UNIVERSIDADE D
COIMBRA

Diana Castro Peixoto

Relatório de Estágio e Monografia intitulada "The Role of Nanoclays in Cancer Therapy" referentes à Unidade Curricular "Estágio", sob orientação da Dra. Maria José Cunha e da Professora Doutora Ana Cláudia Santos, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

Setembro de 2020

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Setembro 2020

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Coimbra, 28 de setembro de 2020.

Diana Castro Peixoto

(Diana Castro Peixoto)

À memória de Carlos Peixoto.

"No matter what anybody tells you,

words and ideas can change the world."

Dead Poets Society

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RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

PARTE I

janeiro a agosto de 2020

Lista de Abreviaturas

DCI	Denominação Comum Internacional
DP	Doença de Parkinson
DPOC	Doença Pulmonar Obstrutiva Crónica
FMJ	Farmácia Maria José
MICF	Mestrado Integrado em Ciências Farmacêuticas
MNSRM	Medicamentos Não Sujeitos a Receita Médica
MSRM	Medicamentos Sujeitos a Receita Médica
PVA	Preço de Venda a Farmácia
PVP	Preço de Venda ao Público
SM	Sintoma Motor
SNM	Sintoma Não Motor
SNS	Serviço Nacional de Saúde
SWOT	*Strengths, Weaknesses, Opportunities, Threats*

Resumo

O Estágio Curricular em Farmácia Comunitária, como parte integrante do ciclo de estudos do Mestrado Integrado em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade de Coimbra, é uma componente imprescindível na formação de futuros farmacêuticos. O presente relatório, realizado sob a forma de uma análise *SWOT* (*Strengths, Weaknesses, Opportunities, Threats*), tem como objetivo apresentar os prontos fortes e fracos, bem como as oportunidades e ameaças sentidas no decorrer do Estágio Curricular na Farmácia Maria José, entre os meses de janeiro e agosto de 2020.

Palavras-Chave: Estágio Curricular, *SWOT*, Farmácia Comunitária, Farmacêutico.

Abstract

The Curricular Internship in Community Pharmacy, as part of the Master's Degree in Pharmaceutical Sciences of the Faculty of Pharmacy of the University of Coimbra, is of utmost importance in the education of future pharmacists. The present report, conducted in the form of a SWOT analysis (Strengths, Weaknesses, Opportunities, Threats), has the objective to present the strengths and weaknesses, as well as the opportunities and threats experienced during the Curricular Internship at Maria José Pharmacy, from January to August 2020.

Keywords: Curricular Internship, Community Pharmacy, Pharmacist.

1. Introdução

A farmácia comunitária, área nobre do saber farmacêutico, surge atualmente como um espaço multidisciplinar privilegiado no contacto com a população, sendo, por isso, frequentemente a porta de entrada no Serviço Nacional de Saúde (SNS). Desta forma, o farmacêutico assume um papel preponderante para assegurar o Primado da Saúde, regendo-se sempre por princípios deontológicos que garantem que a vida humana é o valor máximo a promover e a salvaguardar em quaisquer circunstâncias [1].

No âmbito do Mestrado Integrado em Ciências Farmacêuticas (MICF), o Estágio Curricular surge, com conotação de Unidade Curricular, de forma a proporcionar ao estudante não só a possibilidade de aplicar os seus conhecimentos científicos como também a oportunidade para adquirir um vasto leque de conhecimentos capazes de o dotar de ferramentas únicas que o diferenciam enquanto futuro profissional de saúde.

Neste sentido, o meu estágio em Farmácia Comunitária, realizado na Farmácia Maria José (FMJ), localizada na Vila de Arões São Romão, decorreu entre os meses de janeiro e agosto, sob a orientação da Diretora Técnica, a Dra. Maria José Cunha. Esta oportunidade assumiu uma especial relevância tanto a nível profissional pelos conhecimentos adquiridos, como também a nível pessoal pela experiência complexa que experienciei, contactos que estabeleci e desafios que tive de superar diariamente. Definitivamente, esta é uma das etapas mais enriquecedoras do MICF, visto que, é um momento ímpar de aprendizagem e evolução, é a interface entre o mundo académico e o mundo profissional onde podemos, finalmente, ser aquilo que sempre sonhamos ser, farmacêuticos.

O presente relatório é relativo ao estágio efetuado e tem como objetivo elucidar sobre a reflexão do mesmo, tendo em conta a sua relevância, bem como o enquadramento e a pertinência do plano de estudos do MICF no que concerne às perspetivas profissionais. Assim, sob a forma de uma análise *SWOT* (*Strenghts, Weaknesses, Opportunities, Threats*), pretendo analisar, tanto da perspetiva interna (Strenghts, *Weaknesses*) como da perspetiva externa (*Opportunities, Threats*), aquilo que foi a minha experiência enquanto estagiária na FMJ.

2. Análise SWOT

A Figura 1 representa o esquema-resumo da análise *SWOT* no que diz respeito ao Estágio Curricular realizado na FMJ.

Figura 1: Esquema da Análise *SWOT* do Estágio Curricular realizado em Farmácia Comunitária

A. Pontos Fortes (*Strengths*)

A.1. Integração na equipa

Durante todo o período do Estágio Curricular tive o privilégio de ser a única estagiária na FMJ Se numa fase inicial esta circunstância poderia ser algo constrangedor, rapidamente se assumiu como um dos pontos mais positivos do estágio. Ao longo do meu percurso enquanto estagiária, toda a equipa centrou a sua atenção na minha evolução com o propósito de alcançarmos a minha confiança nas decisões tomadas, tanto a nível interno, como com os utentes, e, consequentemente, na minha autonomia enquanto futura farmacêutica. Deste modo, o auxílio ao meu desempenho foi uma constante, existindo sempre uma transmissão de conhecimentos contínua e construtiva, pois em todas as tarefas por mim realizadas as minhas dúvidas eram esclarecidas e os meus lapsos corrigidos. Para além da colaboração constante, foi notório ainda o interesse nas minhas sugestões de melhoria, tendo sido demonstrado cuidado e atenção a estas. Na FMJ tive a oportunidade de observar que a qualidade dos serviços prestados e o seu sucesso afirmam-se através do trabalho em equipa. Juntamente com 8 colaboradores incansáveis, aprendi que o trabalho em equipa potencia a criatividade de todos os elementos e permite não só uma complementaridade de funções, mas também a partilha de conhecimentos e esforços.

A.2. Aprendizagem gradual

Apesar de não ter um plano de estágio restrito e premeditado, a aquisição de conhecimentos através das tarefas que me foram propostas foi extremamente gradual, permitindo-me adquirir as competências necessárias em cada etapa para avançar para a seguinte.

A fase inicial do meu estágio destinou-se ao trabalho de *backoffice*, onde desempenhei, fundamentalmente, tarefas de receção de encomendas e respetiva verificação (produtos, estado das embalagens, quantidades e prazos de validade), bem como armazenamento dos produtos nos respetivos locais de acordo com o método *first in-first out*. Estas funções proporcionaram um importante contacto com os medicamentos e produtos comercializados na farmácia e, simultaneamente, uma indispensável familiarização com os nomes comerciais e com os respetivos locais de armazenamento que me seriam úteis, posteriormente, durante o atendimento. Por outro lado, esta fase do estágio providenciou-me a possibilidade de consolidar conhecimentos relativos ao preço de venda a farmácia (PVA) e preço de venda ao público (PVP) tendo em conta as margens definidas, quer para os medicamentos não sujeitos a receita médica (MNSRM), quer para outros produtos de saúde e bem-estar comercializados na farmácia. Permitiu-me, ainda, fomentar conhecimentos de gestão da farmácia por análise dos

fornecedores, nomeadamente dos produtos que advinham de cada um, respetivas quantidades e estratégia na realização das encomendas.

Ao longo do estágio tive também oportunidade de desempenhar outras funções, como a gestão e regularização de devoluções, a regularização de notas de crédito, catalogar as listagens de vendas mensais de medicamentos psicotrópicos e respetivo envio atempado para a entidade reguladora, observar o registo semanal das condições de humidade e temperatura nas instalações e temperatura nos frigoríficos, organizar o *dossier* com as informações referentes aos medicamentos manipulados efetuados na farmácia, observar o encerramento da faturação mensal no último dia de cada mês e gerir o receituário de acordo com os diversos subsistemas de saúde e respetivo envio para as entidades competentes.

A execução de todas as tarefas mencionadas preenche grande parte do período de trabalho da farmácia e, por isso, assume um papel de extrema importância no sentido em que é a base para o bom funcionamento e manutenção da sustentabilidade financeira da mesma.

Simultaneamente, foi também possível iniciar a aplicação prática de conhecimentos junto do utente através da análise de parâmetros antropométricos e bioquímicos constituindo assim o primeiro contacto direto junto dos utentes da farmácia. Durante estas semanas que sucederam o início do estágio foi possível integrar-me plenamente e de forma gradual nas dinâmicas da equipa e da farmácia, o que se demonstrou imprescindível para que o atendimento ao público fosse realizado com a devida qualidade. Desta forma, considero que um atendimento personalizado e focado nas necessidades do utente, apenas é alcançado se o profissional de saúde tiver conhecimentos de base bem consolidados no que diz respeito às matérias de *backoffic*e.

A.3. Adequação do plano curricular do MICF

Estando o ato farmacêutico associado a uma enorme responsabilidade e exigência, é imperativo que os estudantes adquiram todas as bases que lhe permitam o exercício do mesmo na sua plenitude. No decorrer do meu estágio senti que a robustez científica do plano curricular do MICF foi imprescindível, por isso, não poderia deixar de o reconhecer nesta análise. Considero que o plano curricular do MICF está de acordo com a atualidade e que se caracteriza por uma abrangência científica singular, em virtude da diversidade de unidades curriculares que comtempla e que são capazes de facultar todas as ferramentas necessárias. Deste modo, dou particular relevo às unidades curriculares de Farmacologia (desde a geral à II), Farmacoterapia, Indicação Farmacêutica, Dermofarmácia e Cosmética, Organização e Gestão Farmacêutica e Farmácia Galénica, uma vez que os conteúdos mencionados pelas

mesmas ao longo da minha formação foram aqueles que considerei de maior utilidade no decorrer do estágio. Assim, não esquecendo a importância de todas as outras unidades curriculares que constituem o MICF, toda a formação que adquiri ao longo de nove semestres contribuiu para o sucesso das tarefas desempenhadas durante o meu estágio.

A.4. Preparação de medicamentos manipulados

Atualmente os avanços científicos no sector da tecnologia farmacêutica convergem na criação de novas fórmulas farmacêuticas, o que, consequentemente, diminui a atual procura e preparação de medicamentos manipulados. No entanto, para casos de ajuste posológico em formulações de uso pediátrico, preparações não existentes no mercado e patologias específicas que requeiram uma terapêutica personalizada, a preparação de medicamentos manipulados continua a ser uma realidade [2]. A FMJ prepara medicamentos manipulados com alguma frequência, possuindo instalações e equipamentos adequados, matérias-primas devidamente arrumadas e identificadas, documentos oficiais e obrigatórios. Considero a minha participação na preparação de alguns medicamentos manipulados um importante ponto positivo do meu estágio, por me ter permitido colocar em prática os conhecimentos teóricos e laboratoriais adquiridos em Farmácia Galénica. Realço a preparação de uma solução alcoólica de ácido bórico à saturação, cuja ficha de preparação se encontra no Anexo III, indicada para o tratamento de uma otite externa devido ao seu efeito antissético e desinfetante, apresentando ação bacteriostática e fungistática. Também participei na preparação de uma pomada propriamente dita que contém Dermovate®, cuja ficha de preparação se encontra no Anexo IV, um corticosteroide de aplicação tópica indicado no tratamento de patologias da dele em que se desenvolvem processos inflamatórios, nomeadamente lúpus eritematoso, eczema e psoríase (exceto psoríase generalizada em placas) [3].

B. Pontos Fracos (*Weaknesses*)

B1. Tipologia dos utentes

Fundada no dia 11 de junho de 1984, a FMJ é uma farmácia local com história e de localização estratégica, atualmente apresenta-se como farmácia de passagem para a esmagadora maioria dos utentes a frequentar unidade de saúde familiar local. Desde o dia de abertura que a direção técnica e os seus colaboradores primam por ajudar incessantemente todos os utentes criando um ambiente de proximidade e de confiança que considero que foram, sem dúvida, a chave para o vasto leque de clientes fidelizados, principalmente para a dispensa de medicamentos sujeitos a receita médica (MSRM). No entanto, aquela que aparentemente é uma vantagem, por vezes, no decorrer do meu estágio tornou-se uma amarga realidade. Frequentemente, vários utentes, tentaram beneficiar da humildade e disponibilidade

da equipa, para tentar comprar MSRM sem a devida prescrição, ameaçando, inclusivamente, perante uma resposta negativa, a deslocação a outra farmácia para a devida aquisição. Além disso, após solicitação de MNSRM ou de produtos de saúde e bem-estar, geralmente os utentes demonstravam insatisfação às questões colocadas para o correto aconselhamento. Este tipo de situações foi extremamente recorrente ao longo do estágio, causando incómodo não só no meu desempenho, como também para os utentes presentes no estabelecimento aquando destes episódios.

B.2. Imagem do estagiário perante a população

No decorrer do estágio, várias foram as vezes que os utentes demonstraram descontentamento e desconfiança por serem atendidos por uma estagiária, mesmo com vigilância do orientador. Notei que para muitos utentes um estagiário é um colaborador sem competência e conhecimento e, por isso, solicitavam um atendimento por parte de outros elementos presentes na farmácia. Perante esta dificuldade, procurei desmistificar aquela que é a imagem de um estagiário, explicando que este possui um vasto conhecimento científico que precisa de ser complementado pela prática em cedência de medicamentos e aconselhamento. Além disso, esforcei-me para que reconhecessem o meu valor no momento de atendimento e que me vissem como uma figura capaz de satisfazer os seus pedidos da melhor forma.

C. Oportunidades (*Opportunities*)

C.1. Participação em formações internas

A formação contínua dos colaboradores de uma equipa técnica é fundamental para se manterem atualizados face à introdução permanente de novos produtos no mercado. Durante o meu estágio tive oportunidade de participar em diversas formações internas facultadas por delegados de informação médica de diferentes laboratórios. As formações eram geralmente de curta duração, durante o período de funcionamento da farmácia e destinavam-se essencialmente à apresentação das indicações terapêuticas, vantagens e técnicas de venda para os produtos já comercializados ou para as novidades. Pude, assim, participar em formações providenciadas pela Aboca® sobre o dispositivo médico GOLAMIR 2 ATC®, pela Bayer® sobre a gama Bepanthene® e pela Mylan ® sobre a solução antisséptica Betadine®, o Venoparil® para a insuficiência venosa, o Pyralpen® no tratamento sintomático de aftas e, por fim, sobre o EndWarts PEN® indicado para as verrugas cutâneas. De facto, estas formações assumem-se como uma ferramenta de suporte ao aconselhamento dos utentes, não só pelo seu carácter informativo, como também por possibilitarem o esclarecimento de dúvidas por parte dos colaboradores.

C.2. Dinamismo crescente da farmácia

A farmácia é um espaço público de saúde onde o utente formula uma opinião sobre esta desde que vê a montra e entra até ao momento que sai da farmácia. Tendo isto em conta, procurei dinamizar as montras da FMJ, pois para além de serem o primeiro contacto comercial com o utente, também refletem a personalidade da equipa técnica e a qualidade do serviço. Deste modo, numa fase inicial do meu estágio decidi, após aprovação da direção técnica, utilizar as montras como um meio para transmitir mensagens no âmbito da intervenção cívica e promoção para a saúde, alertando os utentes, por exemplo, para a importância da cessação tabágica, para o atual consumo excessivo de açúcar e para a relevância de um estilo de vida saudável (Anexo VI). Foi notória uma satisfação por parte dos utentes e, por isso, as montras também começaram a ser dinamizadas em datas comemorativas como o Dia dos Namorados, Carnaval e o Dia Mundial da Criança (Anexo VII).

Adicionalmente, percebi que o espaço interior da farmácia era pouco explorado, por isso, sugeri uma nova organização da zona de atendimento ao público, de forma a criar um espaço organizado de forma lógica, acolhedor e cativante, com a capacidade de despertar a atenção para as compras por impulso. Nesta fase, os conhecimentos adquiridos nas unidades curriculares de Organização e Gestão Farmacêutica e Comunicação e Marketing foram essenciais para fundamentar as minhas ideias durante a apresentação do plano de melhoria a todos os colaboradores, que foi aprovado e implementado. Após todas as alterações serem efetuadas, participei ativamente na renovação da decoração da farmácia, tendo sempre em conta a sazonalidade das patologias mais frequentes, as campanhas promocionais das várias marcas e as datas comemorativas (Anexo VIII). A nível da organização interna foi também possível verificar uma evolução, nomeadamente a nível da organização do *backoffice* (Anexo IX).

Preocupada com a atualização da farmácia face ao mercado e à concorrência, propus uma dinamização superior na rede social *Facebook* e a criação de uma página na rede social *Instagram*, visto que são meios de comunicação úteis tanto para atrair o público mais jovem, como para transmitir as mais variadas informações (Anexo IX).

Participei no processo de desenvolvimento de um programa para auxiliar na preparação e administração de medicação de forma fácil e segura, direcionado para utentes institucionalizados em lares. De um modo geral, o programa inclui *stocks* de medicamentos por utente e um registo rápido e detalhado das terapêuticas de cada utente. É, ainda, essencial no

auxílio da preparação individualizada da medicação, pois permite a visualização do blister no momento da preparação da medicação (Anexo XI).

Durante o período em que estive a estagiar na FMJ, foi notória uma evolução na dinâmica da farmácia. Esta evolução foi muito importante na minha aprendizagem por poder ter participado nela ativamente, pelas minhas ideias poderem ter sido debatidas internamente, e, acima de tudo, por me fazer sentir a verdadeira satisfação de poder contribuir de forma positiva para a sustentabilidade da farmácia.

D. Ameaças (*Threats*)

D.1. Medicamentos esgotados

Foram inúmeras as situações em que, ao longo do estágio, fui confrontada com o desagrado e frustração utentes, perante a impossibilidade satisfazer as suas necessidades devido à quantidade de medicamentos esgotados ou rateados. A realidade dos medicamentos esgotados é cada vez mais frequente graças às imposições que reduzem os preços dos medicamentos em Portugal e, por conseguinte, tornam mais rentável a sua exportação para outros países onde os preços são expressivamente superiores. Para além de condicionar negativamente a saúde dos utentes, esta situação reflete-se num enorme transtorno para as farmácias, uma vez que os colaboradores consomem grande parte do tempo do atendimento na tentativa de explicar o sucedido e encontrar alternativas para aos utentes afetados, bem como a analisar todas as vias alternativas possíveis para obter os medicamentos em causa. Decorrente desta situação, o tempo de atendimento aumenta consideravelmente, levando ao descontentamento dos utentes que, na maioria dos casos, não compreendem que a responsabilidade desta situação não é da farmácia e, consequentemente, perdem a confiança que haviam depositado na mesma.

Recordo o caso marcante da indisponibilidade do medicamento Victan®, substância ativa loflazepato de etilo, indicado para a ansiedade e sintomas ansiosos [4]. A toma deste medicamento induz, frequentemente tolerância, dependência física e psíquica, estes efeitos colaterais aliados à falta deste em *stock* provocaram ansiedade e preocupação nos utentes e, consequentemente, em toda a equipa técnica [5]. Os colaboradores responsáveis pela gestão e receção das encomendas de tudo faziam para repor os *stocks*, mas sem sucesso. Logicamente que esta situação se assumiu como uma ameaça ao meu estágio, sobretudo no ato da dispensa, por me impedir de satisfazer as necessidades dos utentes.

D.2. Estatuto do medicamento genérico

Atualmente, a prescrição de um medicamento inclui a respetiva denominação comum internacional (DCI) da substância ativa, a forma farmacêutica, a dosagem, a apresentação, a quantidade e a posologia [6]. Desta forma, os utentes podem optar por adquirir medicamentos genéricos ou medicamentos originais, devendo ser sempre questionados, pela sua preferência durante o ato da dispensa [7]. O medicamento genérico é um medicamento com a mesma substância ativa, forma farmacêutica, dosagem e com a mesma indicação terapêutica que o medicamento original que serviu de referência, de marca. Contudo, apesar de os medicamentos genéricos apresentarem comprovadamente a mesma segurança e eficácia traduzidas por estudos de bioequivalência adequados, ou quando estes não forem adequados, equivalência terapêutica por meio de estudos de farmacologia, vários utentes permanecem hesitantes quanto à sua utilização.

Esta realidade constituiu uma ameaça durante o meu estágio pela dificuldade que senti em dispensar medicamentos genéricos, sobretudo a utentes idosos. Saliento, ainda, que muitos recusavam adquirir a medicação crónica por indisponibilidade do medicamento original, mesmo tendo explicado o conceito de medicamento genérico. Esta problemática requer, evidentemente, uma maior atenção por parte do setor farmacêutico, para que se promova o conhecimento e desmistifiquem os falsos receios relativamente aos medicamentos genéricos.

D.3 Limite na venda de medicamentos

Na última semana do meu estágio, a farmácia passou a estar limitada a dispensar no máximo duas embalagens de cada medicamento, por linha de prescrição, ou 4 embalagens, no caso de estas serem de dose unitária, por mês e por utente. A restrição surgiu de uma portaria de 2016 que apenas foi implementada informaticamente no dia 3 de agosto do presente ano. Mediante justificação, a farmácia pode dispensar quantidades superiores, particularmente quando a quantidade de embalagens necessária para cumprir a posologia é superior a 2 embalagens por mês, no caso de extravio, perda ou roubo de medicamentos, se houver dificuldade de deslocação à farmácia ou em caso de ausência prolongada do país [8]. O objetivo da medida é minimizar ocorrência de fraude nos MSRM e, paralelamente, possibilitar um controlo mais rigoroso da despesa do SNS, sem que a mesma tenha a ver com falta de medicamentos ou dificuldades na gestão de *stocks*. A medida pode ainda revelar-se positiva no combate ao desperdício devido a prazos de validade expirados neste tipo de medicamentos.

Os utentes, na sua plenitude de direitos enquanto clientes, questionavam sempre esta problemática, não entendendo muitas vezes a inimputabilidade da farmácia neste caso.

Situações como esta colocaram em risco o meu desempenho durante o atendimento pela incompreensão dos utentes.

3. COVID-19: Uma ameaça ou uma oportunidade?

A 2 de março do presente ano foi confirmado o primeiro caso de COVID-19 em Portugal, um vírus que eclodiu a partir da China e que rapidamente se disseminou por todo o globo. Os dados relativos à forma de transmissão do COVID-19, ainda, não estão totalmente estabelecidos, embora se saiba que a doença tem capacidade de transmissão de pessoa para pessoa. Não existe, à data, nenhuma vacina ou tratamento específico para a infeção por SARS-CoV-2, sendo que o tratamento é sintomático e adequado a cada caso. Assim, a forma mais eficaz de combater o COVID-19 é a prevenção do contágio [9].

Numa fase inicial ocorreu uma corrida à farmácia, verificando-se uma procura desmedida de material de proteção individual, máscaras e luvas, de soluções antissépticas de base alcoólica bem como de medicamentos. Um cenário de gestão complicada pois a farmácia tentava adotar medidas de prevenção, que eram desvalorizadas pelos utentes, e simultaneamente tentava responder de forma equitativa a todos os utentes [10]. Nesta fase ainda não tinham sido determinadas as boas práticas e estratégias a adotar pelas farmácias, pelo que, o atendimento ao público começou a revelar-se um pouco difícil, pois a maioria dos utentes ainda não reconhecia a necessidade da adoção das devidas medidas de prevenção e entravam em conflito com os colaboradores. Face a esta situação, o meu estágio foi interrompido no dia 16 de março. Nesse mesmo dia, a autoridade nacional do medicamento e produtos de saúde, Infarmed, emitiu a Norma 003/2020 que veio reforçar e regulamentar medidas de prevenção, indicando que estas tinham de ser cumpridas pelas farmácias de forma garantir a segurança dos seus colaboradores e de todos os cidadãos. As medidas a adotar pela farmácia incluem, a elaboração de protocolos de limpeza e intensificação das rotinas de higienização, a disponibilização de solução antissética de base alcoólica quer para os utentes quer para todos os colaboradores, a utilização de equipamentos de proteção e realização do atendimento através do postigo, entre outras [11]. A 19 de março, foi decretado o Estado de Emergência Nacional, o que obrigou a um confinamento até 2 de maio [12]. No final deste período Portugal passou para a situação de calamidade iniciando-se o desconfinamento [13], foi nesta fase que retomei o meu estágio na FMJ sob o regime de turnos, por forma a limitar o contacto entre colaboradores e, por conseguinte, assegurar a manutenção do serviço farmacêutico. Neste regresso deparei-me com um cenário completamente diferente. O número de utentes era limitado na zona de atendimento, sendo solicitado aos restantes que aguardassem na zona

exterior da farmácia. O chão da farmácia estava marcado de forma a garantir a distância mínima de pelo menos 1 a 2 metros entre os colaboradores e os utentes.

Depois de um longo período a "suster a respiração" a farmácia começou a deparar-se com uma nova "normalidade", onde tive a possibilidade de assumir, tal como todo os colaboradores um papel informativo. Aquando do atendimento para além do esclarecimento de dúvidas dos utentes, referia sempre a importância da adoção das medidas de prevenção com destaque para a lavagem correta das mão e adoção das medidas de etiqueta respiratória, salientando que estas medidas são complementares, não uma alternativa. Adicionalmente, para reforço da informação transmitida verbalmente, entregava suportes informativos. Ainda no contexto do atendimento, a interrupção de tratamentos de doenças crónicas, o aumento de problemas do foro psicológico e a diminuição significativa da procura de produtos de saúde e bem-estar tornaram-se uma realidade. Perante estas situações, a farmácia tudo fez para garantir a continuidade dos tratamentos e para transmitir calma e segurança a todos os seus utentes.

No entanto, do caos provocado pela COVID-19 também emergiram várias oportunidades, nomeadamente a adoção de medidas mais corretas para evitar a transmissão do vírus Influenza e o destaque para a importância da vacinação. Realço, ainda, possibilidade de cedência em ambulatório de medicamentos de dispensa exclusiva hospitalar e a dispensa ao domicílio (Anexo XII) [11]. Serviços que já estavam a ser debatidos há algum tempo e que estavam à espera de uma oportunidade.

Se esta nova realidade que surgiu no meio de tantos outros desafios, inicialmente se apresentou como uma ameaça ao meu estágio, devido à interrupção do mesmo. Mais tarde, demonstrou ser uma oportunidade de contactar com um significativo volume legislativo dirigido ao setor farmacêutico e de experienciar a motivação e o grande sentido de missão e responsabilidade de todos os colaboradores a trabalhar incansavelmente numa farmácia a ser testada ao limite. Esta fase, sem dúvida, foi o pináculo do meu estágio, pois considero que em todos os momentos da prática farmacêutica é sempre bom aprender.

