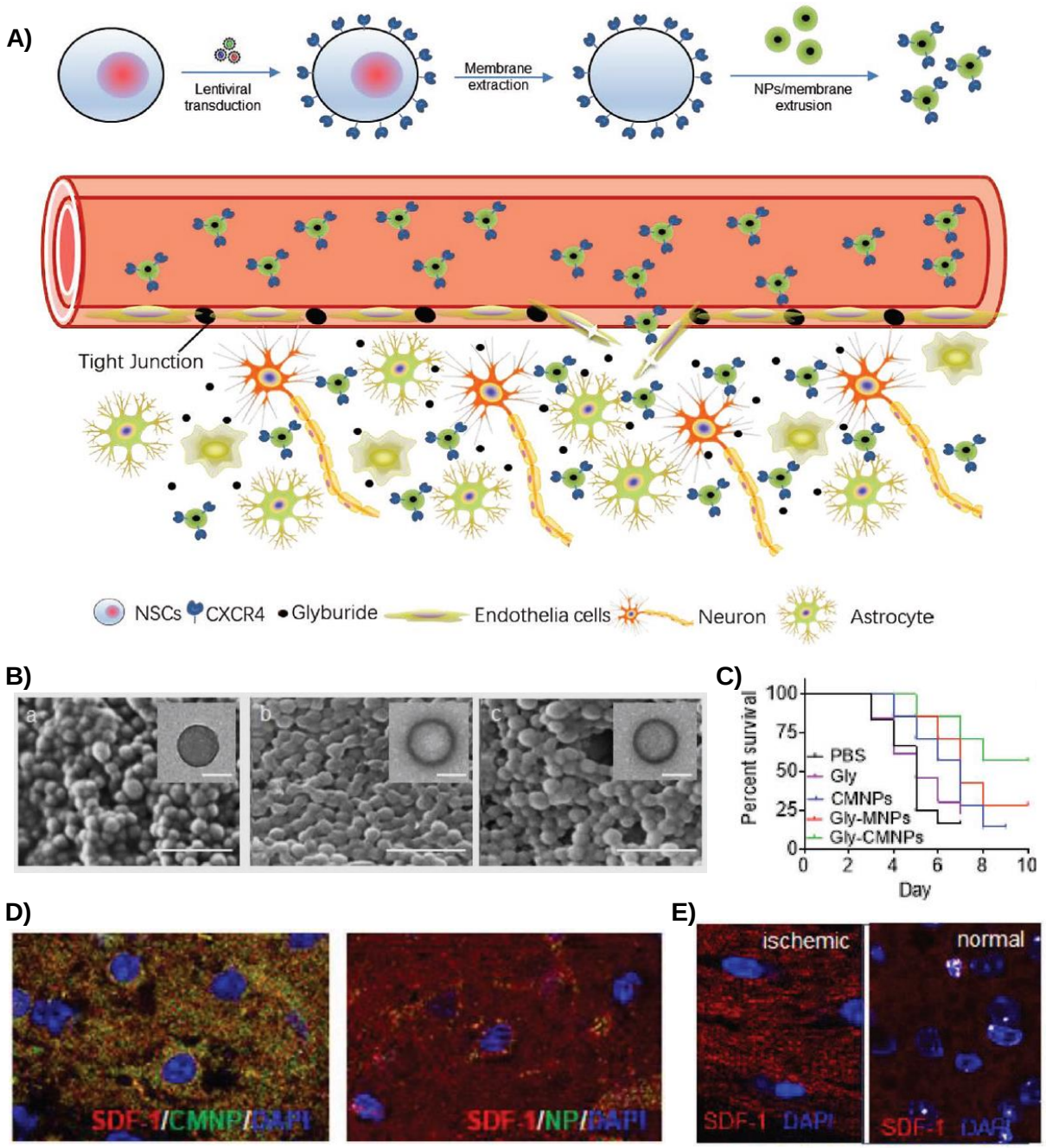
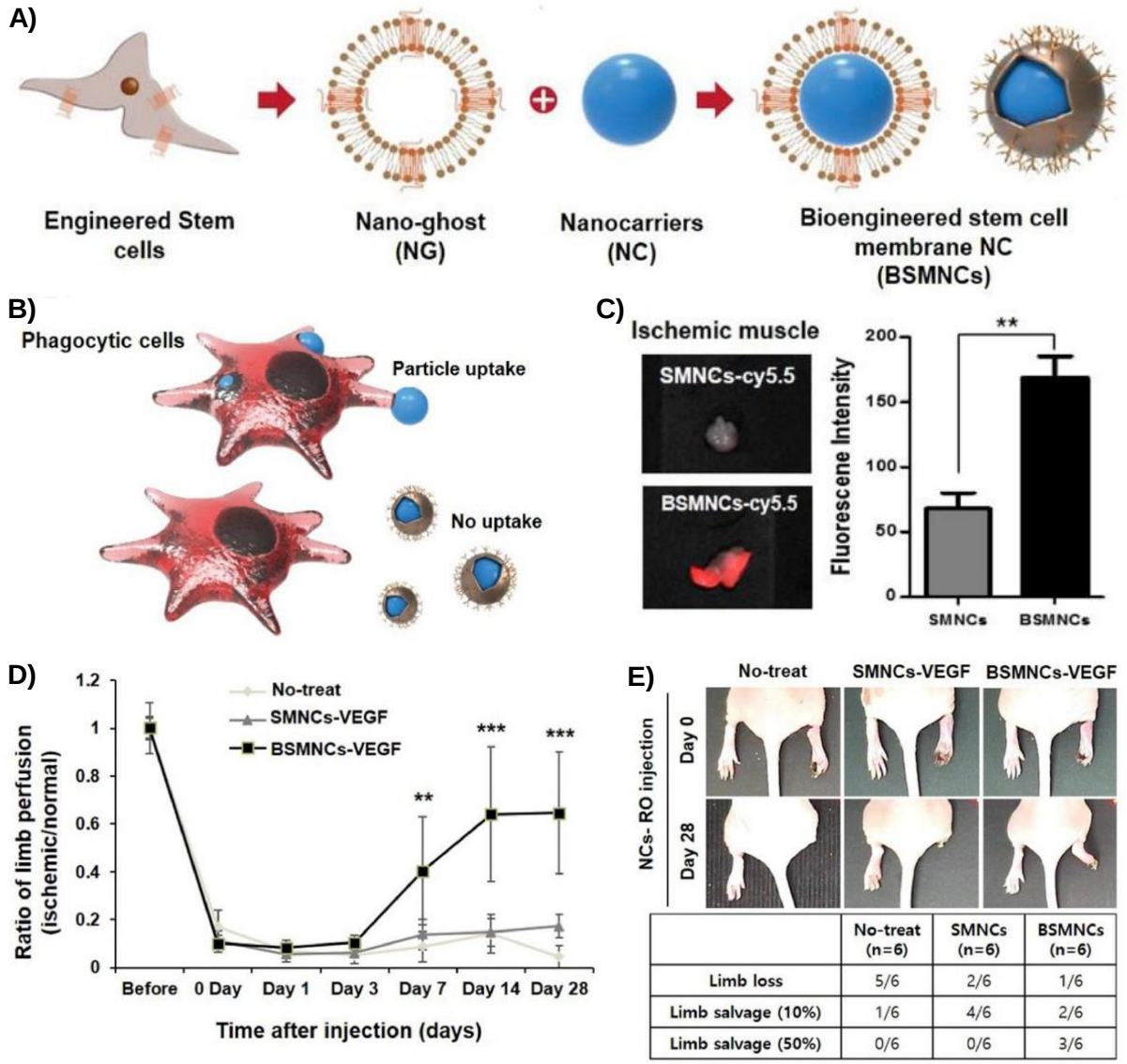