4. Considerações Finais

Terminado o estágio curricular na FMJ, reconheço importância que este tem enquanto etapa final do plano curricular do MICF. Esta é a etapa final em que os conhecimentos técnicos e científicos adquiridos até então são aperfeiçoados, através da experiência a nível profissional. Mais do que adquirir e aperfeiçoar conhecimentos enquanto futura farmacêutica, este estágio proporcionou-me uma enriquecedora aprendizagem a nível pessoal. Devido a uma equipa de enorme singularidade e profissionalismo, sempre focada na minha aprendizagem, fui gratificada com uma evolução constante que me deu conhecimento daquilo que é o quotidiano da farmácia comunitária, das dificuldades, bem como dos desafios que me esperam no futuro, permitindo-me agora encarar o ambiente profissional com um maior otimismo e segurança.

Ser farmacêutico, definitivamente, é ser mais do que um mero vendedor de medicamentos, é ser um especialista do medicamento e verdadeiro conhecedor das pessoas e da doença. A excelência da profissão farmacêutica centra-se no indubitável contributo que esta tem na saúde da população e na evidente preocupação com o seu bem-estar e com a sua qualidade de vida não só no dia-a-dia, como também numa situação de surto e pandemia.

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Anexos

Anexo I - Receita para preparação de Medicamento Manipulado.

Receita Médica Materializada Eletrónica para preparação de uma solução alcoólica de ácido bórico à saturação.

Anexo II -Tabela alcoométrica da Farmacopeia Portuguesa.

Preparação de Misturas de Etanol e Água com Diferentes Graduações

Quantidades (em massa) de álcool de um determinado grau e de água purificada para a obtenção de 1kg de mistura hidroalcoólica

Graduação do álcool a diluir (Grau centesimal)	Graduação Pretendida (Grau centesimal)																	
	95		90		85		80		75		70		65		60		55	
	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água	Álcool	Água
100	925	75	855	145	795	205	730	270	675	325	620	380	570	430	510	490	460	540
99	942	58	871	129	810	190	743	257	690	310	631	369	581	419	519	481	468	532
98	954	46	885	115	823	177	755	245	707	293	643	357	588	412	529	471	477	523
97	968	32	895	105	836	164	768	232	716	284	654	346	595	405	538	462	485	515
96	983	17	911	89	848	152	782	218	723	277	665	335	603	397	547	453	493	507
95	-	-	926	74	860	140	795	205	733	267	676	324	610	390	556	444	501	499
94	-	-	941	59	871	129	808	192	745	255	687	313	620	380	565	435	509	491
93	-	-	957	43	885	115	822	178	758	242	698	302	631	369	575	425	518	482
92	-	-	973	27	910	90	836	164	770	230	710	290	641	359	584	416	526	474
91	-	-	988	12	913	87	847	153	781	219	719	281	650	350	592	408	534	466
90	-	-	-	-	929	71	858	142	791	209	729	271	658	342	600	400	541	459
85	-	-	-	-	-	-	924	76	851	149	784	216	708	292	670	330	582	418
80	-	-	-	-	-	-	-	-	921	79	849	151	767	233	698	302	630	370
75	-	-	-	-	-	-	-	-	-	-	921	79	832	168	757	243	683	317
70	-	-	-	-	-	-	-	-	-	-	-	-	903	97	822	178	741	259
65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	910	90	821	179
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	901	99

Anexo III - Ficha de preparação de Medicamento Manipulado: Solução alcoólica de ácido bórico à saturação.

FARMÁCIA MARIA JOSÉ. UNIP., LDA.
NIF: 510 481 361
Av. da Torre, 260 Arões - S. Romão
4820-758 FAFE
Tel. 253 593 197 - Fax: 253 593 056

Ficha de Preparação

Medicamento: Solução alcoólica de Ácido Bórico à saturação

Teor em substância(s) activa(s): 100 g (ml ou unidades) contêm 5.0 g (ml) de ácido bórico

Forma farmacêutica: Solução Data de preparação: 04.03.2020

Número do lote: 13620 Quantidade a preparar: _____

Matérias-primas	Lote nº	Origem	Farmacopeia	Quantidade para 100 g (ou ml, ou unidades	Quantidade calculada	Quantidade pesada	Rubrica do Operador e data	Rubrica do Supervisor e data
Ácido Bórico		Laboratório Reagente		5.0g	0.5g	0.5g	Diana P.	
Álcool Etílico 70% (V/V)		AGA		q.b.p 10ml	q.b.p 50ml	q.b.p 50ml	Diana P.	

Preparação

	Rubrica do Operador
1. Verificar estado de limpeza do material.	Diana P.
2. Preparação de álcool a 60º partindo de álcool a 70º através da consulta da tabela alcoométrica da Farmacopeia Portuguesa.	Diana P.
3. Confirmar grau alcoólico (60º) com alcoómetro	Diana P.
4. Colocar em proveta rolhada de 100ml uma quantidade de álcool a 60º correspondente a cerca de 3/4 da quantidade total da solução a preparar.	Diana P.
5. Pesar 0.5g de ácido bórico em papel de pesagem	Diana P.
6. Adicionar 3 porções de ácido bórico, ao álcool a 60º agitando fortemente durante 90 segundos após cada adição	Diana P.

Rubrica do Director Técnico	Data
	04.03.2020

FGP 2001 – 1ª Adenda (2005) 1/5

7. Perfazer volume de álcool a 60° (30 mL) e agitar.	Diana P.
8. Deixar proveta em repouso durante 1 hora, agitando-a, durante as seguintes, de 15 em 15 minutos.	Diana P.
9. Filtrar solução obtida em 8, utilizando um filtro de pregas, para um frasco conta-gotas de vidro âmbar.	Diana P.
10. Rotular devidamente	Diana P.
11. Lavar material utilizado.	Diana.
12.	
13.	
14.	
15.	
16.	

Aparelhagem usada:

Balança analítica
Proveta rolhada de 100 mL
Funil de vidro
Papel de filtro
Frasco de vidro âmbar
Copo de precipitação
Alcoómetro
Esguicho com água destilada

Embalagem

Tipo de embalagem: Frasco de vidro âmbar, tipo III (Ph. Eur.)

Capacidade do recipiente: 30 mL

Material de embalagem	Nº do lote	Origem
Vidro âmbar	(?)	Glicatt

Operador: Diana P.

Rubrica do Diretor Técnico	Data
	04.03.2020

Prazo de utilização e Condições de conservação

Condições de conservação:

conservar em frasco de vidro âmbar, bem fechado, à temperatura ambiente.

Operador: Diana P.

Prazo de utilização:

3 meses nas condições de conservação referidas anteriormente.

Operador: Diana P.

Rotulagem

1. Proceder à elaboração do rótulo de acordo com o modelo descrito em seguida.
2. Anexar a esta ficha de preparação uma cópia, rubricada e datada, do rótulo da embalagem dispensada.

Modelo de rótulo

Identificação da Farmácia
Identificação do Director-Técnico
Endereço e telefone da Farmácia

Identificação do Médico prescritor
Identificação do Doente

DENOMINAÇÃO DO MEDICAMENTO

Teor em substância(s) activa(s) 100 mL de sol. contém 1 g de ácido bórico
Quantidade dispensada 60 mL
Referência a matérias-primas cujo conhecimento seja eventualmente necessário para a utilização conveniente do medicamento contém álcool a 60°
Posologia 3 gotas em cada ouvido, 8x
Via de administração Medicamento para aplicação auricular

Data da preparação 04.03.2020
Prazo de utilização 3 meses (04.06.2020)
Condições de conservação conservar em frasco de vidro âmbar, bem fechado, à temperatura ambiente
Nº do lote 19590
Manter fora do alcance das crianças
Advertências (precauções de manuseamento, etc.) Não ingerir
Uso externo (caso se aplique) (em fundo vermelho)

Operador: Diana P.

Verificação

Ensaio	Especificação	Resultado	Rubrica do Operador
1. características organolépticas 1.1. Aspeto	Solução límpida e transparente	conforme	Diana P.

Rubrica do Director Técnico	Data
	04.03.2020

Ensaio	Especificação	Resultado	Rubrica do Operador
1.2. Odor	Odor característico a álcool etílico	conforme	Diamp.
1.3. Cor	Incolor	conforme	Diamp.
2. conforme com a definição da monografia da farmacopeia portuguesa		conforme	Diamp.

Aprovado [X] Rejeitado [ ]

Supervisor _____ 04/03/2020

Nome e morada do doente

Nome do prescritor

Anotações

Graduação do álcool a diluir	Graduação pretendida		Ácido Bórico: dissolve-se facilmente em água bacteriostático e fungistático. Álcool a 70% (V/V): solúvel em água em todas as proporções.
	Quantidade de álcool	Quantidade de água	
90 %	82.8 g	17.2 g	

Rubrica do Director Técnico	Data
	04-03-2020

Cálculo do preço de venda

MATÉRIAS-PRIMAS:

matérias-primas	embalagem existente em armazém		preço de aquisição de uma dada quantidade unitária (s/IVA)		quantidade a usar	factor multiplicativo	valor da matéria-prima utilizada na preparação
	quantidade adquirida	preço de aquisição (s/IVA)	quantidade unitária	preço			
Ácido Bórico		0.60	30 g	0.02	x 2.5 g	x 2.0	= 0.19
Álcool Etílico		3.96	250 mL	0.01584	x 30 mL	x 2.0	= 1.28
					x	x	=
					x	x	=
					x	x	=
					x	x	=
					x	x	=
						subtotal A	1.86

HONORÁRIOS DE MANIPULAÇÃO:

	forma farmacêutica	quantidade	F(€)	factor multiplicativo	valor
valor referente à quantidade base	Solução	30 mL	3.03	x 3	= 13.09
valor adicional			x	x	=
				subtotal B	13.09

MATERIAL DE EMBALAGEM:

materiais de embalagem	preço de aquisição (s/IVA)	quantidade	factor multiplicativo	valor
Exsam monta - gotas	1.13	x 1	x1,2	= 1.356
		x	x1,2	=
		x	x1,2	=
		x	x1,2	=
			subtotal C	1.356

PREÇO DE VENDA AO PÚBLICO DO MEDICAMENTO MANIPULADO: (A + B + C) x 1,3 = 18.1368

IVA = 23% + IVA = 6.1

D = 24.50

DISPOSITIVOS AUXILIARES DE ADMINISTRAÇÃO:

dispositivo	preço unitário	quantidade	valor
			E =

PREÇO FINAL: D + E = 24.50

Operador Diana P.

Supervisor

Rubrica do Director Técnico	Data
	04.03.2020

Anexo IV - Ficha de preparação de Medicamento Manipulado: Pomada que contém Dermovate®.

FARMÁCIA MARIA JOSÉ, UNIP, LDA.
NIF: 510 481 361
Av. da Torre, 260 Arões · S.Romão
4820-758 FAFE
Tel. 253 493 187 - Fax: 253 993 056

Ficha de Preparação

Medicamento: Pomada que contém Dermovate®

Teor em substância(s) activa(s): 100 g (ml ou unidades) contêm 0.0185 g (ml) de propionato de clobetasol

Forma farmacêutica: Pomada propriamente dita Data de preparação: 20.06.2020

Número do lote: 13520 Quantidade a preparar: 160g

Matérias-primas	Lote nº	Origem	Farmacopeia	Quantidade para 100 g (ou ml, ou unidades	Quantidade calculada	Quantidade pesada	Rubrica do Operador e data	Rubrica do Supervisor e data
Dermovate® creme		GSK		37.5g	60g	60g	Diana P.	
ATC creme hidratante		Edol		60.5g	100g	100g	Diana P.	

Preparação Rubrica do Operador

1. Verificar estado de limpeza do material.	Diana P.
2. Descondicionamento das especialidades farmacêuticas Dermovate® e ATC creme hidratante	Diana P.
3. Colocar Dermovate® no centro da pedra de marmore	Diana P.
4. Adicionar, gradualmente, ATC creme hidratante, espatulando cuidadosamente, de modo a obter uma pomada homogenia, de superfície lisa e macia ao tato	Diana P.
5. Acondicionar em frasco de plástico opaco	Diana P.
6. Rotular devidamente.	

Rubrica do Director Técnico	Data
	20.06.2020

7.	
8.	
9.	
10.	
11.	
12.	
13.	
14.	
15.	
16.	

Aparelhagem usada: Espátula
Pedra de ametista
Frasco de plástico opaco

Embalagem

Tipo de embalagem: Frasco de plástico opaco (FP VIII) "Tapitec®"

Capacidade do recipiente: 250 g

Material de embalagem	Nº do lote	Origem
Plástico		

Operador: Diana P.

Rubrica do Director Técnico	Data
	20.06.2020

Prazo de utilização e Condições de conservação

Condições de conservação:

conservar em frasco de plástico opaco, bem fechado à temperatura ambiente.

Operador: Diana P.

Prazo de utilização: 01/2022 ← data do produto industrializado

6 meses nas condições de conservação mencionadas anteriormente.

Operador: Diana P.

25% do tempo que resta para expirar o prazo de validade do produto industrializado, mesmo mencionadas

Rotulagem

1. Proceder à elaboração do rótulo de acordo com o modelo descrito em seguida.
2. Anexar a esta ficha de preparação uma cópia, rubricada e datada, do rótulo da embalagem dispensada.

Modelo de rótulo

Identificação da Farmácia
Identificação do Director-Técnico
Endereço e telefone da Farmácia

Identificação do Médico prescritor
Identificação do Doente

DENOMINAÇÃO DO MEDICAMENTO

Teor em substância(s) activa(s) 10mg da pomada contém 0,018mg de propionato de clobetasol
Quantidade dispensada 10g
Referência a matérias-primas cujo conhecimento seja eventualmente necessário para a utilização conveniente do medicamento
Posologia
Via de administração cutânea

Data da preparação 20.06.2020
Prazo de utilização 20.12.2020
Condições de conservação conservar em frasco de plástico opaco, bem fechado à temperatura ambiente.
Nº do lote 13626
Manter fora do alcance das crianças
Advertências (precauções de manuseamento, etc.)
Uso externo (caso se aplique) (em fundo vermelho)

Operador: Diana P.

Verificação

Ensaio	Especificação	Resultado	Rubrica do Operador
1. Características organolépticas 1.1. Aspeto	Homogéneo	conforme	Diana P.

Rubrica do Director Técnico	Data
	20.06.2020

Ensaio	Especificação	Resultado	Rubrica do Operador
1.0. cor	Branco	conforme	DiamP.
1.3. Odor	característico do creme hidratante AtL	conforme	DiamP.

Aprovado [X] Rejeitado [ ]

Supervisor _____ 20/06/2024

Nome e morada do doente

Nome do prescritor

Anotações

Dermovate é um corticosteroide de aplicação tópica que pertence ao grupo dos anti-inflamatórios esteroides. Está indicado no tratamento de patologias na pele em que se desenvolvem processos inflamatórios, nomeadamente lúpus eritematoso, eczema e psoríase (exceto psoríase generalizada em placas).
AtL creme hidratante → Base hidratante, principalmente parafinas com propriedades emolientes e hidratantes

Rubrica do Director Técnico	Data
	20.06.2024

Cálculo do preço de venda

MATÉRIAS-PRIMAS:

matérias-primas	embalagem existente em armazém		preço de aquisição de uma dada quantidade unitária (s/IVA)		quantidade a usar	factor multiplicativo	valor da matéria-prima utilizada na preparação
	quantidade adquirida	preço de aquisição (s/IVA)	quantidade unitária	preço			
Dermovate® creme		3,42	30g	0,11€	x 2	x	= 0,22 €
AT2 creme		5,3€	100g	0,053€	x 1	x	= 0,053 €
					x	x	=
					x	x	=
					x	x	=
					x	x	=
					x	x	=
						subtotal A	0,283 €

HONORÁRIOS DE MANIPULAÇÃO:

	forma farmacêutica	quantidade	F(€)	factor multiplicativo	valor
valor referente à quantidade base	Pomada pré-preparamente dita	100g	5,03	x 3	= 15,09
valor adicional		60g	x 5,03	x 1,010	= 0,0503
				subtotal B	15,14

MATERIAL DE EMBALAGEM:

materiais de embalagem	preço de aquisição (s/IVA)	quantidade	factor multiplicativo	valor
	4,05	x 1	x1,2	= 4,86
		x	x1,2	=
		x	x1,2	=
		x	x1,2	=
			subtotal C	4,86

PREÇO DE VENDA AO PÚBLICO DO MEDICAMENTO MANIPULADO:

(A + B + C) x 1,3	26,34
+ IVA	6%
D	27,90

DISPOSITIVOS AUXILIARES DE ADMINISTRAÇÃO:

dispositivo	preço unitário	quantidade	valor
		E	

→ **PREÇO FINAL: D + E** 27,90

Operador Diana P. Supervisor

Rubrica do Director Técnico	Data
	20.06.2020

Anexo V - Casos Práticos

CASO 1

Utente de 60 anos, género masculino, dirigiu-se à farmácia com uma prescrição de Bretaris Genuair® 322 µg para iniciar terapêutica. Queixou-se de cansaço e falta de forças e solicitou uma caixa de Fluimucil®, referindo que é o único medicamento que o faz sentir melhor todas as manhãs.

Questiono se tem alguma patologia diagnosticada e que medicação toma, ao que me responde que apresenta DPOC (Doença Pulmonar Obstrutiva Crónica) e utiliza diariamente e indiscriminadamente vários tipos de inaladores. Esclareci que, tratando-se de DPOC, seria expectável tanto a presença de um padrão de sintomas persistente apesar do tratamento, neste caso com Fluimucil®, bem como a manifestação de dispneia de esforço.

Após uma análise do perfil farmacoterapêutico verifiquei que utilizava há muito tempo 3 corticosteroides concomitantemente com vários broncodilatadores, nomeadamente 3 agonistas adrenérgico β2 de longa duração de ação, 1 agonista adrenérgico β2 de curta duração de ação e 2 anticolinérgicos (Tabela 1).

Tabela 1 - Perfil farmacoterapêutico do utente

MEDICAMENTO	FÁRMACO	GRUPO FARMACOLÓGICO
Ventilan® 5 mg/mL SOS [15]	Salbutamol	Agonista adrenérgico β2 (curta duração de ação)
Bufomix Easyhaler® 320 µg/ 9 µg [16]	Budesonida + Fumarato de Formoterol	Corticosteroide + Agonista adrenérgico β2 (longa duração de ação)
Gibiter Easyhaler® 160 µg/ 4.5 µg [17]	Budesonida + Fumarato de Formoterol	Corticosteroide + Agonista adrenérgico β2 (longa duração de ação)
Incruse Ellipta® 5.5 µg [18] Atrovent® 20 µg [19]	Umeclidínio Brometo de Ipratrópio	Anticolinérgico
Revinty Ellipta® 184 µg/ 22 µg[20]	Furoato de Fluticasona + Vilanterol	Corticosteroide + Agonista adrenérgico β2 (longa duração de ação)

Assim, comecei por explicar que a utilização concomitante de um anticolinérgico e de um agonista adrenérgico β2 de curta duração de ação é uma associação sinérgica no tratamento da DPOC estável, permitindo uma broncodilatação constante. Por conseguinte, não se justifica a utilização em SOS do Ventilan® (salbutamol) nesta patologia, tal como o doente está a utilizar, devendo sim a terapêutica com este fármaco ser contínua. Para além disso,

esclareci que a terapêutica para DPOC deve ser orientada pela gravidade da doença, sendo os doentes classificados de acordo com a intensidade dos sintomas e da incidência das exacerbações. Deste modo, no caso de o utente apresentar duas ou mais agudizações por ano, recomenda-se a substituição do agonista adrenérgico β2 de curta duração de ação por um agonista adrenérgico β2 de longa duração de ação presente, por exemplo, no Bufomix Easyhaler®, no Gibiter Easyhaler® e no Revinty Ellipta®. Se apresentar um perfil de alto risco associado a poucos sintomas recomenda-se a associação de um corticosteroide com um agonista adrenérgico β2 de longa duração de ação (Revinty Ellipta® ou Gibiter Easyhaler® ou Bufomix Easyhaler®) ou com um anticolinérgico (Atrovent® ou Incruse Ellipta®). Por outro lado, com um perfil de alto risco associado a muitos sintomas, a sua terapêutica deverá incluir um corticosteroide, um agonista adrenérgico β2 de longa duração de ação e/ou um anticolinérgico. Após este esclarecimento, alerto o doente para a existência de potenciais interações medicamentosas, nomeadamente duplicação da terapêutica do Bretaris Genuair® com o Atrovent® e o Incruse Ellipta® e duplicação da terapêutica do Bufomix Easyhaler® com o Gibiter Easyhaler® e no Revinty Ellipta®. Destaquei, ainda, a possibilidade de as interações aumentarem a toxicidade dos fármacos por sinergismo farmacodinâmico, podendo resultar num aumento de efeitos colaterais, como por exemplo palpitações, taquicardia e tremores. Face a isto, decidi não ceder o Bretaris Genuair® e expliquei ao utente a necessidade de se dirigir ao médico para este avaliar a relação benefício/risco do perfil terapêutico atual, de modo determinar se são necessários ajustes.

Ao percecionar a recetividade do utente ao meu aconselhamento e a demonstrar compreensão face ao referido, recorri a produtos placebo para ensinar o utente a proceder a uma administração correta para cada produto e pedi uma demonstração. Ao obter maior esclarecimento acerca da utilização correta de cada produto, o utente mostrou-se mais descansado e agradeceu por toda a atenção e cuidado prestados. Terminei o atendimento pedindo ao utente que dispusesse qualquer dúvida adicional que pudesse ter.

Considero este caso prático importante de referir pois um dos deveres do farmacêutico é promover a adesão à terapêutica, garantindo a efetividade e segurança do tratamento de modo a melhorar a qualidade de vida do utente. Não obstante, este caso prático representa que um atendimento não tem necessariamente de culminar na cedência de um produto, sendo o farmacêutico um agente de saúde pública com dever cívico de prestar os seus serviços à comunidade onde se insere.

CASO II

Utente de 23 anos, género feminino, dirigiu-se à farmácia apressadamente pedindo aconselhamento farmacêutico para o alívio de sintomas associados a queimadura solar no dorso. Refere que a sua pele tem uma coloração avermelhada e que se apresenta quente ao tato, com dores e alguns sinais de inflamação. Solicita uma solução urgente, alertando para a inconveniência da situação por motivos profissionais.

De forma a proceder a uma correta avaliação e posterior aconselhamento, questionei se detetou a presença de bolhas ou sinais de infeção na zona lesada, se sente febre, vómitos, cefaleias ou tonturas e se tem sinais de desidratação, nomeadamente boca seca, sede, diminuição do volume de urina e fadiga. Após respostas negativas, aconselhei a utente a aplicar duas vezes por dia nas zonas afetadas o gel hipoalergénico Caladryl®, visto que proporciona uma sensação imediata de frescura, diminuindo a inflamação, a dor e o rubor. Expliquei que estes efeitos se devem ao facto de este produto ser especialmente formulado para ter uma tripla ação, visto que associa um anti-histamínico (cloridrato de difenidramina), um analgésico (cânfora) e um adstringente (calamina). Mencionei, ainda, a possibilidade de conservar o gel no frigorífico (5°C) para potenciar a sua ação refrescante.

Alertei para a importância de evitar a ingestão de bebidas alcoólicas e açucaradas, devendo privilegiar a ingestão de líquidos como água ou sumos de frutos naturais sem adição de açúcar. De forma complementar, recomendei medidas de proteção solar para prevenir futuras queimaduras solares, como evitar a exposição solar durante as horas de maior intensidade dos raios UV, utilizar sempre protetor solar de acordo com o fototipo da pele, não dispensar o uso de chapéu e óculos de sol, bem como de roupas de algodão largas e escuras que protejam o corpo dos raios UV.

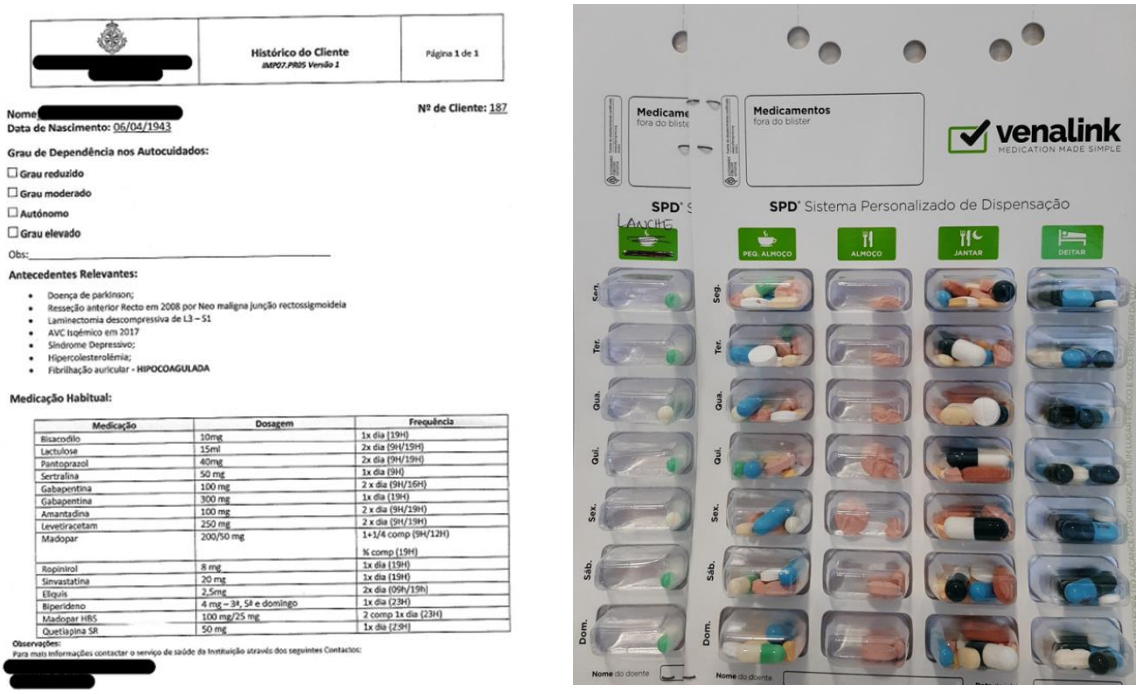
Considero que este caso prático demonstra a necessidade do farmacêutico em prestar atenção aos pormenores e a prezar pela recolha de toda a informação necessária para poder dar resposta às necessidades do utente e garantir o seu bem-estar.

CASO III

Nos últimos anos, em Portugal tem-se vindo a verificar uma inversão da pirâmide etária. O envelhecimento da população portuguesa, a acumulação gradual de doenças crónicas após os 55 anos de vida e a polimedicação dos idosos são uma realidade para o Serviço Nacional de Saúde.

No que concerne aos idosos, estes encontram-se muitas vezes polimedicados por apresentarem diversas comorbilidades. Este contexto aliado às alterações fisiológicas associadas ao envelhecimento e que condicionam a farmacocinética e a farmacodinâmica, faz com que o idoso esteja sujeito a um maior risco de reações adversas que, frequentemente, conduzem à necessidade da toma de mais fármacos por se considerar que constituem uma nova condição clínica. Esta ação não só desencadeia a chamada "cascata de prescrição" como também torna o idoso mais propenso a interações fármaco-fármaco e fármaco-doença.

Consciente desta realidade, decidi analisar o perfil farmacoterapêutico de uma utente de 77 anos institucionalizada num lar há cerca de 8 anos (Figura 2).



[redacted] | Histórico do Cliente IMP07.PR05 Versão 2 | Página 1 de 1

Nome [redacted] Nº de Cliente: 187
Data de Nascimento: 06/04/1943

Grau de Dependência nos Autocuidados:
☐ Grau reduzido
☐ Grau moderado
☐ Autónomo
☐ Grau elevado

Obs:_____

Antecedentes Relevantes:
- Doença de parkinson;
- Ressecção anterior Recto em 2008 por Neo maligna junção rectossigmoideia
- Laminectomia descompressiva de L3 – S1
- AVC Isquémico em 2017
- Síndrome Depressivo;
- Hipercolesterolémia;
- Fibrilhação auricular - HIPOCOAGULADA

Medicação Habitual:

Medicação	Dosagem	Frequência
Bisacodilo	10mg	1x dia (19H)
Lactulose	15ml	2x dia (9H/19H)
Pantoprazol	40mg	2x dia (9H/19H)
Sertralina	50 mg	1x dia (9H)
Gabapentina	100 mg	2 x dia (9H/16H)
Gabapentina	300 mg	1x dia (19H)
Amantadina	100 mg	2 x dia (9H/19H)
Levetiracetam	250 mg	2 x dia (9H/19H)
Madopar	200/50 mg	1+1/4 comp (9H/12H) ¼ comp (19H)
Ropinirol	8 mg	1x dia (19H)
Sinvastatina	20 mg	1x dia (19H)
Eliquis	2,5mg	2x dia (09h/19h)
Biperideno	4 mg – 3ª, 5ª e domingo	1x dia (23H)
Madopar HBS	100 mg/25 mg	2 comp 1x dia (23H)
Quetiapina SR	50 mg	1x dia (23H)

Observações:
Para mais informações contactar o serviço de saúde da Instituição através dos seguintes Contactos:
[redacted]

Figura 2 - Perfil farmacoterapêutico da utente, à esquerda, e respetiva preparação individualização de medicação, à direita.

A utente tem estabelecido um diagnóstico de Doença de Parkinson (DP). Esta doença manifesta-se através de sintomas motores (SM) e não motores (SNM). Frequentemente, os SNM precedem os SM. Alguns SNM mais frequentes são hipósmia, hipotensão ortostática, alterações urinárias, obstipação, alterações do sono, deterioração cognitiva, depressão, apatia, ansiedade, fadiga e dor. No que diz respeito aos SM, estes incluem bradicinécia, rigidez muscular, tremor de repouso e, ainda, alterações posturais e da marcha.

Atualmente não existe cura para a DP, nem nenhuma terapêutica demonstrou até à data capacidade clara apara atrasar a progressão da doença, pelo que o tratamento atual está indicado apenas para o alívio dos sintomas. A escolha do tratamento deve ser fundamentada nos sintomas específicos, idade e estado cognitivo do doente. Assim, considerando que a doente é idosa, pois tem uma idade superior aos 65 anos, o tratamento de primeira linha mais indicado são formulações que contemplam levodopa associada a um inibidor da dopa-descarboxilase periférica (benserazida), dado que são mais eficazes a controlar SM comparativamente aos inibidores da monoamino-oxidase B (MAO-B). Confirmei que esta terapêutica foi instituída, pois o perfil da utente comtempla o Madopar® 100 mg/ 25 mg. Destaco a necessidade de o Madopar® ser tomado 30 min antes das refeições, para melhorar a sua absorção e passagem pela barreira hematoencefálica [20].

Após alguns anos de tratamento, a farmacodinâmica da levodopa/benserazida altera-se e a doente passa a sofrer de flutuações motoras, nomeadamente *wearing off* (agravamento motor previsível antes da toma seguinte), *delayed-on* (aumento do tempo para o início do benefício clínico), *no-on* (inexistência de resposta à medicação dopaminérgica) e períodos *off* súbitos. Perante estas circunstâncias é necessário aumentar a dose de levodopa/benserazida para corrigir estas flutuações motoras. Possivelmente a utente apresentou significativas flutuações de efeito ao longo do dia, pelo que foi introduzido na terapêutica o Madopar HBS® 200 mg/50 mg, uma formulação de libertação prolongada [21].

A utente poderá não ter registado uma melhoria do controlo motor após alteração do esquema terapêutico levodopa/benserazida, pois incluíram na sua terapêutica um agonista da dopamina, particularmente o ropinirol. Este grupo farmacológico tem um risco acrescido de desenvolvimento de perturbações do controlo dos impulsos. Deste modo, saliento a necessidade de a utente ser regularmente monitorizada relativamente ao desenvolvimento destas perturbações, recomendando uma redução da dose ou uma descontinuação gradual do tratamento caso estes sintomas se desenvolvam. O anticolinérgico biperideno poderá ter sido introduzido como auxiliar no tratamento da rigidez e do tremor. A amantadina pode ter sido incluída para atuar ao nível das discinésias de pico de dose (movimentos involuntários que

surgem no período de resposta à terapêutica dopaminérgica, ou períodos *on*) ou para controlar as perturbações do controlo dos impulsos.

Atento que a terapêutica da DP é bastante complexa, pois os fármacos dirigidos aos SM podem agravar alguns dos SNM, nomeadamente a disfunção autonómica e os distúrbios psiquiátricos. No caso da utente em questão, algumas das intervenções farmacológicas possíveis nos SNM foram: a introdução lactulose e bisacodilo indicados para a obstipação, pantoprazol para a motilidade gástrica errática, quetiapina SR para sintomas psicóticos e sertralina para a depressão. Como a doente é idosa e tem um diagnóstico de DP associado, o tratamento da depressão com inibidores seletivos da recaptação da serotonina é o mais adequado, visto que, o uso antidepressivos tricíclicos ou benzodiazepinas está associado ao risco de quedas, fraturas e hipotensão. Para além disso, a utente toma anticonvulsionantes, gabapentina e levetiracetam para o tratamento da dor neuropática, decorrente da mudança de postura e a falta da mobilidade característica dos doentes com DP.

As medidas não farmacológicas não melhoram o controlo da sintomatologia da doença. No entanto, melhoram a qualidade de vida dos doentes. Assim, considero importante utilizar, sempre que possível, medidas não farmacológicas, tais como fisioterapia, terapêutica ocupacional e uma dieta rica em fibras. Realço, ainda, a importância do fracionamento da ingestão diária de proteínas, sendo recomendada a ingestão de uma maior quantidade ao final do dia, pois as proteínas competem com a levodopa, podendo diminuir o seu efeito.

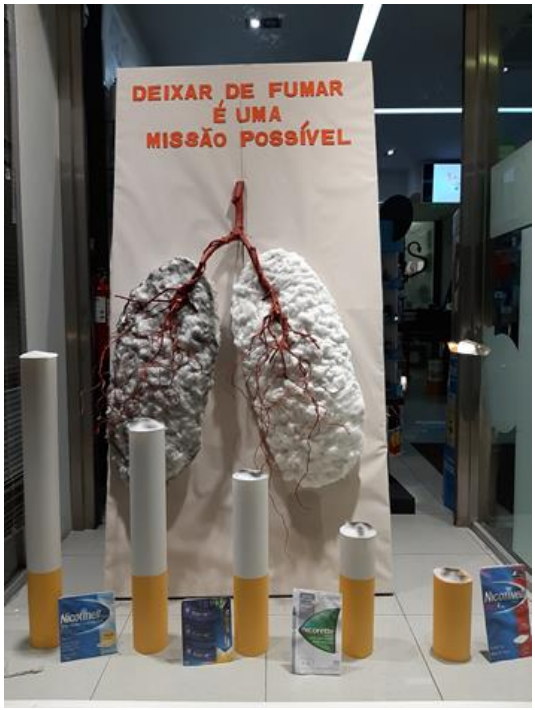
Em 2017 a doente teve um AVC isquémico, ou seja, provocado por uma oclusão súbita de artérias que irrigam o cérebro. Para além disso, apresenta fibrilhação auricular não valvular, um tipo de arritmia que aumenta o risco de fenómenos tromboembólicos e cujos efeitos cardiovasculares aumentam em 5 vezes o risco de AVC. Consequentemente, a utente é hipocoagulada, tomando Eliquis® 2,5 mg (apixabano) 2 vezes por dia para a prevenção de fenómenos tromboembólicos [22]. Esta dose de apixabano é recomendada em situações de comprometimento renal moderado, isto é, quando a *clearance* da creatinina é inferior a 30 mL/min mas superior a 15 mL/ min. Assim sendo, importa referir que é necessária a monitorização, pelo menos anualmente, da hemoglobina, função renal e função hepática da utente.

A utente também toma um anti-dislipidémico do grupo das estatinas, sinvastatina, que deve ser tomado ao deitar pois tem um tempo de semi-vida curto e a produção de colesterol ocorre durante a madrugada.

Em suma, a terapêutica instituída à utente é adequada. Todavia, sempre que que haja um agravamento mais ou menos súbito do quadro clínico devem ser investigadas as potenciais causas para a intercorrência.

Este caso prático foi uma oportunidade de contactar com a heterogeneidade da DP associada a uma terapêutica complexa. Percebi que os maiores obstáculos a uma qualidade de vida satisfatória de um doente com DP são os SNM (sobretudo depressão e psicose) as complicações motoras e outros efeitos adversos farmacológicos.

Anexo VI - Utilização das monstras da Farmácia Maria José no âmbito da intervenção cívica e promoção para a saúde.



Sensibilização dos utentes para a importância da cessação tabágica.

Demonstração da quantidade de açúcar presente em vários tipos de bebidas.

Anexo VII - Dinamização das montras da Farmácia Maria José em dias comemorativos.

Celebração do Dia dos Namorados

Celebração do Carnaval

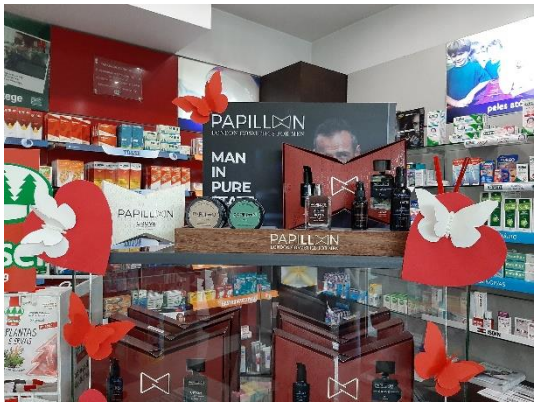
Celebração do Dia Mundial da Criança

Montra da Verão

Anexo VIII - Decoração da zona de atendimento da Farmácia Maria José.

Expositor de porta-máscaras recicláveis

Expositor de porta-máscaras recicláveis



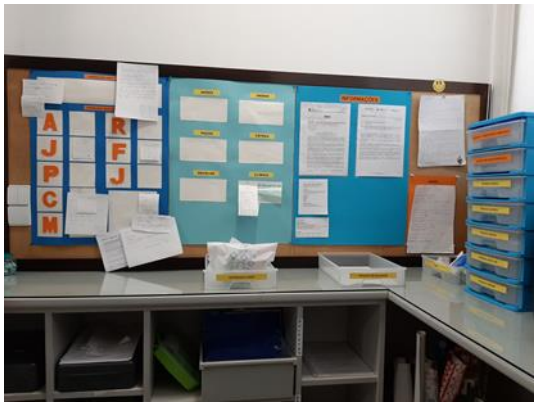
Decoração de expositor no Dia dos Namorados

Decoração de expositor no Dia dos Namorados

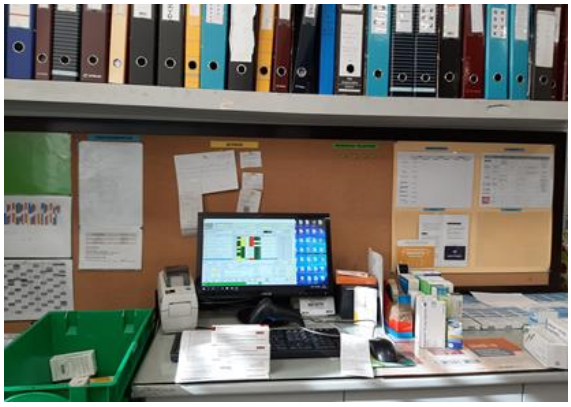
Decoração de expositor no Carnaval

Decoração de expositor no Carnaval

Anexo IX - Organização do *backoffice* da Farmácia Maria José



Criação de espaço para informações



Organização do local de receção de encomendas

Organização da sala destinada à realização de análises de parâmetros antropométricos e bioquímicos

Criação de recipientes para guardar os aparelhos de medição

Organização de vários produtos por categorias

Organização dos suplementos alimentares por categorias

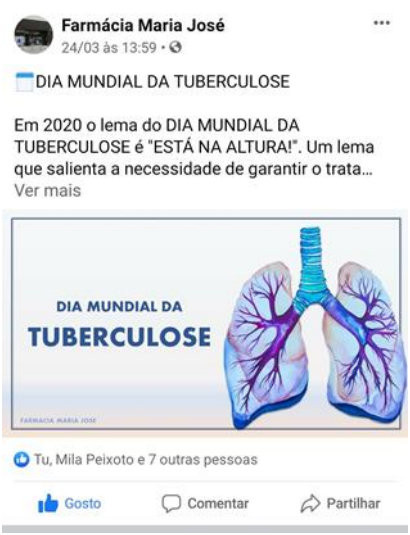
Organização dos produtos presentes no frigorífico por ordem alfabética, de acordo com o nome comercial

Criação de espaços destinados às reservas pagas e não pagas de utentes, às reservas de lares e a produtos ainda não rececionados

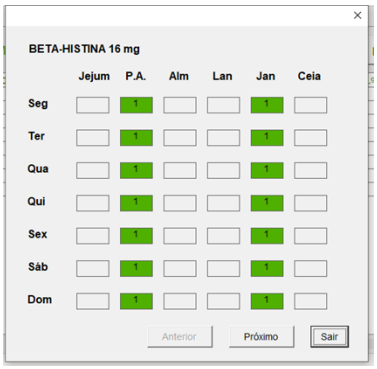
Gestão de reservas pagas e não pagas, bem como de prazos de validade a terminar em que a coloração vermelha é destinada ao mês corrente, a coloração laranja ao mês seguinte ao corrente e coloração amarela para meses posteriores ao mês seguinte ao corrente.

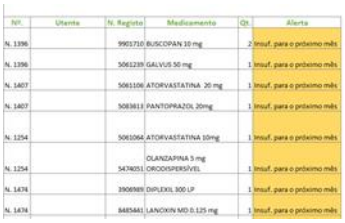
Organização de amostras, caixas de perfumes, cupões, materiais do cartão saúda e materiais das consultas de nutrição.

Anexo X - Dinamização das redes sociais da Farmácia Maria José.



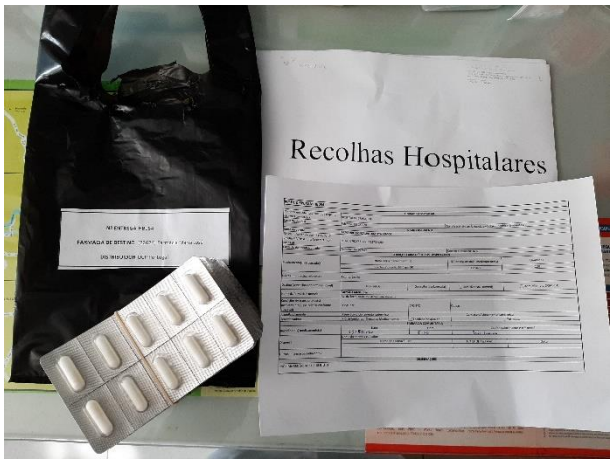
Anexo XI - Desenvolvimento de programa para auxiliar na preparação individualizada da medicação.





N.º Embalagens	Quantidade disponível	Quantidade em falta	Em uso	Stock mínimo
1	42,0 unidade(s)	14,0 unidade(s)	Sim	Sim
2	34,0 unidade(s)	0,0 unidade(s)	Sim	Sim
2	72,0 unidade(s)	0,0 unidade(s)	Sim	Sim
2	65,0 unidade(s)	0,0 unidade(s)	Sim	Sim
2	36,0 unidade(s)	0,0 unidade(s)	Sim	Sim
1	18,0 unidade(s)	10,0 unidade(s)	Sim	Sim
1	59,0 unidade(s)	0,0 unidade(s)	Sim	Sim
0	0,0 unidade(s)	28,0 unidade(s)	Sim	Não

Anexo XII - Dispensa de medicamentos hospitalares.



THE ROLE OF NANOCLAYS IN CANCER THERAPY

PARTE II

Orientada por Professora Doutora Ana Cláudia Santos

Abbreviations

5-FU	5-Fluorouracil
6-MP	6-Mercaptopurine
ACN	Acrylonitrile
AFM	Atomic Force Microscopy
APTES	γ-Aminopropyltriethoxysilane
ASODN	Antisense Oligodeoxynucleotide
ATV	Atorvastatin
AUC	Area Under Curve
BENT	Bentonite
BSA	Bovine Serum Albumin
CAM	Camptothecin
CEC	Cation Exchange Capacity
CEL	Celecoxib
CIP	Ciprofloxacin
CLSM	Confocal Laser Scanning Microscopy
$C_{máx}$	Maximal Concentration
CMC	Carboxymethyl Cellulose
COS	Chitosan Oligosaccharide
CPT	Cisplatin
CRC	Colorectal Cancer
CS	Chitosan
CTAB	Cetyl Trimethyl Ammonium Cromide
CUR	Curcumin
DL	Drug Loading
DOX	Doxorubicin
EC_{50}	Half Maximal Effective Concentration
ECM	Extracellular Matrix
EE	Entrapment Efficiency
FA	Folic Acid
FDA	Food and Drug Administration
FR	Folate Receptor
GEM	Gemcitabine

GFP	Green Fluorescence Protein
GNR	Gold Nanorod
GUV	Giant Unilamellar Vesicle
HDTMA	Hexadecyltrimethylammonium
HEC	Hydroxyethyl Cellulose
HNT	Halloysite Nanotube
HPMACS	Hydroxypropyl Methylcellulose Acetate Succinate
HSA	Human Serum Albumin
IC_{50}	Half Maximal Inhibitory Concentration
ICG	Indocyanine Green
IRN	Irinotecan
KI	Potassium Iodide
LAP	Laponite
LbL	Layer-by-Layer Assembly
LSPR	Localized Surface Plasmon Resonance
MDR	Multidrug Resistance
MMT	Montmorillonite
MNP	Magnetic Nanoparticle
mRNA	messenger RNA
MSC	Mesenchymal Stem Cell
MTX	Methotrexate
NIR	Near-Infrared Light
NSCLC	Non-Small Cell Lung Cancer
OSA	Osteosarcoma
PAA	Poly(acrylic acid)
PAL	Palygorskite
PCL	Poly-(ε-caprolactone)
PDT	Photodynamic Therapy
PEG	Polyethylene Glycol
PEI	Polyethylenimine
PHEMA	Poly(2-hydroxyethyl methacrylate)
PLGA	Poly(D,L-lactide-*co*-glycolic acid)
PMMM	Poly(methacrylic acid-*co*-methyl methacrylate)
PMVEMA	Poly(methyl vinyl ether-co-maleic acid) Polymer

PSS	Poly(sodium-p-styrene sulfonate)
PTT	Photothermal Therapy
PTX	Paclitaxel
PVP	Polyvinylpyrrolidone
QUE	Quercetin
ROS	Reactive Oxygen Species
RSV	Resveratrol
SAP	Saponin
SCLC	Small Cell Lung Cancer
SEM	Scanning Electron Microscopy
SIL	Silibinin
siRNA	small interfering RNA
siVEGF	Vascular Endothelial Growth Factor siRNA
SSA	Specific Surface Area
ß-CD	ß-cyclodextrin
T3	Triiodothyronine Hormone
T4	Thyroxine Hormone
TEM	Transmission Electron Microscopy
TME	Tumor Microenvironment
TMR	Mean Residence Time
TMX	Tamoxifen
VEGF	Vascular Endothelial Growth Factor

Resumo

O cancro continua a ser uma das doenças mais mortais demonstrando diversos desafios no que concerne ao seu tratamento, particularmente eficácia reduzida, toxicidade inespecífica e resistência à quimioterapia.

Há um aumento de interesse no uso de nanoargilas como nanotransportadores multifuncionais na área de terapia de cancro devido à sua natureza moldável e propriedades desejáveis adicionais, nomeadamente o tamanho de partícula, a forma, a capacidade de adsorção, a área de superfície específica, a capacidade de troca iónica, e a carga de superfície.

As nanoargilas apresentam diferentes morfologias de acordo com a organização das suas camadas. As mais representativas são a montmorilonite, a laponite, a bentonite, a caulinite, os nanotubos de haloisite, a sepiolite, a paligorsquite e a alofana. Estas estruturas constituem nanotransportadores atrativos uma vez que são capazes de melhorar a solubilidade, estabilidade, promover uma libertação controlada, promover um transporte eficiente até à localização do tumor, e, portanto, aumentar a biodisponibilidade de um vasto leque de fármacos anticancerígenos, levando ao aumento da ação anticancerígena inerente, ou seja, da eficácia terapêutica. As nanoargilas também apresentam potencial para transportar genes e proteínas, bem como servir como molde para vários agentes fototerapêuticos. Várias funcionalizações e tratamentos têm sido investigados para desenvolver nanotransporadores com potenciais vantagens, não só em formulações farmacêuticas, mas também na prática clínica. As vantagens mais significativas consistem na redução de efeitos secundários severos, e a possibilidade de utilização de vias de administração mais convenientes.

Este trabalho de revisão tem como objetivo analisar os ensaios *in vivo* e *in vitro* que abordam a utilização de sistemas de libertação baseados em nanoargilas contra vários tipos de cancro, destacando as diversas funcionalizações efetuadas em cada sistema.

Palavras-Chave: nanoargila, sistema de libertação de fármacos, terapia génica, fototerapia, co-libertação.

Abstract

Cancer remains one of the deadliest diseases providing several challenges in terms of treatment, particularly limited efficacy, unspecific toxicity and chemoresistance.

There is an increasing interest in the use of nanoclays as multifunctional nanocarriers in the field of cancer therapy due to their tunable nature and remarkable additional properties, namely the particle size, the shape, the adsorption ability, the specific surface area, the cation exchange capacity, and the layer charge.

Nanoclays present different morphologies according to their layer arrangements. The most representative are the montmorillonite, the laponite, the bentonite, the kaolinite, the halloysite, the sepiolite, the palygorskite, and the allophane. These structures compose attractive nanocarriers as they are able to improve the solubility, stability, promote a controlled release, promote an efficient transport to the tumor location, and, consequently, increase the bioavailability of a wide range of anticancer drugs, which increases the inherent anticancer activity, corresponding to the therapeutic efficacy. Nanoclays also present potential to carry genes and proteins, as well as serve as templates for several phototherapeutic agents. Several functionalizations and treatments have been investigated to develop nanocarriers with potential advantages, not only in pharmaceutical formulations but also in clinical practice. The main advantages consist in the reduction of the severe side effects, and the possibility to use more convenient administration routes.

This review work aims to explore the *in vivo* and *in vitro* experiments regarding the use of nanoclays-based delivery systems against several types of cancers with an emphasis on the several performed functionalizations on each delivery system.

Keywords: nanoclay, drug delivery system, chemotherapy, gene delivery, phototherapy, co-delivery.

I. Introduction

Cancer is a disease that has been accompanying mankind throughout history[1]. Nowadays, cancer continues to be a major challenge in health field, affecting the population in all parts of the world[2]. Every year, 14 million new cases of cancer emerge and 8.9 million deaths occur, where the overwhelming majority are metastatic cancers[3]. Non-metastatic cancer treatments present a high chance of success. Surgery and radiotherapy are the most effective and valuable treatments for local and non-metastatic cancers, but are inefficient when the cancer has spread throughout the body[4],[5],[6]. The use of anticancer drugs (chemotherapy, hormone and biological therapies) is the current choice for the treatment of metastatic cancers, since those are able to reach every organ in the body via the bloodstream[7]. However, the available treatments for metastatic cancers come with unresolved problems. The mechanism of action of anticancer drugs is characterized by an indiscriminate destruction of cells. Treatments inhibit the fast proliferation of the cancer cells, but it also affects normal cells endowed with fast proliferation rates, such as hair follicles, bone marrow and gastrointestinal tract cells, generating the characteristic side effects of chemotherapy[4],[8]. Furthermore, frequent administration is required for most of the drugs to maintain high efficiency to kill cancer cells and minimize side effects. Consequently, this kind of therapy leads to tumor multidrug resistance (MDR) and significantly increases the cost of the treatment, which represents a waste of medical resources[9],[8]. In order to avoid these problems, new effective targeted treatments are urgently needed.

The combination of small particle size and high surface area of nanomaterials is the key for the challenge of selecting and removing affected cell populations with greatest precision in cancer therapy[10],[11]. These nanostructures represent an opportunity to achieve sophisticated targeting strategies and multi-functionality. In the last three decades, a notable progress in the development of advanced nanomaterials that have revolutionized cancer treatment has been assisted[12]. The focus was to development nanomaterials with features, such as longer circulation time, slower elimination, higher tumor accumulation, and capability to promote controlled drug release. Subsequently, the second generation of nanomaterials emerged with additional capabilities, including co-administration of different drugs, targeting of tumor cells, and triggered drug delivery. New functions were added to nanomaterials, which gave away to the third generation of nanomaterials, characterized by immune system modulation, biological barrier penetration, and "self-recognition"[12]. Therefore, these three generations of nanomaterials resulted from the effort to enhance the performance of anticancer drugs regarding bioavailability, safety and specificity[13]. The enormous potential of nanomaterials in

the field of cancer treatment is due to their recognized unique properties, such as nanoscale particle size, high surface area, and adjustable surface chemistry, which facilitates their use in clinical endeavors[10],[11],[14]. Nanomaterials can present an organic or inorganic nature. Among the organic nanomaterials are liposomes, dendrimers, solid-lipid nanoparticles, and polymeric nanoparticles[10]. Inorganic nanomaterials comprise gold, silver and silica, magnetic particles, quantum dots, and carbon particles, for instance[13], [15].