Figure 17. A) Schematic representation of the production of Gly-CMNP and its targeted delivery to the ischemic brain. B) Scanning electron microscopy images of PLGA NPs (a), MNPs (b) and CMNPs (c). Scale bar = 500 nm. In the insets are transmission electron microscopy images. Scale bar = 100 nm. C) Evolution of survival of mice submitted to middle cerebral artery occlusion surgery after different treatments. D) Distribution in the ischemic area of SDF-1 and the indicated NPs. Red: SDF-1. Green: NPs. Blue: DAPI. E) SDF-1 expression in the normal and ischemic brain. Red: SDF-1. Blue: DAPI. Reproduced with permission from reference [138].

Abbreviations: CMNP – neural stem cell membrane-coated nanoparticle overexpressing CXCR4; DAPI – 4′,6-diamidino-2-phenylindole; Gly – glyburide; Gly-MNP – neural stem cell membrane-coated glyburide-loaded poly(lactic-co-glycolic acid) nanoparticle; Gly-CMNP – neural stem cell membrane-coated glyburide-loaded poly(lactic-co-glycolic acid) nanoparticle overexpressing CXCR4, referred to in the text as Gly-PLGA@STCM-CXCR4; MNP – neural stem cell membrane-coated nanoparticle; NP- nanoparticle; NSC – neural stem cell; PBS – phosphate-buffered saline; PLGA – poly(lactic-co-glycolic acid).

Appendix XII



	No-treat (n=6)	SMNCs (n=6)	BSMNCs (n=6)
Limb loss	5/6	2/6	1/6
Limb salvage (10%)	1/6	4/6	2/6
Limb salvage (50%)	0/6	0/6	3/6

Figure 18. A) Schematic representation of the production of BSMNCs overexpressing CXCR4. B) Schematic representation of the uptake by phagocytic cells of PLGA nanoparticles, in contrast to SMNCs that are not uptaken. C) Fluorescence intensity of ischemic muscles of mice with hindlimb ischemia after intravenous injection of SMNCs and BSMNCs, both stained with Cy5.5. D) Ratio of limb perfusion (ischemic/normal) in mice with severe ischemic hindlimb on days after injection containing different treatments. E) Photographs of mice with severe ischemic hindlimb before and 28 days after RO injection. Reproduced with permission from reference [142].

Abbreviations: BSMNC – bioengineered stem cell membrane-coated poly(lactic-co-glycolic acid) nanoparticle; BSMNC-VEGF – bioengineered stem cell membrane-coated vascular endothelial growth factor-loaded poly(lactic-co-glycolic acid) nanoparticle; Cy5.5 – cyanine 5.5; PLGA – poly(lactic-co-glycolic acid); RO – retro-orbital; SMNC – stem cell membrane-coated poly(lactic-co-glycolic acid) nanoparticle; SMNC-VEGF – stem cell membrane-coated vascular endothelial growth factor-loaded poly(lactic-co-glycolic acid) nanoparticle; VEGF – vascular endothelial growth factor.