Nanoclays belong also to the group of inorganic nanomaterials and they are easily found in nature[16]. Approximately 80% of sedimentary rocks present in the earth's crust are clay rocks. Clay rocks are constituted by materials whose dimensions are inferior to 3.90 μm, in which clay minerals smaller than 2 μm are predominant. This predominant group includes a fraction designated by nanoclays[17]. Naturally, due to the significant presence of clay minerals at the surface of the earth, humans have been collecting this kind of materials since the pre-history, and using them in several applications, particularly in medicine[18],[19],[20],[21]. Currently, terms associated to clays have their own monographs in worldwide renowned Pharmacopeias[19]. Furthermore, clay minerals have been attracting a lot of attention in cancer treatment due to the synergy between their unique biopharmaceutical and technological properties, such as biocompatibility, particle size, morphology, specific surface area (SSA), density of charge, among others. These properties are specifically conditioned by clay mineral type and crystalline structure[22], [23].

The present review examines the recent literature describing the potential applications of clay-based nanomaterials on cancer treatment.

2. An overview of clay chemistry

Clay minerals are end products that result from chemical weathering of several minerals and present stability under conditions on the surface of the earth. These minerals are laminar silicates that belong to the phyllosilicate family, which is characterized by two main structural units: tetrahedral and octahedral sheets (Appendix I)[24],[25]. Tetrahedrons are regular polyhedral composed by four vertices. Regarding clay minerals structure, each tetrahedron has a central cation, frequently silicon (IV) cation (Si^{4+}), coordinated with four oxide anions (O^{2-}), placed at the vertices (Appendix I (A)). As a result, each tetrahedron presents three basal O^{2-} and an apical O^{2-} (Appendix I (B)). Through three shared basal O^{2-}, tetrahedrons tend to polymerize in order to form a tetrahedral sheet with variable length. The apical O^{2-} is the point of connection with an octahedral sheet[20],[24],[25]. Octahedrons are regular polyhedral composed by six vertices. Each octahedron contains a central metal cation (M^{n+}), frequently aluminum cation

(Al^{3+}) (Appendix I (C)), coordinated with six O^{2-} located at the vertices (Appendix I (D)). Adjacent octahedrons interact by sharing their edges (two O^{2-} or OH), forming an octahedral sheet[20],[24],[25]. Under some conditions, the central the Si^{4+} of tetrahedral sheet and the central Al^{3+} of the octahedral sheet are replaced by lower valence ions with similar atomic radius. Si^{4+} and Al^{3+}can be exchanged for Al^{3+} or magnesium cation (Mg^{2+}) and lithium cation (Li^{+}) or (ferrous cation) Fe^{2+}, respectively[17]. Due to symmetry and dimensional similarities of tetrahedral sheets and octahedral sheets, an octahedral sheet can establish a connection with one (1:1) or two (2:1) tetrahedral sheets forming crystalline structures through electrostatic interactions, Van der Waals forces, interlayer cations or through hydrogen bonding[21],[26]. The formed structures, with a variable length, have approximately 1 nm of thickness and are able to self-organize into stacks with a regular gap, called the interlayer space[20-22].

Clay minerals can be either electrically neutral or display a negative charge[20]. The 1:1crystalline structure is electrically neutral because strong bonds are established between the octahedral sheet and the tetrahedral sheet, which leads to the absence of counterbalancing cations in the interlayer space. Consequently, these type of clay minerals are not hydrated and are considered non-swelling (Appendix II (A))[26]. On the other hand, the charge of 2:1 crystalline structure is variable because in this structure type, weaker bonds are formed between the tetrahedral sheets of two layers. Given this varying nature, the interlayer space may contain counterbalancing cations if the structure has a negative charge; or may not contain any counterbalancing cations if the structure is neutral. As result, negatively charged structures can get hydrated, ergo they have the capability to swell (Appendix II (B))[17],[20]. Considering that the cation exchange capacity (CEC) of clay minerals is directly related to the quantity and type of counterbalancing cations, it is possible to exchange counterbalancing cations for other molecules, such as metal cations, polymers, or therapeutic compounds. This possibility allows the modification of clay minerals properties in order to facilitate their clinical applicability[20].

Generally, clay minerals may be classified into two categories, natural or synthetic[27]. Natural clays include crystalline and non-crystalline minerals (Appendix III). Crystalline minerals contain both planar hydrous phyllosilicates and non-planar hydrous phyllosilicates[17]. Considering the different ratio between the tetrahedral and octahedral sheets and the layer charge that may arise all over the structure, planar hydrous phyllosilicates can be divided into three categories: 1:1, 2:1 and 2:1:1 phyllosilicates[20]. The 1:1 phyllosilicates have one tetrahedral sheet stacked on top of another octahedral sheet. When it comes to 2:1 clay minerals, each layer consists of one octahedral sheet amid two tetrahedral sheets[24]. Lastly, the 2:1:1 phyllosilicates are constituted by an octahedral sheet contiguous to a 2:1 layer[20]. On the other

hand, non-planar hydrous phyllosilicates can only be divided into two categories: 1:1 and 2:1 phyllosilicates. Regarding the non-crystalline minerals, this group includes minerals, like allophane, imogolite and hisingerite[17]. Synthetic clay minerals have the same structure and physicochemical properties as natural clay minerals. However, synthetic clay minerals are able of generating more interest in the industry, due to the fact that industries see the opportunity to produce clay minerals on a large scale and with desired a reproducibility[20].

3. Nanoclays and their main characteristics

The enormous potential of nanoclays in cancer therapy, arises from their unique properties of particle size, shape, adsorption ability, SSA, CEC, and layer charge[24]. In this research field, most studies revolve around the application of specific nanoclay groups, such as smectite, kaolin, palygorskite, sepiolite and allophane.

Generally, smectite group is known for its swelling behavior in the presence of water. Under these conditions, the interactions between interlayer cations and the charged clay layers are replaced for the interactions between cations and water. The hydration of cations allows the passage of water to the interlayer space, and, consequently, the structure swells. Regarding the surface charge, the structure of smectite clay minerals combines two types of charges. Their surface evidences stable negative charges that result from isomorphic substitutions, and their edges show pH-dependent charges, which are created by dangling hydroxyl end-groups. Also, the smectite group is characterized for a high CEC, a large SSA and an increased adsorbability. All these characteristics result in an extensive pool of possible interactions with several molecules, such as drugs or polymers. The smectite group has two key clay minerals: dioctahedral montmorillonite (MMT), and trioctahedral laponite (LAP) (Appendix IV)[24],[25],[28]. LAP has a lath-like shape, and it is notable for its small particle size (Appendix IV (C), (D)). Considering that the optimal particle size for this mechanism of cellular internalization is between 25 nm and 30 nm, this clay, which has a diameter of 25 nm and thickness of approximately 1 nm, can be internalized by cells through endocytosis. Additionally, the small particle size is responsible for its high SSA, which is equal to 370 m^2/g[28],[29],[30],[31]. On the other hand, MMT has a plate-like shape, distinguished for its superb absorption capability, due to its high SSA (756 m^2/g) (Appendix IV (A), (B))[32]. This mineral is a powerful detoxifier, presents mucoadhesive properties, and can intercalate several types of molecules in its adjustable interlayer space. Firstly, the antioxidant properties contribute to the reduction of side effects, such as nausea, vomiting and diarrhea, associated with chemotherapeutics with anticancer drugs[33]. Secondly, the mucoadhesive properties of MMT are advantageous to prolong the drug

delivery through all non-parenteral routes of administration. MMT interacts with mucous membrane and establishes connections with oligosaccharide chains of mucin. Thus, the establishment of these bioadhesive connections makes drug transport across the mucous membrane of gastrointestinal tract easier, thereby increasing their bioavailability[34]. It should be noted that the bioadhesiveness between MMT and epithelial cells of mucous membrane changes the properties of these cells, namely its membrane thickness, reinforcing the protection of the mucous membrane of gastrointestinal tract. As a result, MMT can contribute with an active protection against disturbances in the course of gastrointestinal transportation of anticancer drugs[35]. Additionally, the characteristic bio-adhesiveness of MMT enables an enhanced cellular uptake of some drugs. MMT can create London Van der Waals forces and hydrogen bonding with cells. For example, the hydrogen bridges can be formed between the hydrated cations of MMT and the cellular glycoproteins. The formation of these bonds increases the cellular interactions with MMT, consequently increasing drugs internalization by cellular uptake, which is a crucial factor for an effective drug delivery[32]. As MMT is the core component of bentonite (BENT), the properties of this clay mineral are dependent on the amount and CEC of MMT[36]. As a result, BENT is distinguished for its good absorption capability, high CEC, and swelling behavior. There are only a few types of BENT whose denomination is in accordance with the main exchangeable cation type present in MMT. Therefore, some well-known examples of BENT types are calcium BENT and sodium BENT[37]. The lamellar structure of this smectite clay mineral is irregular, and it depicts a small dimension associated with a high SSA. Frequently, this type of clay tends to form thick and large agglomerates. Moreover, the structure of BENT can be submitted to chemical treatments (including alkaline or acid activation) and organic treatments, being, therefore, categorized as active BENT and organic BENT, respectively. Acid activation of BENT with HCl and H_2SO_4 results in an enhanced absorption capability. Also, with this type of treatment it is possible to increase the global negative charge in BENT, given the fact that BENT charges in its edges are pH dependent, as it happens in MMT. The combination of all these properties allows BENT to interact with biomolecules and polymers[37],[36].

Similarly to smectite group, kaolin group is constituted by layered clay minerals (Appendix V). Kaolinite is the most common clay mineral of the kaolin group. Usually, kaolinite appears as stacked pseudohexagonal platelets with a particle size inferior to 2 μm (Appendix V (A), (B)). Each platelet is considered as an arrangement of two sheets, namelly an aluminum octahedral sheet (aluminol surface) with a silicon tetrahedral sheet (siloxane surface), and stacked one above the other. The negatively charged siloxane surface evidences a hydrophobic

nature, while the positively charged aluminol surface is hydrophilic. Together, these sheets create a strong dipole, being the individual platelets of kaolinite strongly bonded by hydrogen bridges and dipolar bonds. However, the edges of these platelets show a pH dependent charge, due to the protonation or deprotonation of the OH. Given that little or no ionic substitution takes place in kaolinite structure, this clay mineral shows two types of charges, the permanent charges in its surfaces, and pH dependent charges. The total layer charge only takes into account the latter. Consequently, the CEC of kaolinite is typically low, ranging from 3 to 5 meq/100 g. The low adsorption capacity of kaolinite is a result of the charge of the structure and its low SSA, which is between 8 m^2/g and 15 m^2/g. Nonetheless, kaolinite does not display a swelling behavior like clay minerals of the smectite group[24],[26],[38]. Recently, kaolinite showed anticancer activity, reducing metastasis and tumor mass in mice. Kaolinite is capable to affect the electron transport in the course of superoxide radical generation by hepatocyte mitochondria and immunocompetent blood cells of mice inoculated with Lewis Lung Carcinoma cells (LLC cells)[39]. Another clay mineral, which belongs to kaolin group is halloysite. Halloysite present the same chemical structure as kaolinite. However, halloysite is an exception once it presents an unique hollow tubular structure where layers are separated by water molecules. This structure result from rolling sheets of kaolinite around 15 to 20 times and for this reason it is a hydrated polymorph of kaolinite. Halloysite clay nanotubes (HNTs) have a lumen diameter that ranges from 15 nm to 20 nm, an outer diameter that varies from 50 nm to 700 nm and a length between 400 nm and 800 nm. The quite large lumen (10 vol % to 20 vol %) enables the encapsulation of different sized molecules. A notable feature of HNTs is the different chemistries of their inner and outer surfaces: the inner surface (lumen) is composed of an aluminum octahedral sheet, while the outer surface is composed of a silicon tetrahedral sheet. This arrangement confers a negatively charge outer surface and a positively charged inner lumen, which allows a selective immobilization of charged molecules, such as drugs, proteins and DNA. Another interesting feature of HNTs is the presence of two types of hydroxyl groups: those that are located between the layers, inner hydroxyl groups, and those that are located on the surface, outer hydroxyl groups. Surface hydroxyl groups of HNTs have a low density compared to other nanoclays, which makes them relatively hydrophobic. The unique one-dimensional nanoporous structure and the reactive external and internal surfaces make HNTs a promising nanomaterial in encapsulation and release of several compounds, namely drugs[40],[41],[42],[43],[44].

Sepiolite and palygorskite (PAL) are distinguished from other clay minerals due to their fibrous morphology. Sepiolite and PAL distinguish themselves from other clay minerals due to

their fibrous morphology. Structurally, this type of clay minerals consists of an octahedral sheet between two tetrahedral sheets (Appendix VI). In contrast with remaining clays, the apical O^{2-} in tetrahedral sheets of fibrous clays are inverted. This different disposition leads to the interruptions in octahedral sheet, which form rectangular channels that are fully or partially available for several molecules (Appendix VI (A))[16]. Given these properties, sepiolite and PAL occur as crystals in the shape of flexible elongated needles with high SSA associated and high absorption capability (Appendix VI (B))[24]. In addition, a discontinued octahedral sheet leads to the presence of regularly arranged silanol groups (Si-OH) in the external surface of the structure. These groups have a preponderant role in the functionalization of sepiolite and PAL[16]. When compared to the smectite clays, sepiolite and PAL have a lower CEC and are stable under different pH conditions. Therefore, all these characteristics allow fibrous clays to co-deliver different types of active compounds[24].

However, not all clay minerals present a crystalline structure. Allophane is an amorphous clay mineral (short range order) with a hollow spherical shape, with a diameter that ranges from 3.5 nm to 5 nm and it is delimited by a wall with a thickness varying between 0.6 nm to 1.0 nm (Appendix VII). The wall of the hallow sphere is composed of two sheets, an external aluminum octahedral sheet and an internal silicon sheet. Nevertheless, allophane does not have a fixed composition, thus the ratio Si/Al can range from 1 to 2. When ratio Si/Al is close to 1 the spherical structure has a higher proportion of Al (Al-rich allophane). On the other hand, when it is near to 2, the Si sheet has a high expression (Si-rich Allophane)[45]. The CEC and surface charge of allophane change according to pH conditions as a result of its reduced or absent isomorphic substitution. The charge variation in the surface of allophane is caused by $(OH)Al(H_2O)$ groups exposed in perforations, which are present through the wall of the hallow sphere. Moreover, due to its reduced particle size, allophane has a high SSA. The SSA has a value of 1000 m^2/g, which is determined for the internal (intrasphere) and external (intersphere) areas considering the particle size and density. However, when SSA is calculated through the holding capacity of ethylene glycol and ethylene glycol monoethyl ether, it ranges from 700 m^2/g to 900 m^2/g[17]. The reduced particle size and high SSA of allophane are determinant for its adsorption capacity.

In summary, a successful application of nanoclays in cancer therapy requires the mastery of its physicochemical properties, as these properties strongly influence all interactions between the nanoclays and cancer environments that will emerge along the treatment.

4. Applications of nanoclays in cancer therapy

In cancer, the importance of the tissue and cell type to the origin of the disease is clear. However, it is also essential to consider the tumor microenvironment (TME), which results from the interaction between cancer cells and both multiple stromal cell types and non-cellular elements (Appendix VIII (B))[46]. TME is indispensable for tumor generation and metastasis dissemination. Hanahan *et al.* (2011) identified six hallmarks of cancer, which are sustaining proliferative signaling, evading growth suppressors, avoiding programmed cell death, enabling replicative immortality, inducing angiogenesis and active invasion metastasis. These unique characteristics result from cooperation between the components of TME and are the main reason that conventional delivery systems of anticancer drugs are often not effective. TME has also been shown to have different characteristics from normal tissues, which can be vascular defects, higher expression of given enzymes, elevated glutathione, hydrogen ion and reactive oxygen species (ROS) concentrations. This evident differentiation allows the opportunity to create smart nanocarriers that can deliver anticancer drugs to the tumor location efficiently[47],[48].

4.1 Anticancer drugs

Anticancer drugs indicated in current cancer treatments, typically have a narrow therapeutic index, present low bioavailability, lack of specific action toward cancer cells and require a highly dosage to avoid the emergent resistance to therapy. Therefore, the combination of available anticancer drugs with nanoclays aims to reduce side effects and improve therapeutic efficacy by minimizing limitations and maximizing benefits from anticancer drugs[11],[49]. When anticancer drugs are loaded in clay minerals, they constitute a modified drug delivery system. This strategy differs from the conventional dosage forms because it improves drug bioavailability and decreases side effects by modifying the rate, shelf-life and target location of drug release. Also, this strategy can be useful to enhance the stability of drugs through their protection against chemical and enzymatic degradation. Taking in consideration that clay minerals are not inert additive ingredients, they are innovating the concept of excipient[23]. For this reason, clay minerals have been proposed as excipients once they are abundant in nature, economically viable and present unique characteristics, such as swelling capacity and low toxicity[21],[20].

In cancer treatment, to create a nanocarrier with a good performance, it may be necessary to improve some clay characteristics, such as SSA, type of exchangeable cations,

hydrophilic nature, zeta potential, interlayer space, or porosity. In this regard, sometimes clay minerals are submitted to several modifications (Appendix VIII (A))[13],[2].

4.1.1 Lung cancer

Regarding global cancer outlook, lung cancer leads cancer incidence and mortality. There are two main types of lung cancer, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which involve different therapies. NSCLC is the most common lung cancer and the anticancer drugs often used for its treatment include gemcitabine (GEM) and cisplatin (CPT)[50].

GEM, a pyrimidine analog, acts specifically in the phase S of the cell cycle, where the semiconservative replication of DNA occurs. In certain conditions, GEM, also blocks the progression of cells in transition between phases G1 and S. Typically, GEM is administrated intravenously and presents unspecific action and inefficient distribution. HNTs are a possible solution to overcome these problems, since they can carry GEM through cell membranes using several pathways. In the cell cycle assay, the authors demonstrated that GEM loaded HNTs had the capability to decrease phase S of human lung cancer cells (A549 cells). Consequently, they led to the inhibition of cell division and growth of mentioned cells[51]. HNTs also demonstrated effects on A549 cells using other anticancer drugs or strategies. For example, water dispersible HNTs loaded with doxorubicin (DOX) induced structural defects in the cellular organization of A549 cells and allowed the sustained released of DOX over 2 weeks without any initial burst release[52]. Furthermore, HNTs loaded with a model anticancer drug and coated with tube-end stoppers of dextrin, exhibited the ability to be selectively internalize and accumulate only in cells with high proliferation rates such as A549 cells[53].

Only approximately 10% to 15% of all lung cancers are SCLC, which tends to grow and spread faster than NSCLC. Since this cancer grows quickly, it tends to respond well to chemotherapy and radiation therapy. Generally, SCLC is treated with combinations of chemotherapy drugs that often include CPT plus one other drug[50]. CPT is a platinum-based anticancer drug that interacts with DNA, forming cross-links. Although the main side effect of CPT is bone marrow suppression, this anticancer drug can also be responsible for nephrotoxicity, neurotoxicity, nausea, and severe vomiting. Allophane has been studied as a carrier for CPT to reduce its side effects in clinical applications. Toyota *et al.* (2017) investigated the complexation of allophane nanoparticles with CPT on A549 cells. The release profile of CPT from the complex showed that the CPT could be released not only at the conditions of endosome media of cancer cells (pH 5.6) but also at the conditions of blood circulation. The

release was higher at acidic media, where approximately 60% of CPT was released in the initial 50 hours, whereas, at pH 7.4, about 44% of CPT is released in 70 hours, comprising a sustained release. Through fluorescent analysis, the cellular uptake of allophane nanoparticles by A 549 cells was studied, which showed that agglomerates of allophane nanoparticles were internalized by the treated cells. Additionally, the complex presented a lower toxicity than free CPT at comparable doses, due to the increase of biocompatibility[54].

4.1.2 Colorectal cancer

Colorectal cancer (CRC) is the third most frequent cancer globally, accounting for 10.2% of the estimated 18.1 million new cancer cases registered in 2018[3]. The rectum and colon have different embryological origins, anatomies and functions, thus primary rectal and colon cancer require different procedures, treatments, and surgical approaches. However, metastasized rectal and colon cancer are normally considered as one entity and treated in the same manner[55]. Oral administration is the preferential route of administration of anticancer formulations into the colon or rectum[56]. Thus, pH-responsive and time-dependent carriers are the best choice to perform the drug delivery, since they maximize drug release in the intestinal tract and minimize the early drug release in the gastric region (Appendix IX)[35]. 5-fluorouracil (5-FU), camptothecin (CAM) and paclitaxel (PTX) are anticancer drugs normally used in different treatment regiments for colon or rectal cancer[3],[57],[58],[59].

5-FU is an antimetabolic drug indicated for metastatic CRC and neo-adjuvant treatments. Although the oral route represents the main via of administration, 5-FU shows incomplete and unpredictable absorption due to its degradation on the gastrointestinal tract. In addition, the intravenous administration of 5-FU leads to severe collateral damage to normal cells[59],[60]. Lin *et al.* (2002) suggest MMT as a carrier system to overcome the problem of oral administration of 5-FU in CRC, releasing 5-FU only in the cancer location. In this study, 5-FU has been embedded into the interlayers of MMT whether by free surface adsorption or sodium and OH substitution[59]. Although MMT is a great nanocarrier for delivery of 5-FU, its loading capacity is only ca. 8%. In order to increase the loading capacity of MMT, amino-modified MMT was functionalized with ß-cyclodextrin (ß-CD) by a nucleophilic substitution reaction. The obtained nanocomposite self-assembled into supramolecular dendrimer-like structures with high loading capacity, which were further loaded with 5-FU. *In vitro* drug release experiments showed that the 5-FU loaded supramolecular dendrimer-like structures were able to sustain the drug release over 120 hours (Appendix X)[61]. In an alternative approach, Rao *et al.* (2014) prepared a new type of nanocomposite hydrogel that proved to be potentially useful for 5-FU delivery. Initially, hydrogels were prepared using sodium hyaluronate (SH) with poly(2-

hydroxyethyl methacrylate) (PHEMA), then 5-FU was loaded both in the hydrogel network and in the lumen of HNTs. The resulting nanocomposite hydrogel exhibited a pH-responsive performance, acquired from the presence of SH. *In vitro* studies demonstrated that the release of 5-FU was less than 10% in the gastric conditions proceeded by a controlled release for about 70 h in the intestinal conditions[60].

CAM is an anticancer drug predominantly indicated for metastatic CRC, which is recognized as a topoisomerase-I inhibitor and, as 5-FU, its administration is marked by poor water solubility and toxicity to healthy tissues. Therefore, clay-based nanocarriers, such as HNTs can be applied as nanocarriers for CAM delivery to avoid these drawbacks. C. Rizzo *et al.* (2017) developed an innovative supramolecular gel for sustained release of CAM by self-assembly of fluoromethoxycarbonyl-*L*-phenylalanine in the presence of functionalized HNTs. The presence of HNTs in the hydrogel matrix induced a decrease in thermal stability but simultaneously induced an increase in gel strength. Release assays in physiological conditions exhibited that CAM hydrolysis did not occur and CAM was released from HNTs at a slower rate[57]. Dramou *et al.* (2018) developed nanocomposite with magnetic properties and selectivity for cancer cells through the functionalization of HNTs with magnetic oxides and CS oligosaccharides (COS), and further modification with folic acid (FA). The resulting nanocomposite loaded with CAM showed a sustained release over 60 hours with a higher release of CAM at cancer cells conditions (pH 5) than at both pH 6.8 and 7.4. It was also verified a significant cell growth inhibition against human colon carcinoma cells (Caco-2 cells)[62].

Although PTX is an antimicrotubule anticancer drug used in a broad spectrum of solid cancers, its clinical application is complicated by its extremely poor water solubility and lower oral bioavailability.[63],[64],[56],[65]. There have been several efforts to efficiently carry PTX by incorporating hydrophilic moieties into nanocarriers. This is exemplified in the work undertaken by Dong *et al.* (2005), where an oral bioadhesive drug delivery system for PTX has been developed. PTX was loaded in spherical nanoparticles of poly(D,L-lactide-*co*-glycolic acid) (PLGA) incorporated with MMT and the drug release and cellular uptake of the PLGA-MMT nanoparticles using Caco-2 cells and human colon adenocarcinoma cells (HT-29 cells) was investigated. The obtained drug release profile showed a biphasic pattern with an initial burst release followed by a slow rate release, due to the presence of the copolymer PLGA, which increases the hydrophilicity and stability of the nanocomposite and enables the modulation of the release profile. To increase the residence time in the absorption location and the absorption of PTX, MMT was included to provide mucoadhesive properties to the delivery system. Thus,

regarding uptake cellular results, there was an increase from 57% to 177% for Caco-2 cells and from 11% to 55% for HT-29 cells, in the cellular uptake efficiency of the PLGA-MMT nanoparticles[63]. In another study, Bothijara *et al.* (2014) tried to improve PTX therapeutic efficacy trough the incorporation of PTX into the interlayer space of MMT by an ion exchange reaction and with posterior coating with chitosan (CS). The resulting nanocomposite showed a slow controlled release of PTX that was useful to overcome toxicity limitations of PTX and enhance the treatment efficacy. Also, *in vitro* assays revealed that PTX-MMT-CS showed an improvement of at least 10% in anticancer action toward human colon cancer cells (COLO-205 cells) than free PTX. CS coating lowered the half maximal inhibitory concentration (IC_{50}) value of PTX-MMT-CS nanocomposites due to the presence of amino groups in its structure, that establish hydrogen bridges with glycoproteins present in the cancer cells surface, leading to the formation a of bioadhesive clay cluster[64]. In order to maximize the release of PTX in the intestinal segment, Yendluri *et al.* (2017)loaded PTX in HNTs and then coated with poly(methacrylic acid-*co*-methyl methacrylate) (PMMM), a polymer only soluble in alkaline conditions (pH>6.8). The obtained release profile demonstrated that the formulation is capable to pass the stomach and release PTX only in the intestinal segment at a sustained rate for 24 hours (Appendix XI). Also, they proposed a paclitaxel tablet formulation, where HNTs act as a sustained drug delivery vehicle and as tablet compression excipient, which showed a sustained and controllable release for 250 hours[65].

Atorvastatin (ATV) and celecoxib (CEL) are two drugs, which cooperate on CRC inhibition. Li *et al.* (2017) used a microfluidic technique to encapsulate HNTs in a pH responsive hydroxypropyl methylcellulose acetate succinate (HPMACS) polymer to develop an oral drug delivery system for ATV and CEL. Thus, they prepared gastro-resistant microspheres with ability to control the release of the two drugs, given that these microspheres disintegrated only when exposed to alkaline media (pH 7.4). Hence the premature release of the adsorbed drugs is avoided, and a fast release of ATV and CEL loaded in HNTs is promoted in alkaline media, which provides an improvement of the inhibition of colon cancer cells proliferation[56].

HNTs were also studied as nanocarriers for oral administration of irinotecan (IRN), a potent topoisomerase I inhibitor indicated for CRC treatment. In order to protect the IRN from the stomach conditions and allowing it to be released only in the intestinal media, HNTs were coated with Eudragit S100 anionic copolymer, which is soluble in intestinal fluid and insoluble in gastric fluid. Therefore, the release rate of IRN from the polymer-coated HNTs was very low at stomach conditions, 0.7% in pH 1.2 for 2 hours, and high at intestinal conditions, ca. 70% of IRN was released in 2 hours[66].

All these works showed that nanoclays, namely HNTs and MMT, can be useful to develop oral nanocarriers with ability to protect anticancer drugs from premature release and degradation in the stomach and perform precise a release at colon or rectum locations.

4.1.3 Stomach cancer

Stomach cancer has a significant impact globally. It was responsible for over 1 million new cases in 2018 and an estimated 783,000 deaths, making it the third deadliest cancer[3],[8]. The early diagnosis is fundamental for the success of stomach cancer treatment, given that radical surgery is the only treatment option at is early clinical stage. However, the overwhelming majority of stomach cancer cases are diagnosed when the cancer is in an advanced stage due to the undetectable clinical symptoms at early stage. Consequently, anticancer drugs are necessary to prolong the life of patients and improved their quality of life[8].

In order to improve the anticancer efficacy of DOX and develop an innovative treatment option for gastric cancer, HNTs loaded with DOX were encapsulated by soybean phospholipid, which protects DOX-HNTs from the direct contact with charged ions of blood, avoiding a thrombus occurrence. This was demonstrated in blood and histological experiments, where the nanocomposite evidences a great hemocompatibility. An i*n vitro* drug release profile was determined in acidic (pH 5.4) and physiological conditions (pH 7.4). A faster release profile with almost a linear pattern was observed in acidic conditions. The cumulative release in acidic conditions reached nearly 30% for 4 days. This pH-responsive release ability enables DOX release at acidic conditions of TME reducing its release in health cells with neutral environments. *In vitro* experiments were conducted to study the cytotoxicity of DOX-HNTs-LIP nanocomposite using the MMT assay, and a model of nude mice xenograft tumor was used to evaluate its anticancer activity and *in vivo* distribution. In contrast to free DOX, the nanocomposite exhibited a superior inhibitory efficiency on the growth of MCF mice gastric cancer cells. Furthermore, the survival time of tumor-bearing mice treated with the nanocomposite was significantly prolonged[8].

4.1.4 Breast cancer

Breast cancer is the most frequent and deadliest cancer type among women[3]. The term breast cancer includes different types of neoplasm derived from breast tissue. Given its heterogeneity, breast cancer can be classified into three phenotypes: luminal, non-luminal human epidermal growth factor receptor type 2 positive (HER2+) and triple negative phenotype[33].

Most breast cancers are luminal, meaning that they express estrogen receptors and proliferate in response to estrogen stimulation. These hormone-sensitive cancers respond best to endocrine therapies that inhibit estrogen synthesis. Antiestrogens or aromatase inhibitors are two classes of drugs used in endocrine therapies[35]. Tamoxifen (TMX) is a non-steroidal antiestrogen that competitively binds to estrogen receptors inhibiting estradiol binding. TMX is used both in breast cancer in premenopausal and postmenopausal and its administration is normally recommended during at least 5 years. Considering that TMX implies a long-term treatment, the favored route of administration is the oral route. Hence, to achieve a successful treatment, it is necessary to overcome the challenges associated with the oral route, reduce the side effects and increase its efficacy[68]. Kevadiya *et al.* (2012) inserted TMX in the interlayer space of MMT and produced microcomposite particles for oral chemotherapy and the obtained microcomposite particles were further modified with poly-(ε-caprolactone) (PCL). The release profile of microcomposite particles showed a controlled release pattern up to 72 hours (Appendix XII (A)). These results were obtained due to the additional coating with a hydrophobic polymer and the low permeability of water in the inside of microcomposite particles. Regarding the evaluation of the pharmacokinetic profile of microcomposite particles, after single oral administration in a rat model, plasma drug levels were within the therapeutic window in contrast to free TMX (Appendix XII (B)). Additionally, genotoxicity assays showed that the preservation of TMX in interlayer space of MMT and further coating with PCL led to a marked reduction of genotoxicity activity compared with free TMX. It should be noted that MMT was essential to reduce the genotoxicity of TMX and facilitate drug delivery[68].

Steroidal aromatase inhibitors are normally used in antiestrogen refractory patients with breast cancer in postmenopausal. Exemestane is a steroidal aromatase inhibitor that specifically inhibits the aromatase enzyme, responsible for estrogen production, through a permanent connection. However, exemestane presents a reduced bioavailability due to its poor water solubility and susceptibility to first pass effect[35]. In order to enhance the oral bioavailability of exemestane, reduce the fluctuation of plasma drug levels and minimize the side effects, Li *et al.* (2013) developed MMT-PLGA nanocomposites, which showed a reduction of the initial burst release and the rate of drug release, due to its hydrophobicity, elevated surface area and swelling ability. These properties can facilitate the swelling and degradation of PLGA allowing drug diffusion in the course of time. Also, mucoadhesive and detoxifying abilities of MMT improved the adhesive capacity of nanoparticles in the intestinal segment and reduced the side effects caused by exemestane. *In vitro* results on human breast cancer cells (MCF-7

cells), demonstrated that the presence of exemestane-loaded nanoparticles led to a lower cell viability compared with free exemestane[35].

For non-luminal HER2+ cancer, i.e., breast cancer that overexpresses human epidermal growth factor receptor-2 (HER2), the best treatment choice is trastuzumab. Trastuzumab is a humanized monoclonal antibody specifically produced target HER2 receptors, which are transmembrane receptors necessary in the signaling network that cause cell proliferation. Sun *et al.* (2008) developed trastuzumab decorated MMT-PLGA nanocomposites for targeted delivery of anticancer drugs. In these nanoparticles trastuzumab has a dual function, target and treat cancer cells responsible for overexpressing HER2. Their results demonstrated that the functionalization of the nanocomposite with trastuzumab accelerated the drug release and significantly improved the cellular uptake of the nanocomposite. An *in vitro* cytotoxicity assay in HER2-overexpressing breast cancer cells (SK-BR-3 cells) demonstrated that the therapeutic efficacy of nanocomposite decorated with trastuzumab was 13.11 times higher than free paclitaxel, the model drug[33].

Triple negative breast cancer is associated with poor prognosis and presents a better response to anticancer drugs, such as DOX, docetaxel and CPT. CPT is a platinum based anticancer drug that binds to DNA intervening in its replication. The use of CPT has some limitations due to its nonspecific biodistribution and severe side reactions. Kouser *et al.* (2018) adopted a strategy to form a nanocomposite hydrogel film with hydroxyethyl cellulose (HEC), carboxymethyl cellulose (CMC), acrylonitrile (ACN), sustainable polyols and sodium MMT (NaMMT) for controlled release of CPT. The CPT-loaded nanocomposite hydrogel obtained acted like a pH-responsive reservoir for the sustained release of CPT and inhibited the growth of MCF-7 cells in an efficient way. It should be highlighted that sustained and efficient release of CPT may be due to the intermolecular interaction created via the dispersion of NaMMT nanoparticles in the polymer matrix[69].

Docetaxel is another anticancer drug frequently used in the treatment of breast cancer with poor prognosis. However, this anticancer drug has poor water solubility, which is a limitation that can be overcome by using appropriate drug carriers. Feng *et al.* (2009) created a formulation based on MMT for the oral administration of docetaxel. MMT was applied to reduce some side effects inflicted by docetaxel. Cell viability analysis in MCF-7 cells demonstrates that montmorillonite-poly(lactide)–vitamin E TPGS nanocomposites (MMT-PLA–TPGS) were more effective when compared to free docetaxel. *In vivo* studies in male Sprague-Dawley rats showed that MMT-PLA–TPGS nanocomposites presented a 26.4 times

higher half-life than the intravenous administration of free docetaxel with the same dose. Additionally, this formulation had better oral bioavailability[70].

Clinical application of DOX is conditioned by its cardiac toxicity, short plasma half-life and low specificity. A strategy to overcome these disadvantages is the targeted delivery of DOX through polymeric materials. Kohay *et al.* (2017) used the amphiphilic copolymer polyethylene glycol-phosphatidylethanolamine (PEG-PE) to create DOX formulations. Initially, DOX was incorporated into the core of PEG-PE micelles, which have been adsorbed by MMT. The combination of PEG-PE with MMT, in which MMT carries both micelles and DOX, is a novel method that simultaneously enhanced the release profile and the bio-effectiveness of the DOX. Two DOX formulations based on different PEG-PE/MMT proportions were studied. In low polymer loading composite (LOW), the surface of MMT was partially coated with polymer and its interlayer space incorporated a monolayer of polymer. In the case of high polymer loading composite (HIGH), a bilayer of polymer covered the surface of MMT, and two layers of polymer were embedded in the interlayer space of MMT. In both cases, the external coat of composites conditioned the interaction between DOX and MMT and, consequently, the rate of DOX release. Thus, in LOW composite a direct interaction between DOX and MMT occurs due to partial polymer coverage, while in HIGH composite the interaction is mediated by the embedded or external bilayer of polymer. The release rate was reversely correlated with the degree of DOX interaction with MMT, meaning that higher and faster releases imply weak interactions (polymer mediation), resulting higher rates of release in HIGH composite when compared to LOW composites. However, the release rate of both composites is inferior to DOX-M micelles and superior to DOX-MMT nanocomposites. *In vitro* experiments evaluate the performance of LOW, HIGH and DOX-MMT formulations in cells sensitive to DOX (MCF-7 cells) and DOX resistant cells (A2780-ADR cells). All formulations presented a better cytotoxic effect when compared to free DOX. On MCF-7 cells, HIGH composite presented the highest cytotoxicity. In the case of A2780-ADR cells, the elevated presence of PEG-PE copolymer delays the internalization of the formulation, thus the LOW composite showed a greater cytotoxic effect despite its slow release. It is possible to conclude that the cytotoxic effect in sensitive cells mainly depends on the release rate of DOX, whereas in resistant cells the interactions between the composite and cells are the most important factor[32].

Another strategy to overcome the limitations of clinical use of DOX is the exploration of target DOX delivery systems via surface folate receptor (FR). FA is known as an important ligand for active targeting of cancer cells due to the strong presence of FR on the surface of numerous cancer cells, including breast cancer cells. Yan-Ping Wu and co-workers developed

folate-targeted PEGylated HNTs loaded with DOX as a delivery system for targeted therapy of breast cancer. The *in vitro* release assay showed a sustained and controlled release of DOX from the carrier up to 35 hours in acidic media (pH 5.3), whereas it remained quite stable in neutral media. In cytotoxicity assays, HNT-PEG-FA nanocomposite inhibited proliferation and led to cell death of cancer cells overexpressing FR, while demonstrating relatively low cytotoxicity for cells with absence of FR. Furthermore, the system triggered more ROS production in MCF-7 cells causing apoptosis. The *in vivo* action of HNT-PEG-FA nanocomposite against breast cancer cells was verified in 4T1-bearing mice. In contrast to free DOX, HNT-PEG-FA nanocomposite effectively decreases the cardiotoxicity of DOX and inhibits solid tumor growth with higher cleaved caspase-3 protein expression in tumor tissue of 4T1-bearing mice (Appendix XIII)[9]. Jing Yang *et al.* (2016) also created a new delivery system for DOX based on HNTs to treat breast cancer. HNTs were functionalized with COS in detriment of CS because of its low molecular weight and to enhance the antitumor efficacy of DOX through the targeting of both mitochondria and nuclei. The nanocarrier exhibited acceptable biocompatibility, reduced hemolysis ratio, and suitable drug release. *In vitro* studies with MCF-7 cells demonstrate that DOX-HNTs-COS nanocarrier had a different action from free DOX, because it successfully induced apoptosis, increased ROS production, led to mitochondrial damage and acted on nuclei level in this cell line. *In vivo* antitumor tests in 4T1-bearing mice, enforce that DOX-HNTs-COS nanocarrier differs from free DOX because it had a better inhibition of tumor accompanied by less ruptured cardiomyocytes. Also, these nanocarriers showed absence of toxicity on tissues of vital organs[71].

The above results presented innovative nanocarriers based on MMT or HNTs for anticancer drugs in breast cancer treatment. All nanocarriers, in a certain way, represent a new approach to release anticancer drugs in a controlled manner and decrease its side effects. Also, they were fundamental not only to protect anticancer drugs from degradation but also to enhance its half-life, solubility of cytotoxic agents and to decrease renal clearance.

4.1.5 Pancreatic cancer

Pancreatic cancer is one of the deadliest cancers accounting for 331000 deaths per year worldwide[3]. Current treatment approaches including surgery, chemotherapy and radiation present a reduced efficacy. Chemotherapy is the optimal option to be used when surgery cannot totally remove the cancer or when cancer is in a metastatic stage[72].

GEM is one of the most common anticancer drugs used for pancreatic cancer treatment[51]. However, GEM presents a very short plasma half-life (ca. 15 min) and is rapidly

metabolized. Thus, elevated doses of GEM are repeatedly administrated to guarantee the desired therapeutic effect. However, it causes high toxicity and patient compliance[73]. Hydrogel matrices are one of many strategies to maximize the effectiveness of GEM and minimize its side effects. Hydrogels are three-dimensional polymeric networks with excellent biocompatibility and biodegradability in physiological conditions, since they are structures mainly composed by water., and present the ability to carry molecules through bloodstream and to permeate tissues[74]. Phan *et al.* (2016) produced a nanobiohybrid hydrogel for controlled release of GEM to overcome limitations of the clinical use of this anticancer drug in pancreatic cancer treatment. GEM was simultaneously inserted into the interlayer spaces of MMT and adsorbed onto the surfaces. Subsequently, the obtained GEM-MMT nanocomposites were dispersed into a temperature-responsive poly(ε-caprolactone-co-lactide)-b-poly(ethyleneglycol)-b-poly(ε-caprolactone-*co*-lactide)(PCLA-PEG-PCLA) hydrogel. The *in vitro* release profile of GEM exhibited that the nanobiohybrid hydrogel can reduce the initial release burst and prolongs it for the remaining time when compared to pristine hydrogel. As a result, GEM-loaded nanobiohybrid hydrogel showed an elevated antitumor efficacy in pancreatic tumor-bearing mice, demonstrating a sustained release of GEM and maintaining an appropriate drug concentration for an extended period (Appendix XIV). It should be highlighted that the presence of MMT was essential for an efficient loading and controlled release of GEM and to considerably increase the stability of the hydrogel[73].

4.1.6 Brain cancer

Glioma is a general designation for tumors that start in nervous tissue, namely at the level of glial cells that include oligodendrocytes, astrocytes, ependymal cells, microglia and Schwann cells. Thus, several tumors can be considered as gliomas, including glioblastomas, oligodendrogliomas and ependymomas[75]. Overall, gliomas presented a poor clinical prognosis in adults and children[76].

Javiera Cervini-Silva *et al.* (2016) studied the effects of bentonite on human glioblastoma cells (U251 cells). *In vitro* tests showed that bentonite specifically interplayed with U251 cells, inhibiting their proliferation by controlling the presence of metabolic growth components played a vital role to inhibit the growth of U251 cells[77].

6-mercaptopurine (6-MP) is an antimetabolic drug with a hydrophobic nature that easily connects with plasmatic proteins. Due to this features, 6-MP demonstrates a short plasma half-life and a lower bioavailability. In order to address these drawbacks, MMT-PLLA microcomposite spheres were explored as a nanocarrier for 6-MP to provide and maintain its therapeutic plasmatic concentration of 6-MP without overdosing or underdosing periods, for

long period. The viability of human neuroblastoma cells (IMR32 cells) was decrease after exposure to 6-MP loaded into MMT-PLLA microcomposite spheres. The release prolife of 6-MP from microcomposite spheres did not exhibited an initial burst release, occurring a controlled release with 22% of 6-MP in 10 hours followed by a sustained release with 52% within 68 hours. Furthermore, the oral administarion of microcomposite spheres in wistar rats leaded to significant decrease in 6-MP toxicity, given that the microcomposite spheres significantly decreased the values of the high peak value of plasma concentration ($C_{máx}$) and the total area under curve (AUC), while increasing the mean residence time (TMR) of 6-MP in plasma[78].

LAP is another nanoclay that can be useful for glioma treatment, due to its stability and the impressively elevated SSA of its nanoscale disks. Studies showed that LAP has a great potential as nanocarrier of anticancer drugs. In the study carried out by Wang *et al.* (2013) in which LAP exhibited a high entrapment efficiency (EE) (98.3%) and a pH-responsive sustained release of DOX, which resulted in better therapeutic effect when compared to free DOX[79]. In order to achieve a target treatment with an elevated anticancer effect, LAP can be modified with several targeting ligands. However, this kind of functionalization can reduce the high drug delivery efficiency of LAP. Mustafa *et al.* (2015) designed a novel method to optimize the performance of LAP-based nanocarriers, without compromising the intrinsic loading capacity of LAP. Thus, poly(amidoamine) dendrimers were attached to LAP nanodisks to form G2 dendrimer-modified LAP nanodisks (LM-G2), which was subsequently loaded with DOX presenting an elevated EE of 98.4%. DOX was and that were applied to carry DOX. DOX released from the DOX-LM-G2 system exhibited a pH-responsive sustained release pattern, with a higher release at acidic media when compared to DOX-LAP nanocomposite, within the same interval of time. Additionally, it was demonstrated that the DOX-LM-G2 system can be significant internalized by cancer cells, showing a stronger inhibitory action when compared to free DOX. Thus, considering that dendrimers do not compromise the elevated EE and presented several functional groups, LM-G2 nanodisks can be used as an adaptable delivery system for several anticancer drugs in cancer therapy[30]. LAP has also proved to be useful to confer morphology persistence in polymer vesicles synthesis, due to the rigid nature of its disks. *In vitro* studies showed that the DOX release from LAP-PLGA-F68 nanocomposite vesicles were successfully internalized by C6 rat fibroblast glioma cells, indicating a possible application of these vesicles for cancer therapy[29].

4.1.7. Skin cancer

Skin cancer is the most frequent type of cancer. There are three principal types of skin cancer: squamous cell carcinoma, basal cell carcinoma, and melanoma. Although melanoma skin cancer (MSC) presents a lower incidence than the other cancer types, it is associated with the highest mortality. MSC begins in melanocytes, which are the cells that produce melanin. However, MSC presents the ability to invade surrounding tissues and disseminate for other parts of the body, which makes it more aggressive than other skin cancers. There are different therapy approaches for MSC and their application depend on the stage of the disease. Chemotherapy with anticancer drugs, such as DOX is one of the standard treatments for advanced MSC[58]. Hosseini *et al.* (2018) used BENT nanoclays to create a nanocomposite for DOX and explore its anticancer action on melanoma cancer cells. The nanocomposite was developed through electrostatic interactions between the DOX molecules with positive charge and the surface of the BENT with negative charge. The authors demonstrate that DOX was slowly released from the DOX-BENT nanocomposite under physiological conditions (pH 7.4) and reached a faster release rate under tumor conditions (pH 6.5). The *in vitro* anticancer action of these nanocomposite was evaluated using highly metastatic mouse melanoma cells (B16F10 cells), and the results showed that its anticancer activity is higher than free DOX. These results demonstrate that DOX-BENT nanocomposite is an alternative approach for local treatment of melanoma tumor and that it may minimize the side effects of DOX. The authors suggest that DOX-BENT nanocomposite aqueous suspension must be injected in and around the melanoma tumor location[36].

4.1.8 Thyroid cancer

Thyroid cancer ranks ninth worldwide when it comes to incidence, being accountable for over 567,000 cases. It is worth noticing that the incidence rate in women is 3-fold higher when compared with men[3]. The thyroid gland is a follicular endocrine gland located in the anterior region to the trachea. It has two main types of cells, follicular epithelial cells and parafollicular cells (C cells), from which distinct cancers develop. It is important to note the differences once they define the seriousness of the cancer and the approach to treat it[80]. The treatment options for thyroid cancer might include surgery, radioiodine therapy, external beam radiation therapy, gene therapy and chemotherapy. Frequently, most of these options involve complex processes and have significant associated side effects. Generally, a combination of chemotherapy with external beam radiation therapy is applied in thyroid cancers with poor prognosis[81]. There are three main types of thyroid cancer, differentiated, medullary and anaplastic. Differentiated cancer (including papillary, follicular and Hürthle cell) develops in

follicular epithelial cells and is the most common. Medullary cancer is responsible for only 4% of thyroid cancers and begins in C cells. Anaplastic cancer is a rare cancer associated to a poor prognostic and can develop from both follicular epithelial cells and C cells[80].

DOX is one of the anticancer drugs most used in the treatment of thyroid cancers. Zhang *et al.* (2016) developed DOX-loaded nanocarrier to target papillary thyroid cancer cells. Firstly, the interlayer space of kaolinite was expanded from 0.72 nm to 0.85 nm by a methoxy modification, in order to increase the loading capacity. Then, after loading DOX, polyethylene glycol (PEG) was conjugated on the external surface of methoxy-intercalated kaolinite (Kaolin $_{MeOH}$) to increase its hydrophilicity, prolonging its circulation time and reducing its opsonization. Considering that follicular epithelial cells have a strong ability to capture iodine ions for synthesis of the thyroid hormones triiodothyronine (T3) and its prohormone, thyroxine (T4), the obtained nanocomposite was coated with potassium iodide (KI) to specifically target papillary thyroid cancer cells (Appendix XV). Regarding DOX release profiles, there was a fast DOX release rate in simulated tumor intracellular conditions (pH 5.5), while in normal physiological conditions (pH 7.4) a slower DOX release occurred. This happens because Kaolin $_{MeOH}$ is positively charged at lower pH values, which reduces electrostatic interactions with positively charged DOX are reduced. Therefore, the DOX-Kaolin$_{MeOH}$-KI nanocomposite presented a pH-responsive controlled release behavior, physiological stability in the physiological conditions and the ability to accelerate DOX release only under the tumor microenvironment conditions. Confocal laser scanning microscopy (CLSMS) and Bio-transmission electron microscopy (TEM) analysis of papillary thyroid cancer cells after incubation with the DOX-Kaolin$_{MeOH}$-KI nanocomposite, showed that nanocomposites were internalized via endocytosis and presented dose-dependent therapeutic action *in vitro*. Additionally, its performance was additionally optimized by the KI functionalization by virtue of the resultant targeting ability. Anti-metastasis evaluation through healing and migration experiments revealed that the nanocomposite almost completely inhibited repair of the damage location and presented an excellent inhibition of cellular migration. In the mentioned work, a biodistribution analysis was conducted through two distinct *in vivo* experiments, active targeting research in white rabbits and passive targeting research in mini-pigs. In the first experiment, after intravenous administration there was a significant accumulation of DOX-Kaolin$_{MeOH}$-KI in the thyroid gland. Similarly, in the second experiment a marked accumulation occurred in the thyroid gland after DOX-Kaolin$_{MeOH}$-KI being directly administrated in the thyroid gland of mini-pigs. These results suggest that DOX-Kaolin$_{MeOH}$-KI can be applied for an efficient and targeted thyroid cancer therapy[81]. Recently, Zhang *et al.* (2018) intercalated

dodecylamine into the interlayer of $Kaoling_{MeOH}$ to prepare dodecylamine modified kaolinite (Kaolin $C_{12}N$). This modification was applied to increase the interlayer space to 4.16 nm and expose the Si-OH groups of the internal surface of kaolinite. Increased space between layers provides specific locations for chemical reactions to occur and the SI-OH groups become more exposed, allowing the dispersion of compounds to be carried. Kaolin-$C_{12}N$ was functionalized with Mn_3O_4 magnetic nanoparticles and further loaded DOX in its interlayer space to form a promising theranostic platform to be applied in thyroid cancer. DOX- Kaolin $C_{12}N$-KI-Mn_3O_4 showed better *in vitro* anticancer action than free DOX toward papillary thyroid cancer cells (TPC-1 cells), 79% of cell inhibitory rate compared to the 43% of cell inhibitory rate promoted by exposure to free DOX. Moreover, images of tumor sections of tumor-bearing mice demonstrated that the theranostic platform caused a significant tumor shrinking (75%) and an accumulation in the tumor tissues[82].

The studies presented above provide evidence that the use of KI in the nanocomposites was essential to achieve a tumor-targeted accumulation with minimal toxicity. Generally, in both studies kaolinite was an efficient and low-toxicity drug deliver carrier, that increases water solubility of DOX, facilitates the interaction with cancer cell membranes and controls DOX release in cancer microenvironment, minimizing the distribution of DOX in healthy tissues. Furthermore, the ability to incorporate MNP and KI into kaolinite-based nanocomposite allows imaging diagnosis, targeted therapy, and drug delivery applications at the same time.

4.1.9 Bone cancer

Osteosarcoma (OSA) is the most frequent type of bone cancer in children and young adults. Surgery is an important step of the treatment to remove all sites of clinically detectable tumor mass. However, the tumor recurrence is very high if the treatment includes only surgery. Thus, adjuvant chemotherapy is often used in combination with surgery, to inhibit micrometastasis before and or after surgery. Despite the progress in the treatment of OSA, innovative treatments and prevention approaches are still needed[83],[84].

HNTs have been studied as carriers of anticancer drugs, such as MTX, quercetin (QUE), taurolidine and artemisinin for the treatment of OSA. Cytotoxic assays exhibited that the nanocomposites can modify OSA cells morphology and inhibit their proliferation. A release test was carried only for the MTX loaded into HNTs, the release profile showed an initial burst release, where 70% of the loaded MTX was release in 1h, and 95% of the loaded MTX was release from HNTs after 24hours[83]. In order to control the release of MTX release, HNTs were alternately modified with positively charged polyvinylpyrrolidone (PVP) polyelectrolyte

and with the negatively charged poly(acrylic acid) (PAA) polyelectrolyte via Layer-by-Layer (LbL) method. *In vitro* experiments showed that polyelectrolyte coatings provided a sustained release of MTX over 160 min, without affect the nanocomposite ability to inhibit the growth of OSA cells[84].

4.2 Natural compounds

Curcumin (CUR) is a phenolic compound obtained from the rhizomes of the *Curcuma longa*. Several studies have revealed that CUR presents anti-inflammatory, antioxidant and anticancer actions, being a pleiotropic compound related to many mechanisms of action with different cellular targets. However, therapeutic application of CUR is limited by its poor water solubility, chemical instability, and rapid metabolization[85]. Nanocarriers based on nanoclays have been created with the aim of extending the therapeutic application of CUR.