Appendix XIII

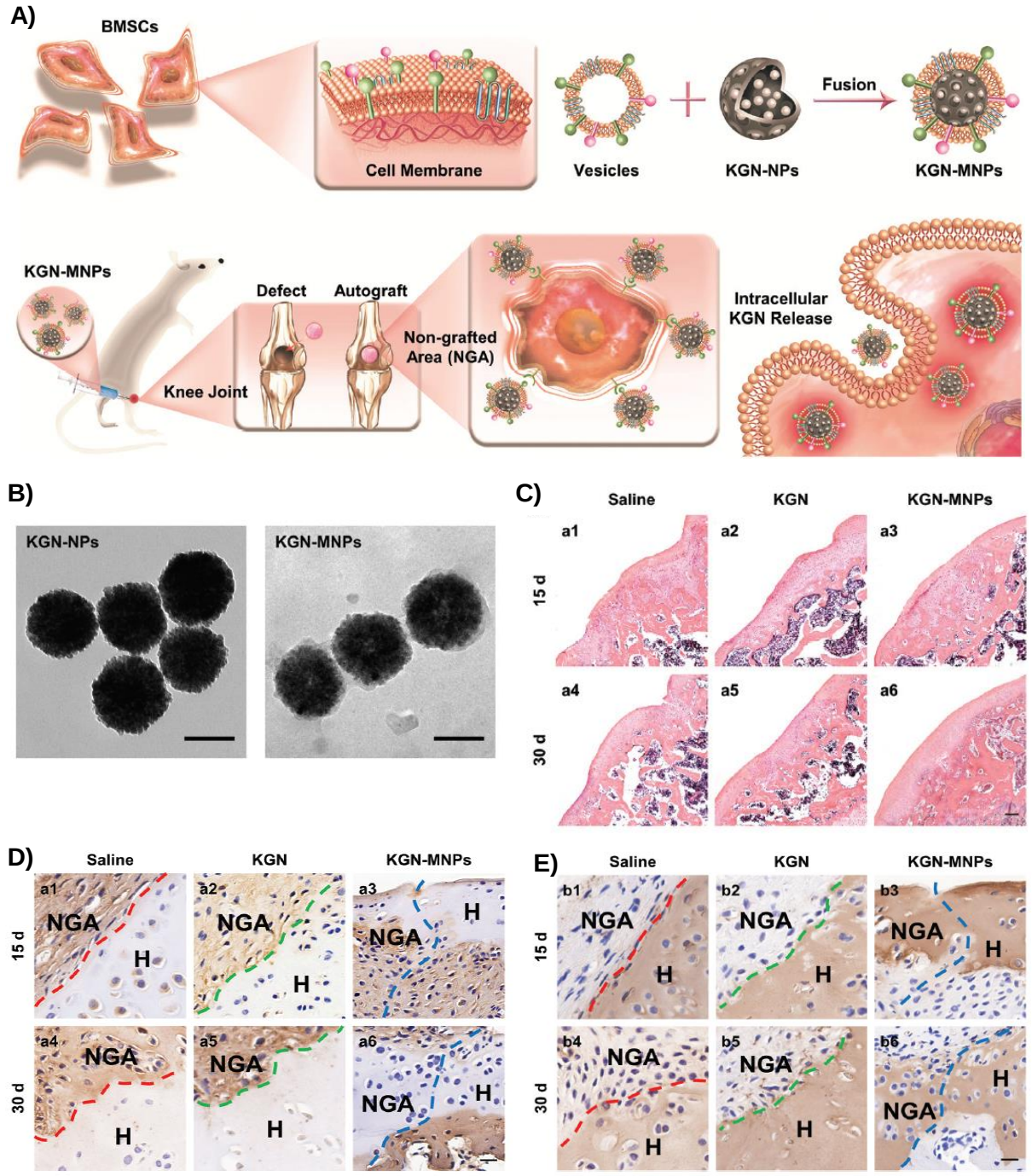


Figure 19. A) Schematic representation of the production of KGN-MNPs and their ability to promote cartilage regeneration in rats with osteochondral autograft transplantation. B) Transmission electron microscopy images of KGN-NPs and KGN-MNPs. Scale bar = 200 nm. C) H&E-stained articular cartilage of rats with osteochondral autograft transplantation 15 and 30 days after intra-articular injection with different treatments. Scale bar = 200 μm. D) and E) Immunohistochemical staining of non-grafted area (NGA) and the host cartilage (H) regarding the formation of type I collagen (D) and type II collagen (E). Scale bar = 20 μm. Reproduced with permission from reference [147].

Abbreviations: BMSC – bone marrow mesenchymal stem cell; H&E – hematoxylin and eosin; KGN – kartogenin; KGN – MNP – bone marrow mesenchymal stem cell membrane-coated kartogenin-loaded iron oxide nanoparticle, referred to in the text as KGN-Fe_3O_4@STCM ; KGN-NP – kartogenin-loaded iron oxide nanoparticle.