MMT has been studied as a potential carrier for CUR. Madusanka *et al.* (2015) reported the use of CMC combined with MMT as nanocarrier for water insoluble CUR. Considering that CMC has emulsifying properties, it was applied as an intermediary that linked CUR to MMT, thus improving the solubility of the drug. Also, curcumin-activated MMT-CMC nanocomposite showed the ability to control the release of CUR, by promoting a release of 60% during 2 hours and 30 min at acidic media (pH 5.4)[86].

Hydrophilic biopolymers have been receiving increased attention due to their ability to make nanocarriers more biocompatible and biodegradable, to increase their water solubility, and to provide colloidal stability in the bloodstream. CS is a hydrophilic biopolymer often used to functionalize nanocarriers, due its biocompatibility, biodegradability and mucoadhesive properties. Furthermore, CS is only ionized in acidic conditions, which makes it the ideal biopolymer to interact with the cancer cells, given that at low pH CS is positively charged and establish electrostatic interactions with negatively charged cellular membranes[9]. Therefore, Khatun *et al.* (2018) used MMT-CS nanocomposites for controlled delivery CUR. After 6 hours of sustained release, the cumulative release reduced with the increase of pH in the experimental conditions. Thus, the release profile of curcumin exhibited a cumulative release rate that ranges from 70% to 90% at acidic conditions (pH 1.2), whereas in alkaline conditions it ranges from 45% to 60%. This cumulative pH-dependent release is a consequence of the difference in the swelling of CS in acidic and alkaline conditions. CS swelled more in acidic media and in these conditions its residual amino groups were protonated, which led to a faster drug release rate. The nanocomposite reduced the viability of both MCF-7 and liver hepatocellular cells (Hep G2 cells)[87].

It has also been proved that HNTs are good candidates for carrying CUR by enhance its oral bioavailability[85],[88],[89] [90],[91],[92],[93]. The surface of CUR loaded HNTs was, subsequently altered with a mucoadhesive poly(methyl vinyl ether-co-maleic acid) polymer (PMVEMA) to increase interactions with the intestinal epithelium and promote a better permeation of CUR over the intestinal cell monolayers. The interactions of PMVEMA modified HNTs with Caco-2 cells and human colon adenocarcinoma cells (HT29-MTX cells), which mimic the absorptive enterocytes and mucus-producing goblet cells, respectively, showed that the modification with the mucoadhesive PMVEMA results in a 13-fold increase of CUR permeability. In addition, the CUR-HNT-APT-PMVEMA nanocomposites were encapsulated with the pH-responsive HPMCAS through microfluidics technique (Appendix XVI (A)). Thus, spherical nanocomposites were produced with homogeneous particle size distribution. *In vitro* drug release studies showed a pH-responsive dissolution and drug release performance (Appendix XVI (B)). This performance is essential to protect CUR from the harsh environment of the stomach and release the CUR only in the intestinal environment[85].

In a study that aims to mimic not only the well-known carbohydrate-lectin complexes but also the glycocluster phenomenon, it was created a new nanocarrier based on HNTs to carry phenolic compounds like CUR. Thus, carbohydrate–cyclodextrin units were covalently attached onto the HNTs surface. Afterwards, CUR was incorporated in the two available cavities, the hydrophobic cavity of cyclodextrins and the lumen of HNTs[88]. In a different approach, CUR was covalently combined with HNTs, through pH-responsive and disulfide linkages. Thus, the resulting dual-responsive prodrug showed a significantly enhanced release of CUR in a lower pH and high concentration of GSH. The CUR-HNT nanocomposite showed a high cytotoxicity towards two hepatocellular carcinoma cell lines after a 48 hours incubation, which indicates potential in hepatocellular carcinoma treatment[89].

Another strategy for the pH-responsive release of CUR was developed through its interaction with the hydroxyl groups present both in the internal and external surfaces of HNTs. In addition, CUR also helps the formation of gold nanoparticles (Au NPs) in HNTs. The obtained HNT nanocomposite was coated with the cationic biopolymer CS (Appendix XVII (A)). The presence of both Au NPs and CS conferred near-infrared (NIR)-light responsive ability and pH-responsive release. In fact, the CUR release was more significant in acidic pH media (pH 5.5) than in alkaline pH media (pH 7.4) (Appendix XVII (B)). Also, the nanocomposite exhibited a better anticancer action towards MCF-7 cells under intracellular tumor cell conditions (pH 5.5) than extracellular conditions (pH 7.4) (Appendix XVII (C)). Therefore, the designed nanoparticles could be applied for a targeted drug release with NIR-

imaging[90]. Similarly, Liu *et al.* (2016) developed an innovative nanocarrier based on HNTs that included CS as a coating. Initially, the hydroxyl groups in the surface of HNTs were converted to carboxyl groups (COOH) in the presence of succinic anhydride. Then, CS was grafted onto the HNTs-COOH to decrease its toxicity, increase its stability and improve the loading efficiency of CUR. Thus, chitosan grafted HNTs (HNTs-CS) presented superior values for the EE (90.8%) and drug loading (DL) (3.4) in contrast pristine HNTs. In addition, HNTs-CS exhibited a satisfactory stability in serum without the presence of significant hemolytic effects. The CUR release from HNTs-CS showed a specific toxicity towards several cancer lines, with a highest anticancer action in EJ human bladder carcinoma cells[91]. CUR was also efficiently encapsulated into cellulose-HNTs composite hydrogels. Cellulose hydrogel in the presence of HNTs showed higher EE (21%) than pure cellulose hydrogel (17%). This may be due to the fact that cellulose-HNTs composite hydrogels are composed by two components, HNTs and cellulose, both with an elevated adsorption capacity. In drug release studies, CUR presented controlled release from the cellulose-HNTs composite hydrogels and reached its maximum release percentage (62.1%) at 20 hours. Moreover, CUR loaded composite hydrogels exhibited a high inhibition action towards MCF-7 cells, while demonstrating a favorable cytocompatibility for mouse embryo osteoblast precursor cells (MC3T3-E1 cells)[92].

HNTs coated with polyelectrolytes multilayers were used to carry CUR. HNTs were coated alternately with positively charged and negatively charged electrolytes, through LbL method. The coated HNTs reduced the release rate of CUR, releasing 20% less of drug than uncoated HNTs in 24 hours Also, they were successfully incorporated by MCF7-cells releasing CUR after 24 hours with a slow pattern[93].

Several studies in the cancer field have paid attention to resveratrol (RSV), that is a polyphenol recognized for being an effective anticancer and antiproliferative agent. However, its pharmacological use has been limited by its reduced instability and poor water solubility. Thus, RSV was encapsulated into HNTs to conserve its bioactivity and avoid its metabolization. The release of RSV was controlled by the functionalization of the external surface of HNTs with a multilayer structure. LbL method was used to coat nanotubes alternatively with a cationic polyelectrolyte, protamine salt (PRM) and with an anionic polyelectrolyte, dextran sulfate sodium (DXS). Hence, the RSV release from coated HNTs exhibited a slow and constant pattern during 48 hours. In addition, *in vitro* experiments in MCF-7 cells showed a slow release of RSV and an increase of apoptosis[95].

Saponins (SAP) are secondary metabolites produced by several plant species to protect them from stressful conditions. SAPs are chemically defined as heterosides with a genin that

can present a triterpenic or steroid nature. Several studies have revealed that steroidal SAPs present a cytostatic and cytotoxic effect towards cancer cells. SAP can interplay with high amounts of cholesterol present in the cell membrane of cancer cells. Given that SAPs produce membrane damage and necrosis, they have a reduced therapeutic index. It should be noted that SAPs have high toxicity after parenteral administration due to its hemolytic action in erythrocytes. Thus, the use of nanocarriers is an option to overcome the toxic effects of SAPs, control its release and avoid its degradation at a physiological media. Akbal *et al.* (2018) utilized MMT modified with human serum albumin (MMT-HSA) nanocomposites to load SAPs and investigated the cytotoxicity of the formulation using human colorectal adenocarcinoma cells (DLD-1 cells) and L929 fibroblast cells. The results revealed that the formulation can provoke a dose-dependent death in cancer cells and absence of toxicity in healthy cells. The use of MMT-HSA nanocomposites not only improved SAPs efficacy but also are an alternative approach for CRC treatment[96].

Serratia marcescens produces prodigiosin, which is a secondary metabolite with anticancer activity against several human cancer cell lines. However, the clinical use of prodigiosin is limited by its hydrophobicity, which results in a limited absorption and low bioavailability. These limitations can be overcome using the HNTs as nanocarriers. Guryanov *et al.* (2020) showed that prodigiosin can be adsorbed in the external surface and simultaneously encapsulated into the lumen of HNTs, resulting in nanocomposites with high bioavailability. Furthermore, the nanocomposite exhibited cytotoxic activity against Caco-2 cells and human colon carcinoma cells (HCT116 cells) without damaging healthy cells[97].

Overall, the strategies listed in this section showed the usefulness of both HNTs and MMT as efficient nanocarriers for natural compounds delivery in cancer cells.

4.3. Biopharmaceutical agents

4.3.1 Enzymes delivery

Proteins are unstable, presenting high susceptibility to enzymatic action and sensibility to extreme pH and temperature values. Moreover, proteins are characterized by a fast clearance and low permeability in biological membranes. Given that they present such unique characteristics regarding their stability, the use of proteins requires special care.

Regarding cancer treatments, there has been an increasing interest in the use of enzymatic therapeutics. As it is necessary to develop more effective and less toxic drugs, enzymes with antiproliferative activity are an option. These specific types of enzymes have a more effective anticancer action during a long period of time and with less risk involved.

Enzymes, such as l*accase*, *glutaminase*, *binase*, *arginase*, *methioninase* and *uricase* were documented for their anticancer properties. The preferred route of administration for enzymes is the parenteral route. However, this route is less desirable given the discomfort and compromised quality of life of the patient. Thus, there is an emerging necessity to develop systems that recur to less invasive routes of administration, for example, the oral route[98],[99],[23]. The administration of enzymes through the oral route presents several difficulties, such as the loss of therapeutic activity, probable degradation in TGI and possible side effects. For this reason, exploring innovative new delivery systems for enzymes is extremely important. A possible strategy is loading enzymes in neutral nanocarriers, which prevent its degradation and possible side effects. Furthermore, nanoscale carriers increase the concentration of the enzyme only in cancer cells through a sustained targeted release[98],[99],[23].

HNTs are promising nanocarriers for targeted enzyme delivery for cancer treatment[99]. The opposite surface charges of HNTs enable a selective charged enzymes adsorption. The nature of the enzymes and the pH conditions in which the adsorption procedure is performed affect the outcome[98]. The pH conditions affect the charge of the amino acids present in the enzymes and, consequently, the electrostatic interactions between enzymes and the HNTs surfaces. Accordingly, if the pH of the enzymatic solution is above the isoelectric points of enzymes, the enzymes are negatively charged and interact more efficiently with the positively charged lumen of HNTs. On the other hand, if the pH of the solution is below the isoelectric points of enzymes, the enzymes are positively charged and interact more with the negatively charged external surface of HNTs (Appendix XVIII). Typically, the charge of the surface area of HNTs is superiorly negative which leads to an inferior adsorption of negatively charged enzymes, that can range from 6 to 7 wt. %. This is a rather low value when compared to the adsorption of positively charged enzymes on the HNTs, that ranges from 15 to 25 wt. %[98]. Concerning the release profile of HNTs, it has been showed that approximately one third of negatively charged proteins will present a prolonged release pattern within the first 5 to 10 hours, preceded by an initial burst, which releases the most part. This means that the remaining two thirds of the corresponding proteins stay functional in the HNTs lumen and present a small release rate. All these features allow the use of HNTs as functional confined enzymatic nanoreactors[98].

Applications of the HNTs as an enzymatic nanoreactor have already been studied for cancer therapy. For example Kim *et al.* (2018) and Khodzhaeva *et al.* (2017) have demonstrated the immobilization of *laccase* and *binase* on HNTs, respectively, for enzymatic cancer therapy[100],[99]. In the first study, a new promising theranostic system that improves efficiency

and specificity of *laccase* delivery is suggested. *Laccase* is a polyphenol oxidase with antiproliferative activity indicated for cancers overexpressing estrogen receptors (cervical or breast cancers), given that *laccase* prevents the interaction between estrogens and estrogens receptors by degrading estrogens, natural phenolic compounds. In order to improve these anticancer properties and achieve a targeted release, *laccase* (pI 3.0) was immobilized recurring to electrostatic interactions in the lumen of HNTs. HNTs were functionalized with CS and Fe_3O_4 nanoparticles before the immobilization of *laccase*. The CS functionalization of the HNTs enables a covalent loading of *laccase* mediated by a crosslinking agent, glutaraldehyde. The modification of HNTs with Fe_3O_4 nanoparticles gives the nanocarrier magnetic features, enabling a specific release mechanism triggered by magnetic stimulus. Additionally, Fe_3O_4 nanoparticles allow treatment process monitoring given that they are stable image elements. The obtained result is a dual actively targeted theranostic system (influenced by external magnetic field and *laccase*-estrogens receptor interactions) with highly efficient antiproliferative and apoptotic properties against cell lines overexpressing estrogen receptors, such HepG2 cells (Appendix XIX)[100]. On the other hand, the second study *binase* immobilized on HNTs is a good illustration of an enzymatic nanoreactor application in CRC treatment. The guanyl-preferring ribonuclease from *Bacillus pumilus*, *binase (*pI 9.5) has a specific antitumor action towards cancers expressing *ras* gene mutations. These mutations are present in more than 30% of all cancers and are more prevalent in 90% of cancers with a poor prognostic, such as the CRC cancer. Mutations in *ras* gene result in a permanent activated state of protein Ras, which is a problematic scenario because cells proliferate in an uncontrolled manner and acquire resistance to the cancer therapy[101]. In order to prevent this scenario, the use of *binase* is a great strategy due to its capability to block *ras* mutation downstream signaling pathway. According to Khodzhaeva *et al.* (2017) *binase* was immobilized onto the HNTs lumen to avoid its decomposition by proteases and prolong its release. Considering *binase* a positively charged enzyme, its loading through electrostatic interactions on the positively charged lumen of HNTs is difficult. Nevertheless, the 3.3 nm in diameter of binase allows its insertion into HNT lumen that has a diameter that ranges from 12 nm to 15 nm. Additionally, dextrin stoppers were inserted at the HNTs ends to prevent *binase* early release from HNTs lumen. The immobilization of *binase* on HNTs significantly increases its cytotoxicity toward colon adenocarcinoma cells due to perfect absorption of *binase* by this cell line and its prolonged release. The *binase*-HNTs complex reduces the cell viability by 60%, whereas free *binase* reduces cell viability by 25%. Considering that HNTs are not biodegradable, a binase enzyme immobilized on HNTs in the form of rectal suppositories may be a promising enzymatic nanoreactor for CRC treatment[99]. These two experiments illustrate that both *laccase* and

binase after immobilization onto HNTs constitute enzymatic nanoreactors, which are highly cytotoxic toward two different cancer cells lines, colon human cervical cancer and adenocarcinoma cells, respectively.

4.3.2 Gene delivery

Gene therapy is an approach that consists of modulating gene expression levels by introducing exogenous nucleic acids into specific cells to treat human monogenic or multifactorial disorders[102]. In order to correct the genetic defect that causes the aforementioned disorders, gene therapy comprises a large number of biologically active nucleic acids, which are applied to control the post-transcriptional or translational regulation of gene expression.

For the last 20 years, several clinical trials have been documented to study the efficacy and safety of gene therapy. Although the monogenic disorders are gaining attention, more than half (67%) of ongoing clinical trials are focused on cancer[103]. Cancer is characterized by an accumulation of gene mutations, innate or acquired, which results in transcription alterations causing the change from a normal cell growth pattern to a malignant cell growth. The occurrence of mutations in proto-oncogenes and tumor suppressor genes promote oncogene formation and loss of function of tumor suppressor genes. These are necessary alterations for the development of cancer once they promote uncontrolled cell proliferation, the ineffective performance of the immune system and an accumulation of driver and passenger mutations[47],[7]. Generally, cancer is a progressive process. Initially accumulation of mutations happens randomly. Then, a selection of mutations that produce an excess of malignant cells takes place. The malignant cells, with the exception of hematological cancers, develop an abnormal cell mass or tumor. Considering the mentioned cancer development process, gene therapy plays an important role in cancer therapy[104].

In gene therapy, the efficacy of exogenous nucleic acids is largely dependent on the compatibility with endogenous cellular components to perform their actions. Naked nucleic acids have a hydrophilic nature, large molecular size and are very unstable due to their easy degradation by nucleases when applied *in vivo*. For this reason, in gene therapy, the main challenges are to overcome the cellular, tissue and enzymatic barriers to deliver genes to the targeted cells, which can be achieved by applying effective gene carriers[104],[105]. Gene carriers can be biological or chemical and they are classified into viral vectors and non-viral vectors, respectively[102], [105],[104]. Viral vectors were the primary molecular carriers with the ability to transfer genes into human cells and have been applied in more than 60% of clinical trials[104].

Viral vectors integrate the viral cargo into the target cell genome, and they are recognized for their capacity to transduce a wide variety of cell types. Although viral vectors remain the most used delivery method for gene therapy, they have associated high risks, including strong immunogenicity and capacity to trigger insertional mutagenic events, which may deregulate the expression of proto-oncogenes or tumor suppressor genes[105]. These associated risks prompted the development of non-virus vectors. In contrast to viral vectors, non-viral vectors present higher biosafety, lower immunogenic characteristics and easier preparation[102]. Unfortunately, they present lower transfection efficiency and absence of self-tacking ability imposing limitations in clinical applications. To circumvent these disadvantages, new strategies are being developed such as the chemical synthesis of inorganic particles to adjust their shape, chemical properties and composition[102],[106]. Given the large size and negative charge of nucleic acids, normally the surface of synthesized inorganic particles is functionalized with positively charged groups or cationic polymers[105]. Regarding natural inorganic nanoparticles, they present satisfactory cell uptake efficiency and good biosafety. However, they are not studied as gene vectors, usually. Nanoclays are a particular case that has shown to be promising in gene therapy as a non-viral vector[105].

Antisense oligodeoxynucleotides (ASODN) and small interfering RNAs (siRNA), compose an important class of widely used effectors for gene therapy, especially in cancer treatment. These strategies show a strong ability in silencing oncogenic factors for cancer gene therapy[107],[48],[108].

ASODNs are single strand DNA molecules with 15 to 30 nucleotides that are released in the cytoplasm to establish a connection with complementary regions of a target gene, present in messenger RNA (mRNA). The gene expression is specifically inhibited by hybridizing ASODNs with the genes of mRNA[107]. Preliminary work on the application of HNTs as a nanocarrier for intracellular delivery of ASODNs was undertaken by Yin-Feng Shi *et al.* (2011). Firstly, to facilitate the loading and delivery of ASODNs, HNTs were functionalized with γ-aminopropyltriethoxysilane (APTES). Then, ASODNs were labelled with fluorescein for their intracellular tracking. The obtained complex showed a high intracellular delivery ability in HeLa cells (98.69%) and better antitumor activity when compared to free ASODNs[107].

Another potential gene therapeutic agent is siRNA, an endogenous small noncoding RNA of ca. 21 nucleotides, with the ability to silence target genes in a precise approach. Through a sequence-specific action, siRNAs degrade the complementary mRNAs and lead to the knocking down of a target protein at a post-transcriptional level. This mechanism of action has been exploited to interfere with specific genes that are important in cancer

propagation[48],[108]. Wu *et al.* (2014) prepared functionalized HNTs to carry siRNA into PANC-1 cells to decrease the levels of surviving proteins with an important role in the inhibition of cell proliferation. In this work, HNTs were functionalized with polyethylenimine (PEI) polymer via LbL-method for loading and delivery of anti-survivin siRNA. Also, mercaptoacetic acid capped (CdSe) quantum dots were conjugated, via noncovalent electrostatic interaction, with anti-surviving siRNA to enable the visualization of the process. This HNTs-based multifunctional nanocarrier exhibited high transfection efficiency in PANC-1 cells (95.6%) indicating that functionalization with PEI had significantly increased the delivery efficiency of siRNA. An i*n vitro* cytotoxicity assay showed an increase in cell apoptosis in the presence of the complexes, which indicates that they can enhance antitumor activity. Furthermore, according to western blot analysis, after 72 hours of exposure to functionalized HNTs nanocarriers, approximately 90% of the surviving protein in PAC-1 cells had been knocked down. All these results suggest that HNTs-based multifunctional nanocarriers silenced the gene of interest (survivin), inhibiting the survival of cancer cells[48]. Similarly, Long *et al.* (2017) transfected green fluorescence protein (GFP) labeled DNA using functionalized HNT nanocarriers. In their study, shortened HNTs with 200 nm length were coated with PEI to enhance the transfection efficiency and avoid cell injury and inflammation. The resulting complexes reached an elevated DNA transfection efficiency (44.4%) in HeLa cells and exhibited good biocompatibility[102]. In another study, Long *et al.* (2018) proposed polyamidoamine grafted HNTs (HNT-PAMAM) for loading and intracellular delivery of vascular endothelial growth factor siRNA (siVEGF). *In vitro* assays, using human breast cancer MCF-7 cells, showed a cellular uptake efficiency of 94.3% for siVEGF-HNT-PAMAM and a reduction of 78.0% for the expression of mRNA of vascular endothelial growth factor (VEGF). The significant reduction of VEGF expression can induce apoptosis which increases antitumor efficacy. Regarding *in vivo* anticancer assays, the results reveal that siVEGF-HNT-PAMAM significantly reduced the tumor volume, showed a high tumor inhibition rate (55.1%) and inhibited angiogenesis (Appendix XX). All these results suggest that siVEGF-HNT-PAMAM is a promising therapeutic method for breast cancer[105]. Liu *et al.* (2019) used HNTs as a nanocarrier for siRNA (siRNA-HNT) to target oncogene RIPK4 for bladder cancer treatment, via RIPK4 reduction. siRNA was encapsulated in HNTs to increase their serum stability and promote a target silencing action. It was shown that HNTs could deliver siRNA in an efficient and specific way into bladder cancer cells. Also, the *in vitro* and *in vivo* results obtained in this study suggest that siRNA-HNT nanocomposites knocked down RIPK4 expression, which results in an inhibition of bladder cancer progression[105].

MMT also presents a great potential to contribute to the development of gene delivery systems[109],[110]. Lin *et al.* (2006) design a vector for gene delivery by the combination of hexadecyltrimethylammonium (HDTMA) and MMT. Given that the surfaces of MMT are negatively charged as the DNA molecules, the functionalization of MMT with HDTMA is essential to increase its affinity with DNA molecules. Furthermore, HDTMA expands the interlayer space of MMT, increasing loading capacity. Electrophoresis analysis showed that the DNA-MMT-HDTMA nanocomposite provided a good physical protection to DNA. *In vitro* assays using human dermal fibroblasts demonstrated that the MMT-HDTMA nanocomposite was successfully transfected into the nucleus of the cells[109]. Sironmani *et al.* (2015) used silver nanoparticles (Ag NPs) stabilized in MMT to carry a plasmid DNA vector combined with complementary DNA of GFP (pcDNA-GFP). The characterization of the pcDNA-GFP-MMT-Ag nanocomposite showed that it can be recognized by a shift in plasmon resonance to a higher wavelength that occurs in the presence of DNA. Thus, DNA molecules are detected as a variation in the absorption probability and a variation in the resonance wavelength. This result suggests that pcDNA-GFP-MMT-Ag nanocomposite can be applied for diagnostic purposes. Moreover, pcDNA-GFP-MMT-Ag nanocomposite showed satisfactory results in transfection assays[110].

4.4 Phototherapy

Currently there is a comprehensive range of therapies under investigation designed for different cancer treatments[111],[112]. Within all these therapies, phototherapy has been showing promising results. Phototherapy implies an administration of light-responsive molecules followed by a light irradiation of tumor location, so that the molecules accumulate in this area and exert the desired anticancer action[113],[114]. This therapeutic action is localized because the molecules that possibly accumulated in other locations will not produce any cytotoxic effects, since those sites are not exposed to light irradiation. *In vivo* application of phototherapy requires the use of radiation with longer wavelengths to facilitate light penetration in tissues. In the human body, the main tissue chromophores (hemoglobin, myoglobin, and melanin) strongly absorb UV and visible light, while water absorbs light with wavelengths above 900 nm. Therefore, the most appropriate phototherapeutic agents are those that have a strong absorption in the near-infrared wavelengths (650 nm to 900 nm), a region well known as the biological transparency window. The use of NIR light guarantees a minimal interaction with biological components and deep tissue penetration, which is fundamental for the activation of phototherapeutic agents accumulated in the tumor area[113],[115]. Generally, phototherapy provides two types of phototherapeutic agents: the photosensitizers indicated for

photodynamic therapy (PDT) and the photothermal agents suitable for photothermal therapy (PTT) (Appendix XXI)[114].

In PDT, upon light irradiation, the administered photosensitizers lead to production of cytotoxic ROS, which cause the destruction of the tumor acting in three main targets: cancer cells, microvasculature, and the inflammatory and immune host system (Appendix XXI (A)). The ROS responsible for the therapeutic effect in PDT are a result of the transference of energy from the excited photosensitizer to the molecular oxygen. PDT differs from anticancer drugs because it does not have any toxic effects on the biological systems and contrary to the ionizing light used in radiotherapy, it does not damage surrounding normal tissues. Additionally, PDT has great functional and cosmetic outcomes, lower long-term morbidity and enhanced quality of life of the patients. It should be noted that the level of cytotoxicity resulting from PDT is dependent on the optical properties and the concentration of the photosensitizers, its localization, the period of time between photosensitizers administration and light activation, and the amount of oxygen in the tumor location[112],[114]. Indocyanine green (ICG) is a photosensitizer approved by the Food and Drug Administration (FDA) for medical applications and has been investigated for PDT employed in cancer treatment, once it produces cytotoxic effects when activated by light irradiation. However, ICG is still lacking in its clinical application due to its instability in physiologically conditions. ICG has poor stability in aqueous solution, particularly when exposed to light. Therefore, application of nanocarriers to encapsulate ICG is a good strategy to overcome some limitations associated with this photosensitizer[112],[114]. Li *et al.* (2019) loaded negatively charged ICG into the positively charged lumen of HNTs through electrostatic interactions, to enhance photochemical stability of ICG. HNTs were functionalized with a polyanion coating of poly(sodium-p-styrene sulfonate) (PSS), which increased its zeta-potential from -32 mV to -52 mV and, consequently, improved its biocompatibility and dispersion stability. The resulting ICG-HNT-PSS nanocomposite provided a higher photosensitization effect than free ICG, demonstrating that when the ICG is encapsulated into HNTs, it is protected from photochemical degradation and shows greater photochemical stability in aqueous media. PDT effect of ICG-HNT-PSS nanocomposite was examined using giant unilamellar vesicles (GUV) as membranal models. When exposed to NIR light, GUV suffered a photodynamic rapid morphological alteration, which confirms the great PDT performance of the nanocomposites in oxidative membrane perturbation[114]. The ICG-HNT-PSS nanocomposite was also decorated with MDA-MB-436 (human cervical carcinoma tumor) cell membranes. This modification increased the biocompatibility of nanocarrier and demonstrated a superior targeting towards MDA-MB-436

cells. *In vitro* PDT was performed by irradiating MDA-MB-436 cells with a NIR light with a wavelength of 808 nm and power density of 300 mW/cm^2 for 160 s. Under these conditions, MDA-MB-436 cells exhibited significant membrane disintegration. The study also reported *in vivo* PDT using membrane-coated ICG-HNT-PSS nanocomposites at 808 nm NIR laser irradiation with a safe irradiance of 300 mW/cm^2 for 300 s. After tail-vein injection of membrane-coated ICG-HNT-PSS nanocomposites, a significant tumor volume reduction (ca. 95%) occurred within 14 days, when compared to control, ICG and uncoated ICG-HNT-PSS groups (Appendix XXII). All these results suggest that membrane coated ICG-HNT-PSS is a promising biomimetic nanocomposite for targeting therapy against breast cancer[114].

In PTT, photothermal agents are administered to convert light into heat inside tumors, resulting in thermal ablation of cancer cells. In this method, cancer cells suffer irreversible damage at high temperatures (Appendix XXI (B)). The damage level depends on the thermal energy that is applied, the rate of application and the thermal sensitivity of the target tissue. When temperatures range from 41°C to 47°C, irreversible cellular damage occurs only after prolonged exposure (above 60 min). On the other hand, at temperatures above 50°C, the required time to achieve irreversible damages decreases exponentially. Above 50°C, protein denaturation occurs and leads to coagulative necrosis. Moreover, hyperthermia promotes mitochondrial dysfunction and inhibition of DNA replication. It is important to highlight that cancer cells are more thermosensitive than healthy cells due to high metabolic stresses, reduced heat-dissipating capacity and the acidity of the TME[116],[117],[118]. In contrast to conventional therapies, PTT is a technique with high selectivity due to the possibility to regulate the position and the irradiation area of the laser to concentrate the laser light on the tumor location. Furthermore, photothermal agents play a key role in PTT, thus their optical features and photothermal conversion efficiency, their availability into TME and their cellular distribution are crucial for the successes of the treatment[116],[118].

Currently, nanomaterials are a possible new generation of photothermal agents because they are much less susceptible to photobleaching and present a higher capacity to accumulate at the tumor local through passive or active targeting. For example, Au NPs have been extensively explored as photothermal agents because of their tunable resonance characteristics, higher absorption ability in the NIR region and more efficient photothermal conversion. Also, it is important to highlight that Au is an element with inert chemical activity and an acceptable biocompatibility. The application of Au NPs is based on an optical phenomenon acknowledged as localized surface plasmon resonance (LSPR). The LSPR absorption of Au NPs could be easily tuned to the NIR region, changing the morphology of

nanoparticles. For example, the LSRP absorption of Au NPs can be adjusted to the NIR region through the prolongation of the structure of nanomaterials along a single direction[113],[119]. Wu *et al.* (2016) used a simple process to prepare Au nanostructures with a tube shape using PAL as a template. Firstly, the negatively charged surface of PAL was adjusted to be positively charged by the sequential deposition of polymers with opposite charges on its surface, using the LbL method. Then, the surface of polymer coated PAL was uniformly decorated with several small Au NPs (diameter of ~15 nm) linked to each other, to originate tube-like Au-PAL nanocomposites (Appendix XXIII). This shape allowed a successful adjustment of the LSPR peak of Au nanoparticles into the NIR region (~670 nm) and the nanocomposites acquired the ability to rapidly adsorb NIR light and efficiently convert light into thermal energy. In this study, the temperature increases from room temperature to 53°C when Au-PAL nanocomposites were exposed to 808 nm NIR irradiation at 0.5 W/cm^2. This indicates an elevated photothermal conversion capacity of Au-PAL nanocomposites. Their photothermal activity was examined using A549 cells and A549-cell-bearing nude mice. A549 cells were incubated with nanocomposites (at 100 μg/mL) and irradiated with 808 nm laser at the power density of 0.5 W/cm^2 for 15 minutes. These conditions induced a notable cell death since the obtained cell viability is nearly 0. It was also revealed that tumors grown in Balb/c nude mice, after intra-tumoral injection with Au-PAL nanocomposites, were effectively ablated when exposed to the 808 nm laser irradiation with a power density of 0.5 W/cm^2 for 15 minutes. Moreover, it was observed an absence of re-growth for at least 10 days, the assay period, suggesting that the PTT ablation of tumors is important for the inhibition of cancer cell metastasis[118].

Tube-like Au-PAL nanocomposites have also been proven to be promising for potential combination of PTT and chemotherapy. Zhao *et al.* (2019) suggested that MTX intercalated and adsorbed onto the channel and surface of PAL associated with Au-PAL nanocomposites. In a cytotoxicity assay carried on HeLa cells, PAL combined with MTX induced a distinct and rapid decrease of cell viability in comparison free MTX and PTT produced a suppression effect after irradiation for 15 minutes after 24 hours of incubation with Au-PAL nanocomposites[119]. Similarly, Zhang *et al.* (2019) presented an innovative chemo-PTT nanocomposite indicated for the treatment of breast cancer, where gold nanorods (GNR) and DOX were simultaneously carried in the lumen and surface of HNTs, respectively. The external surfaces of HNTs were also grafted with targeting ligand FA, via reaction with bovine serum albumin (BSA). This additional functionalization enables a specific recognition of cancer cells overexpressing FR, that leads to a better tumor-targeted efficacy. *In vitro* experiments showed that Au-DOX-

HNTs-BSA-FA nanocomposite exhibited strong chemotherapeutic effects against MCF-7 cells, with a positive FA receptor. Before the laser irradiation, the cell survival rate was 73%. However, when MCF-7 cells were exposed to 808 nm NIR laser irradiation at power density of 1 W/cm^2 for 8 min, their apoptosis rate increased, and the survival rate of cells was only 7.4%. These results suggest that both DOX and GNRs in Au-DOX-HNTs-BSA-FA nanocomposite play a significant role in the apoptosis, under light irradiation. The slow release of loaded DOX led to a low cellular apoptosis, whereas the subsequent laser exposure enhanced cellular apoptosis, given the increase in temperature. Furthermore, 4T1-bearing mice were intravenously injected with Au-DOX-HNTs-BSA-FA nanocomposite (20 mg/kg) and exposed to an 808 nm laser at the power density of 1 W/cm^2. Significant reduction of both tumor volume and weight was achieved with this treatment. The combination of Au-DOX-HNTs-BSA-FA nanocomposite with laser irradiation caused a 71% reduction in the average tumor volume, while in the absence of DOX the reduction of the average tumor volume was approximately 67.4%. When compared to the free DOX, the Au-DOX-HNTs-BSA-FA nanocomposite decreased the tumor volume more prominently and did not cause a decrease in body weight (Appendix XXIV)[120]. Together, the mentioned two studies outline simultaneous delivery of heat and anticancer drugs as a method that leads to a considerable increase of the cancer treatment efficacy with reduced presence of side effects.

Another example of nanomaterials used in PTT are magnetic nanoparticles (MNP). Anirudhan *et al.* (2012) explored the magnetic hyperthermia properties of MNPs for tumor ablation. They studied the *in vitro* heating behavior of MNPS using carboxymethyl chitosan-capped-magnetic nanoparticle intercalated MMT nanocomposites (MMT-CMCS-MNP). When these nanocomposites were exposed to an alternating electromagnetic gradient, a rapid increase of temperature, from room temperature to 40°C, occurred in 20 min. After 40 min, the temperature reaches 45°C and remains constant until the end of the assay. These results demonstrated that MMT-CMCS-MNP nanocomposites can regulate the temperature and heat flux which is a fundamental requirement for PTT applications[94].

All these studies highlight the potential of phototherapy and provide important insights of nanoclays as a template and nanocarriers for several photosensitizer and photo-thermal agents against cancer.

4.5 Co-delivery

Resistance to anticancer drugs has been a true hurdle in cancer treatment. Frequently, conventional treatments with a single therapeutic agent are unable to eliminate all cancer cells,

which gives rise to MDR. One strategy to overcome this problem is to use co-delivery systems for combination chemotherapy. Ideally, combined therapy with two or more drugs promotes a synergism effect between the drugs against cancer cells and suppresses drug resistance via several pathways[121],[122],[31].

Massaro *et al.* (2016) created multicavity nanotubes for simultaneous encapsulation of two natural compounds with distinct characteristics, Silibinin (SIL) and QUE, or SIL and CUR. The group created the multifunctional carrier through modification of the external surface of HNTs with amphiphilic-cyclodextrin units. Spectroscopy and chromatography assays exhibited that SIL connected preferentially with the lumen of the HNT, while QUE and CUR interplayed more with the hydrophobic cavity of cyclodextrins. These distinct interactions allow a targeted release in different environments. *In vitro* release experiments demonstrated an enhanced release of SIL in gastric conditions (pH 1.0), whereas the release of QUE and CUR was higher in intestinal fluid conditions (pH 7.4). The multifunctional carrier showed a higher cytotoxic activity towards anaplastic thyroid cancer cells (8505C cells) in contrast to free drugs. In fluorescence microscopy analysis, it was possible to observe the presence of the carrier near the nuclei of cells, which indicates that it was successfully internalized by cells. It should be noted that the cellular internalization increased when mannose units were added to the core of cyclodextrins. This is due to carbohydrate-receptor-mediated endocytosis, since mannose units specifically bind to conA, a carbohydrate binding protein with a strong presence on the surface of the cell[121],[122].

Given that cancer patients present a weakened immune system, they are more susceptible to infectious diseases. Thus, antibiotics are indicated for these patients, during chemotherapy. Zeynabad *et al.* (2017) reported an innovative antibacterial nanocomposite to be applied in multi-agent cancer treatments. MMT was the nanoclay used as the nanocarrier since metallic ion exchanged MMT has the proven ability to interact with bacteria via electrostatic forces. MMT was further modified with silica-based copolymers, which were inserted in its interlayer space via an ion exchange mechanism. The resulting nanocomposites were loaded with two anticancer drugs, DOX and MTX, and an antibiotic, CIP, revealing an EE of ca. 95% for all drugs. The *in vitro* release test showed an increase of drug release with the decrease of pH value. This behavior is good for cancer treatment due to the high acidity of cancer cells compared with healthy cells. In addition, the simultaneous release of DOX and MTX from the nanocomposite led to higher cell death of breast cancer cells (T47D cells) when compared to free drugs individually. Concerning the CIP release from nanocomposites, it shows a satisfactory antimicrobial action towards both *Escherichia coli* and *Pseudomonas*

aeruginosa bacteria[123]. The same authors developed another pH-responsive co-delivery system, but this time based on LAP. In this work, LAP was modified with two kinds of cationic vinyl monomers by a cationic exchange method. After that, MTX and CIP were loaded separately in the obtained nanocomposite with an EE superior to 90% for both drugs. Besides the higher release in acidic pH the nanocomposite also exhibited a good antimicrobial action towards both *Escherichia coli* and *Pseudomonas aeruginosa* and an enhanced cytotoxicity in MCF-7 cells[31].

Kar *et al.* (2019) evaluated the potential of the MMT organically modified with cetyltrimethyl ammonium bromide (CTAB) as a carrier for both MTX and CUR. The simultaneous co-delivery allows an initial improvement of FR expression with a non-toxic dose of CUR which increases the uptake and deposition of a high concentration of the folate antagonist, MTX, in FA positive cancer cells. Cytotoxic studies performed in FR-positive (HeLa cells) and FR-negative cells (MCF-7 cells) showed a higher reduction of cell viability in HeLa cells (94%) in comparison to MCF-7 cells (77%), when they were exposure to CUR (500 ng/mL). Also, exposing cells to the IC50 drug concentration of MTX, after a pre-exposure of CUR, enhanced cell death occurred (96%). Thus, the co-delivery of CUR and MTX allow the use a lower concentration of MTX, that does not damage healthy cells, while being cytotoxic inside cancer cells (Appendix XXV)[34].

Collectively, these studies outline the, they important role of nanoclays as nanocarriers of two or more drugs. In all cases had the ability to equally enhance therapeutic efficiency and reduce side effect.

Conclusion and future trends

Cancer is a leading cause of death worldwide providing several challenges in drug delivery of anticancer drugs (chemotherapy, hormone, and biological therapies). Given the physiological alterations that occur during the development of a tumor, its resulting characteristics can be diverse and unique, such as vascular defects, high enzyme activity, acidic pH, elevated GSH and ROS concentrations. These conditions often require specific treatment approaches to maximize the efficacy of the delivered therapeutics.

The relevance of nanoclays in cancer therapy is clearly supported by current findings. Their unique properties including particle size, shape, adsorption ability, SSA, CEC, and layer charge, allow the transportation of a wide range of anticancer drugs to the tumor location efficiently, via the modeling of the drug release patterns, which minimizes premature release and maximizes release in the tumor location. They also demonstrated the ability to carry genes

and proteins while protecting them from enzymatic degradation, which ensures their therapeutic action. They also act as templates for photosensitizers and photo-thermal agents, making them less susceptible to photobleaching increasing their ability to act in the tumor location.

The charge of the payload is not a determining factor as both positive and negative payloads have been loaded efficiently in nanoclays, which facilitates the further development of anticancer formulations based on nanoclays. This also allows the efficient co-delivery by nanoclays of two or more drugs with synergistic anticancer effects, presenting the ability to equally enhance therapeutic efficiency and reduce side effects.

However, it should be noted that the successful application of nanoclays in cancer therapy requires the mastery of its physicochemical properties, as these properties strongly influence all interactions between the nanoclays and cancer environments that will emerge along the treatment. Thus, it may be necessary to improve some clay characteristics, such as SSA, type of exchangeable cations, hydrophilic nature, zeta potential, interlayer space, or porosity through an appropriate functionalization, for example with target ligands, cyclodextrins, hydrophilic polymers, magnetic nanoparticles, and pH-responsive polymers. These modifications are especially useful to improve anticancer activity, obtain a controlled release, design stimuli-responsive nanocarriers, and achieve a successful targeting effect.

Although the *in vitro* experiments showed the efficacy of nanoclays-based anticancer formulations, all of them were based in conventional two-dimensional cells cultures, also referred to as 2D models. This methodology exhibits some limitations when attempting to reproduce the *in vivo* behavior of the cells, specially, concerning the mimicking of the TME. In order to surpass these limitations, *in vitro* experiments using three-dimensional *in vitro* tumor models, which mimic the biological and bio-chemistry diversity of TME accurately, are necessary. Furthermore, more preclinical studies need to be performed in animal models to make pre-clinical data more reproducible and translatable to the clinic.

In this monography, a comprehensive review regarding the role of nanoclays in cancer therapy demonstrates that nanoclays are promising materials for cancer therapy and in combination with their low cost and their high availability may be considered as suitable materials for the development of controlled delivery anticancer formulations.

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Appendix I

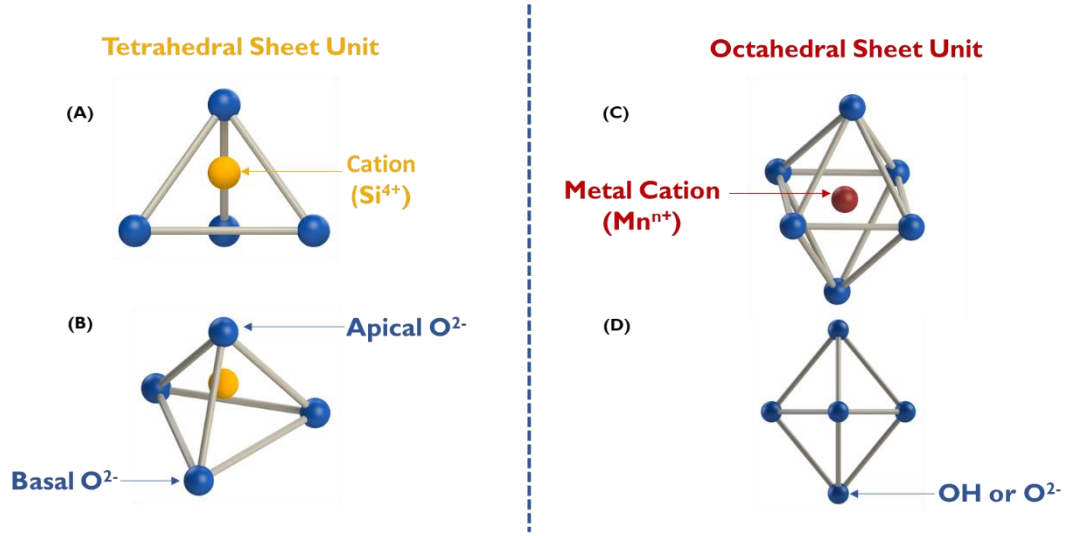

Figure 1: Structural unites of clay mineral sheet structures. Single tetrahedral sheet unit showing the central cation, usually Si^{4+}, **(A)** surrounded by four oxide anions (O^{2-}), three basal O^{2-} and one apical O^{2-} **(B)**. Single octahedron unit showing the central metal cation (M^{n+}) **(C)**, surrounded by six O^{2-} **(D)**.

Appendix II

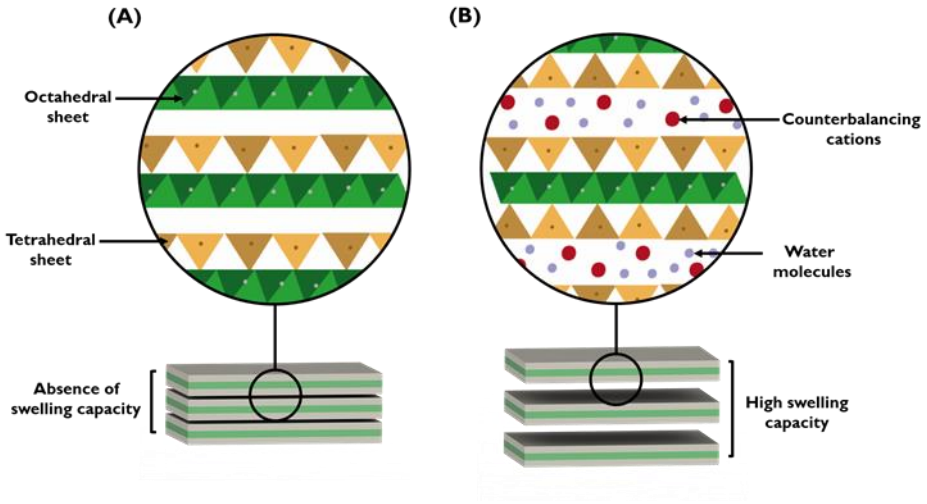

Figure 2: The arrangement of clay mineral layers into particles. **(A)** The 1:1 crystalline structure and **(B)** the 2:1 crystalline structure with red and blue circles representing exchangeable counterbalancing cations and water molecules, respectively. The continuous triangular and rectangular shapes illustrate tetrahedral and octahedral sheets, respectively.

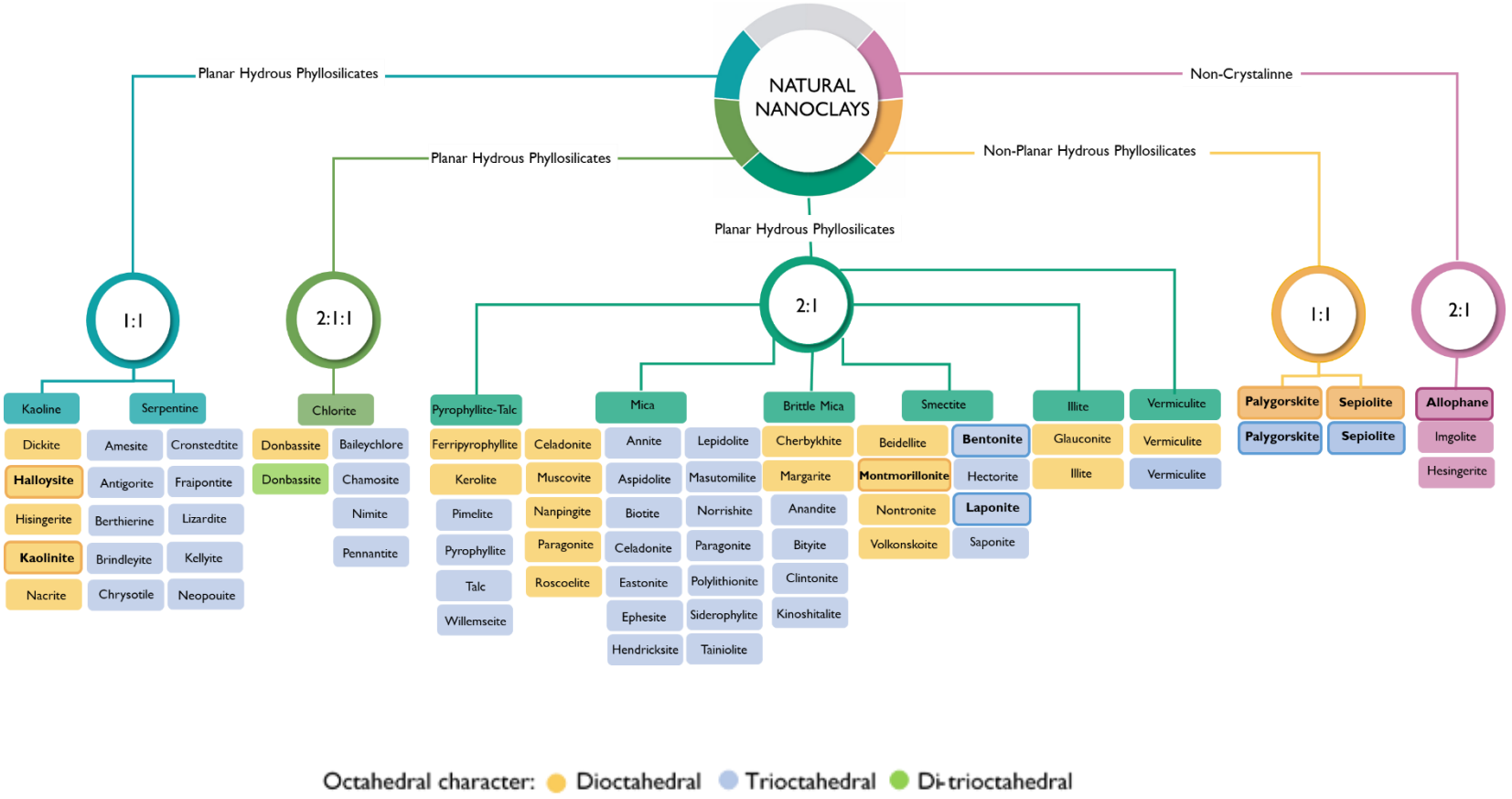

Figure 3: Classification of natural nanoclays into crystalline, composed by planar and non-planar hydrous phyllosilicates, and non-crystalline minerals.

Appendix IV

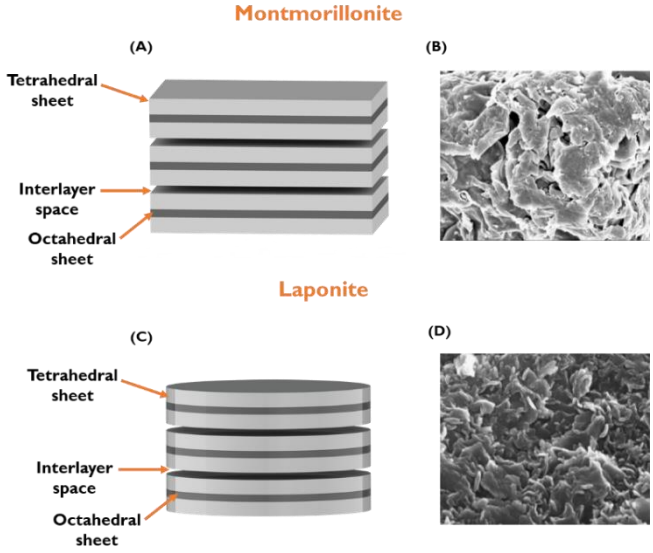

Figure 3: The structure of smectites. **(A)** Molecular simulation of MMT structure showing tetrahedral sheet, octahedral sheet, and interlayer space. **(B)** SEM image of MMT nanoparticles (Adapted from [124]. **(C)** Molecular simulation of LAP structure showing tetrahedral sheet, octahedral sheet, and interlayer space. **(D)** SEM image of LAP nanoparticles (Adapted from [125]).

Appendix V

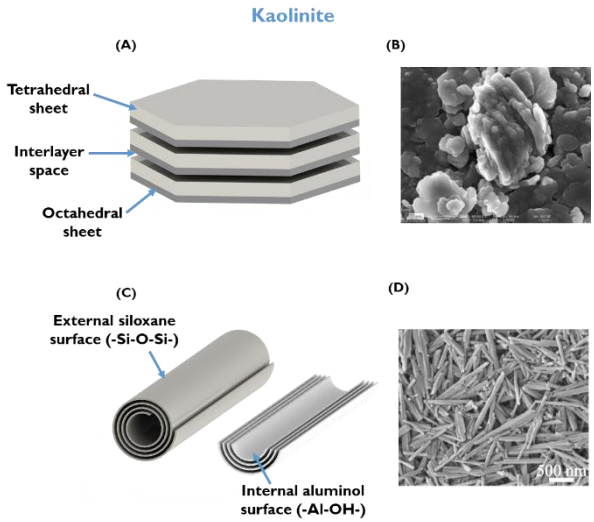

Figure 4: The structure of nanoclays of kaolin group. **(A)** Molecular simulation of kaolinite structure showing tetrahedral sheet, octahedral sheet and interlayer space. **(B)** SEM image of kaolinite nanoparticles (Adapted from [124]). **(C)** Molecular simulation of halloysite nanotube structure showing external siloxane surface and internal aluminol surface. **(D)** SEM image of halloysite nanotubes (Adapted from [92]).

Appendix VI

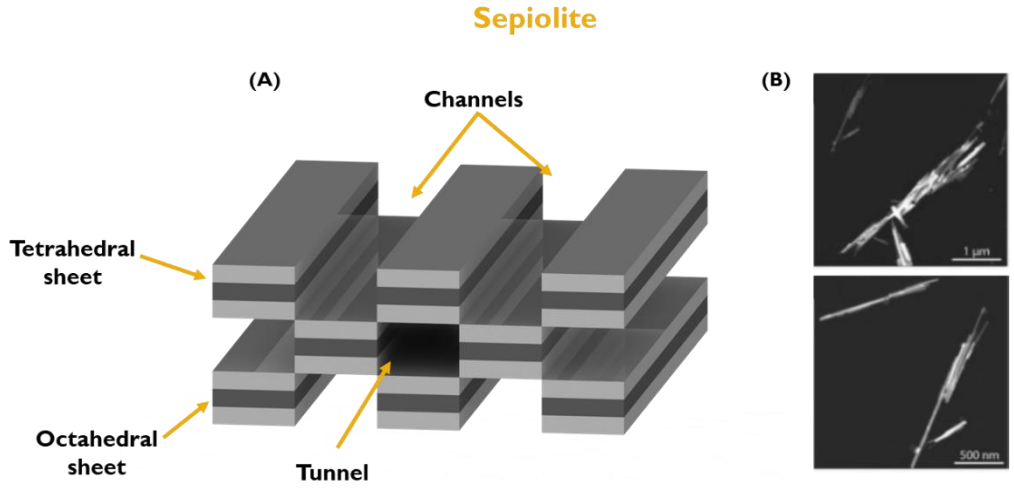

Figure 5: The structure of sepiolite. **(A)** Molecular simulation of sepiolite structure. **(B)** AFM image of sepiolite fibers (Adapted from [16]).

Appendix VII

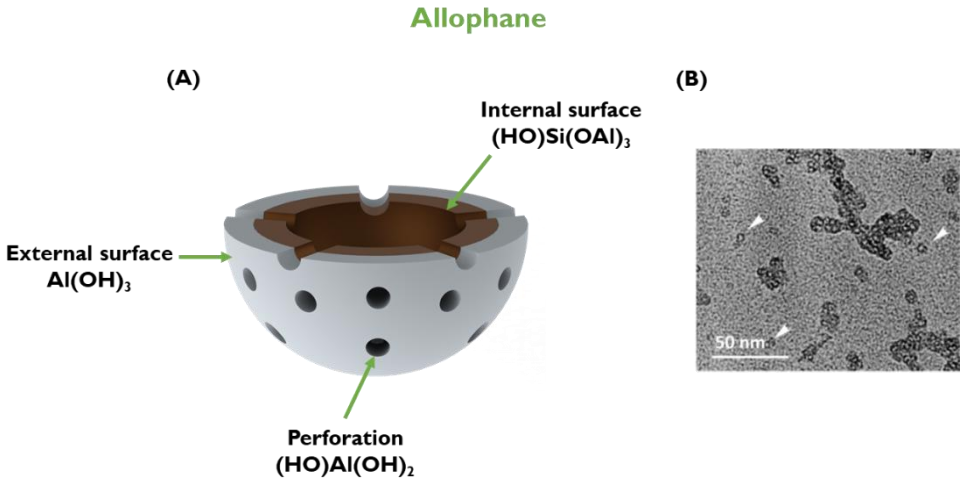

Figure 6: The structure of allophane. **(A)** Molecular simulation of allophane structure showing a hallow sphere structure with perforations. **(B)** TEM image of allophane nanoparticles (Adapted from [45]).

Appendix VIII

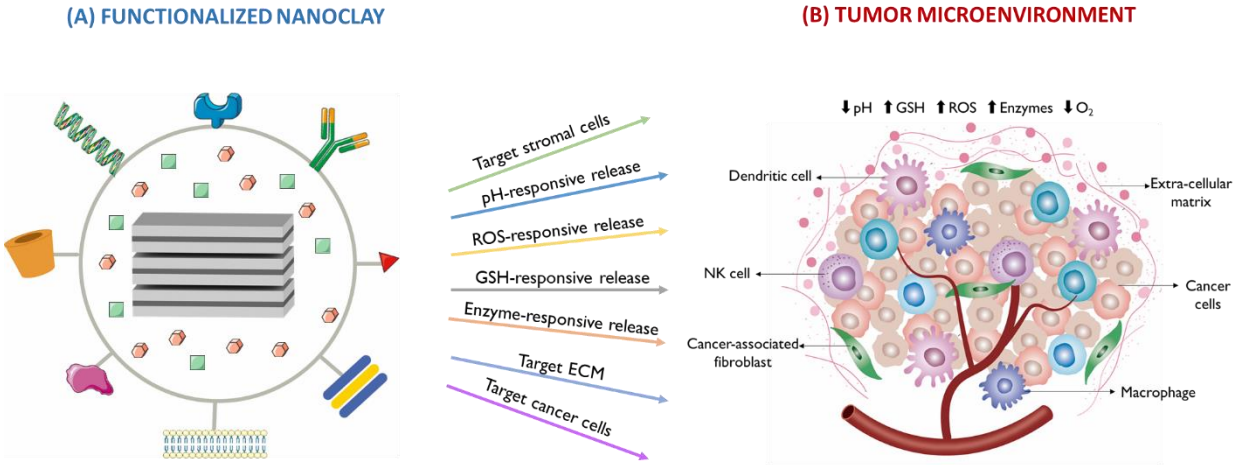

Figure 7: (A) Strategies for expanding the functionalities of nanoclays include surface modification and encapsulation. These existing approaches could be applied for tumor microenvironment intervention in cancer therapy. **(B)** Representation of the cellular and non-cellular components found in tumor microenvironment and that present an active role in the process of carcinogenesis.

Appendix IX

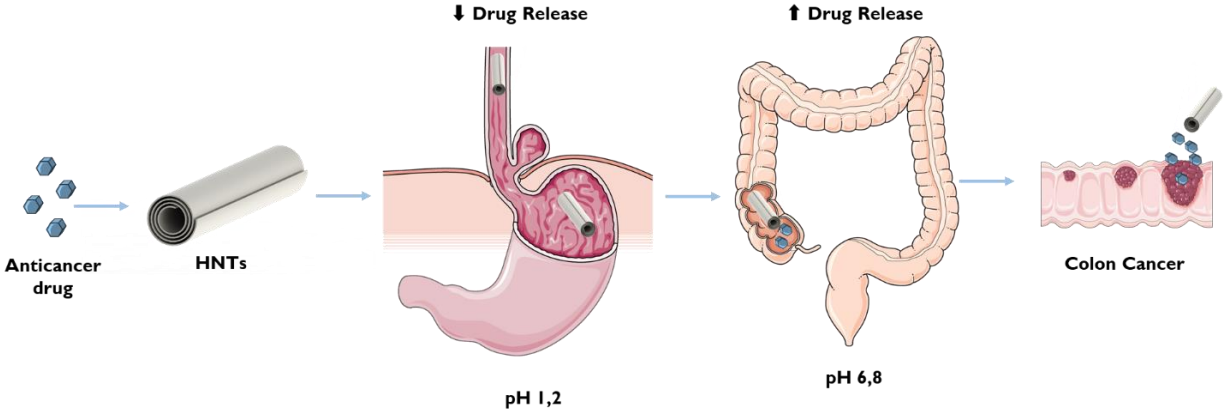

Figure 8: pH-responsive release of anticancer drug from HNTs, with minimal drug release in gastric conditions and maximal drug release in intestinal conditions.

Appendix X

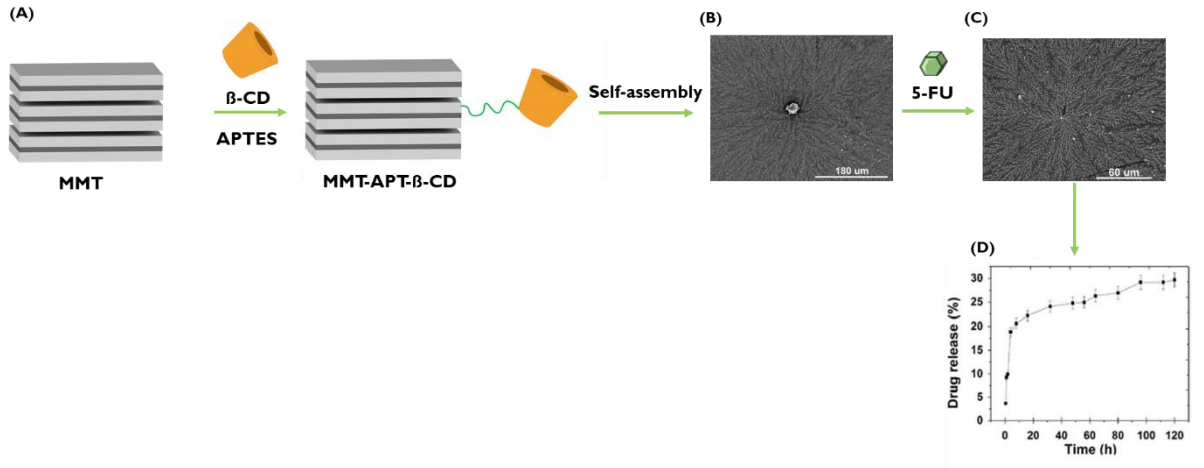

Figure 9: (A) Illustration of formation of ß-cyclodextrin functionalized MMT. **(B)** SEM image of empty supramolecular dendrimer-like structure obtained by self-assembling of ß-cyclodextrin functionalized MMT (Adapted from [61]). **(C)** SEM image 5-FU-loaded supramolecular dendrimer-like structure. (D) 5-FU-loaded supramolecular dendrimer-like structure, obtained by self-assembling of ß-cyclodextrin functionalized MMT, exhibits the ability to sustained drug release over 120 hours (Adapted from [61]).

Appendix XI

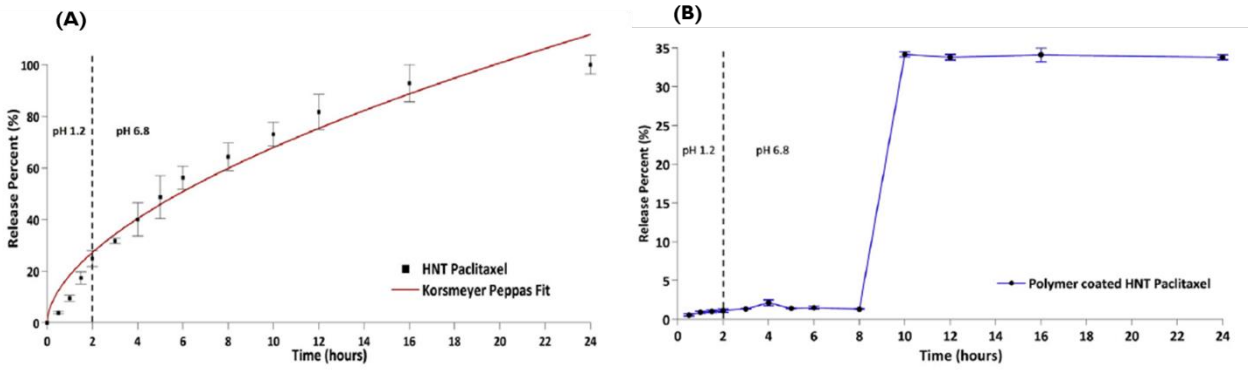

Figure 10: (A) Release profile of PTX in pH 1.2 and 6.8 from HNTs at 37°C. **(B)** pH-responsive release from HNTs functionalized with PMMM. (Adapted from [65]).

Appendix XII

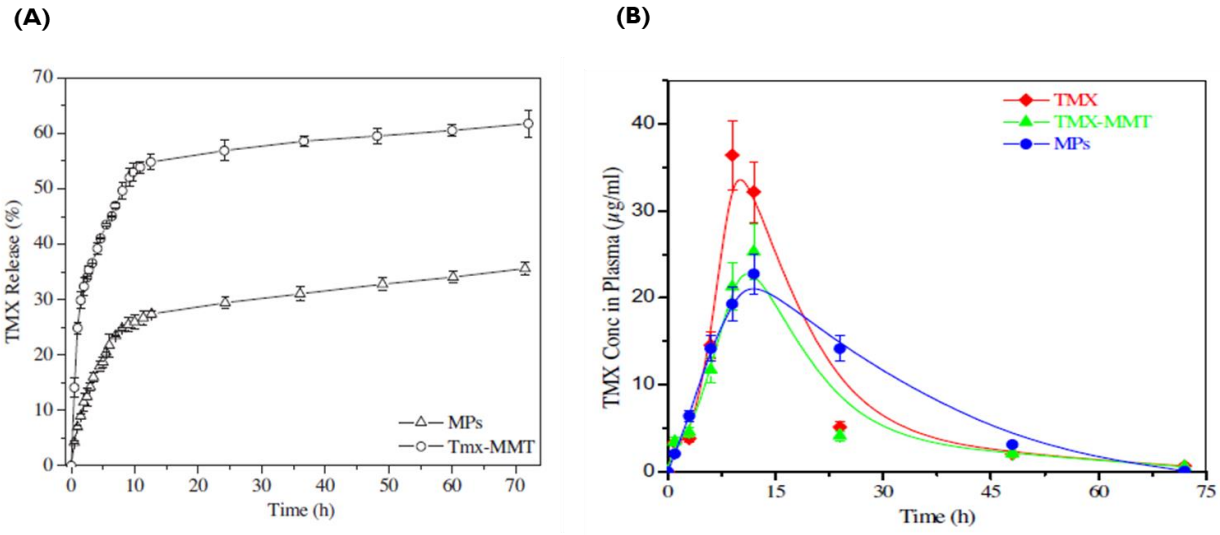

Figure 11: (A) Release profiles of TMX in intestinal conditions (pH 7.4) at 37°C. **(B)** Evaluation of pharmacokinetic profile of TMX after oral administration to female wistar rats (Adapted from [78]).

Appendix XIII

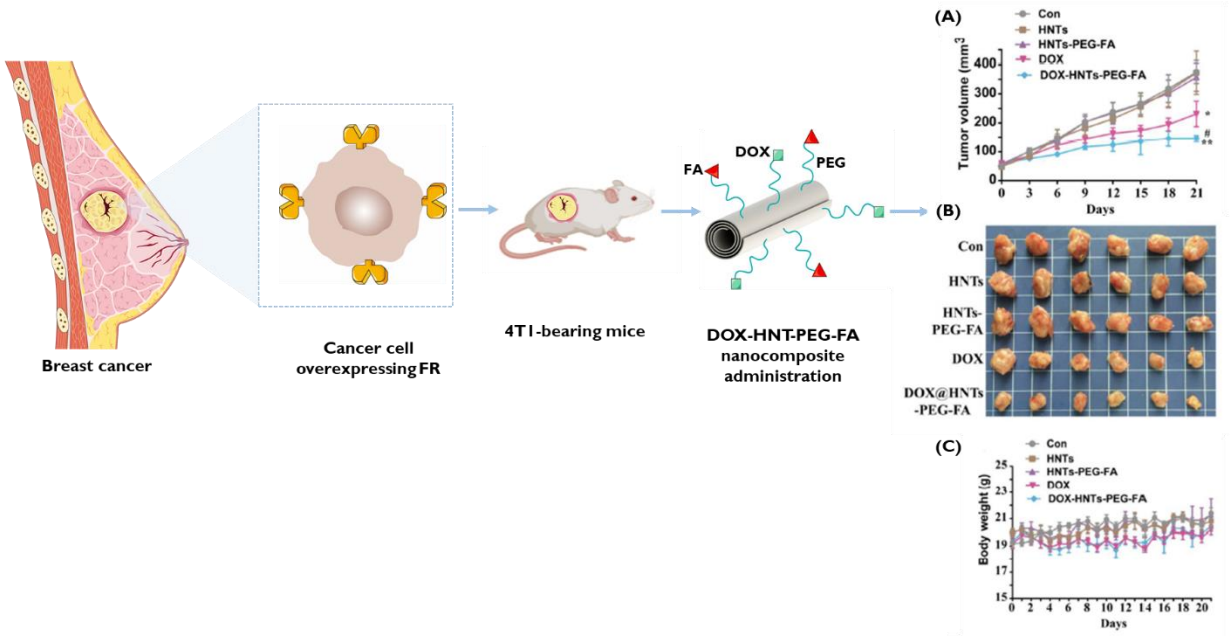

Figure 12: (A) *In vivo* antitumor effect obtained from treatment with DOX-HNT-PEG-FA nanocomposites in 4T1-bearing mice, expressed as tumor volume (mm^3). **(B)** Typical photograph of excised 4T1 solid tumor on the 22nd day after treatment. **(C)** Change in tumor weight of each group during the assay (Adapted from [9]).

Appendix XIV

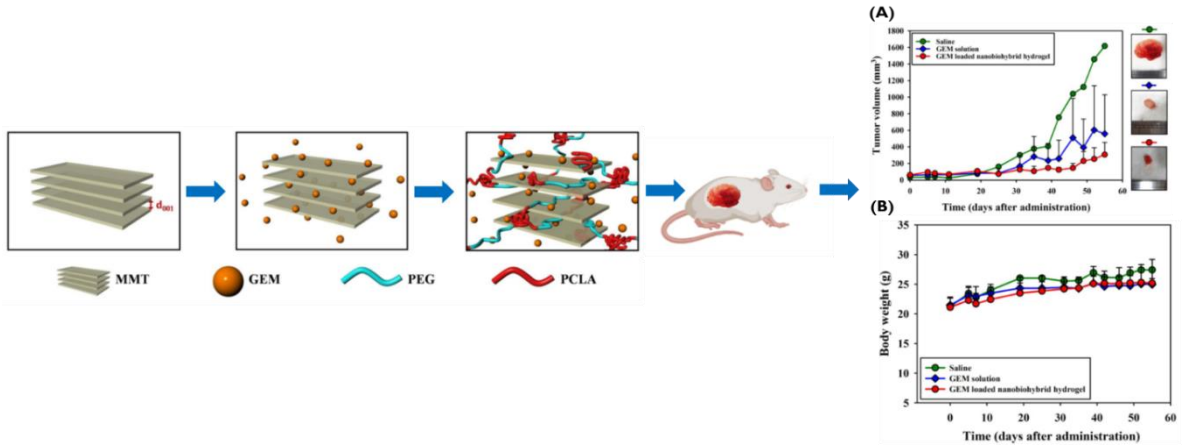

Figure 13: (A) *In vivo* antitumor effect obtained from treatment with GEM-MMT-PCLA-PEG-PCLA nanobiohybrid hydrogel in pancreatic-bearing mice, expressed as tumor volume (mm^3) (Adapted from [73]). **(B)** Alteration in tumor weight of each group during the assay (Adapted from [73]).

Appendix XV

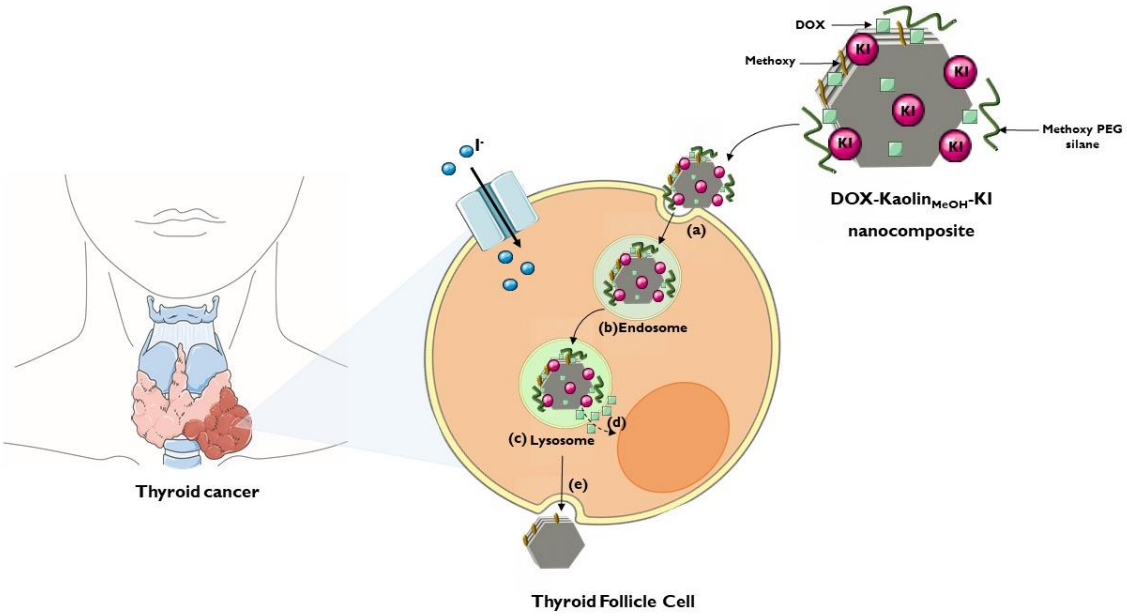

Figure 14: Mechanism of the intracellular delivery of DOX from the DOX-Kaolin$_{MeOH}$-KI nanocomposite. (a) Nonreceptor mediated endocytosis, (b) membrane surrounds the DOX-Kaolin$_{MeOH}$-KI nanocomposite by forming endosomes followed by internalization, (c) formation of a lysosome containing the DOX-Kaolin$_{MeOH}$-KI nanocomposite, (d) release of DOX, (e) Kaolin$_{MeOH}$ leaves the cell.

Appendix XVI

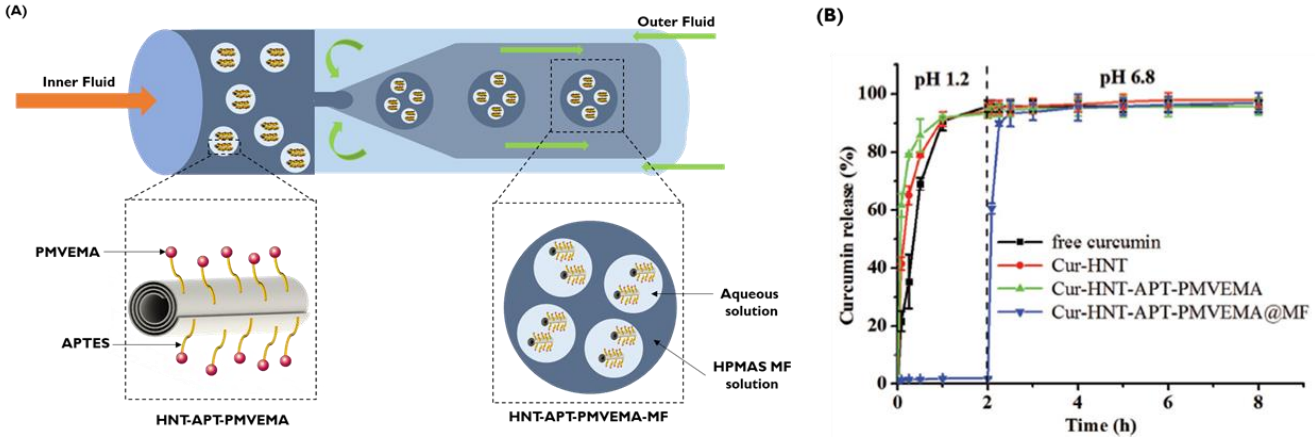

Figure 15: (A) Microfluidic preparation of CUR-HNT-APT-PMVEMA nanocomposites. **(B)** Release profiles of CUR from Cur-HNT, Cur-HNT-APT-PMVEMA, and Cur-HNT-APT-PMVEMA nanocomposites (Adapted from [85]).

Appendix XVII

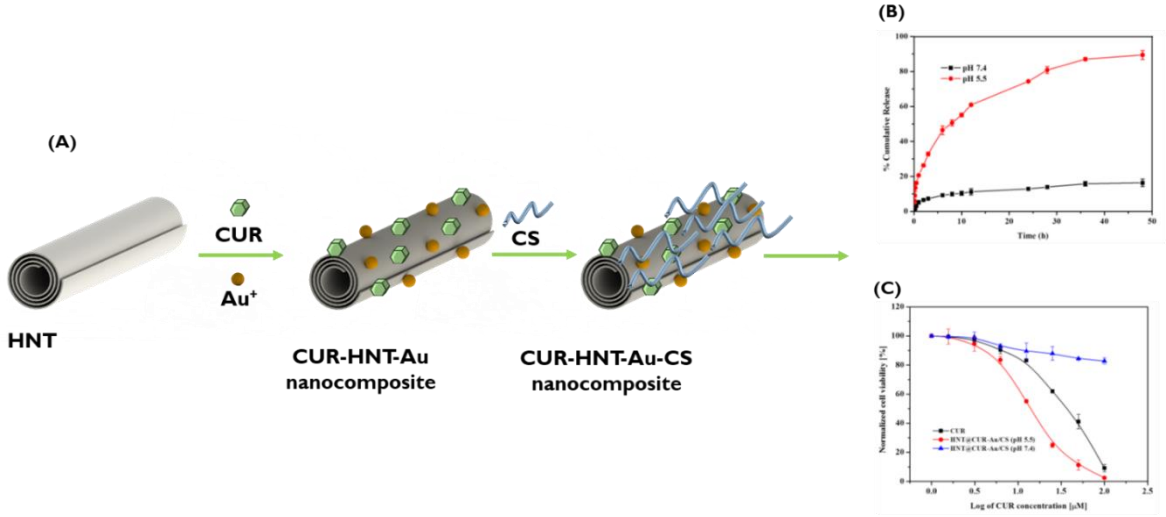

Figure 16: (A) Illustration of formation of CUR-HNT-Au-CS nanocomposite. **(B)** Release profile of CUR in pH 5.5 and 7.4 from CUR-HNT-Au-CS nanocomposite (Adapted from [90]). **(C)** *In vitro* cytotoxicity of CUR release from CUR-HNT-Au-CS nanocomposite towards MCF-7 cells for 48 hours incubation (Adapted from [90]).

Appendix XVIII

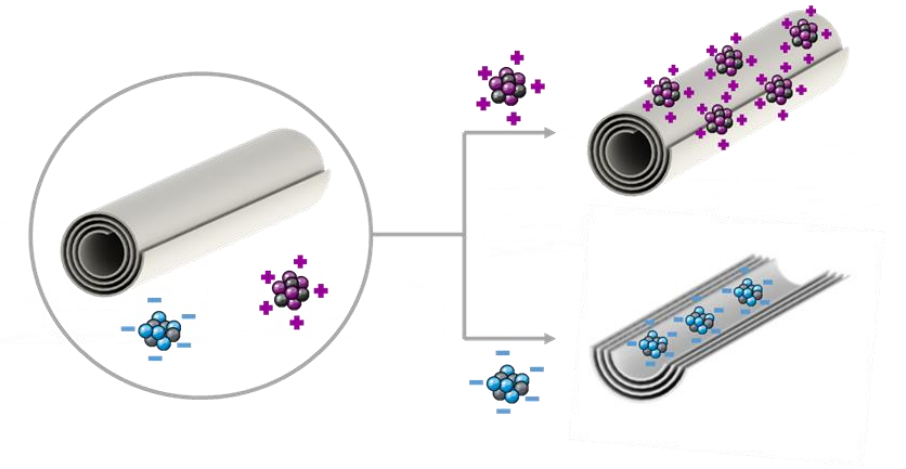

Figure 17: Illustration of enzyme immobilization on the HNTs. Negatively charged enzymes interact more efficiently with the positively charged lumen of HNTs. On the other hand, positively charged enzymes and interact more with the negatively charged external surface of HNTs.

Appendix XIX

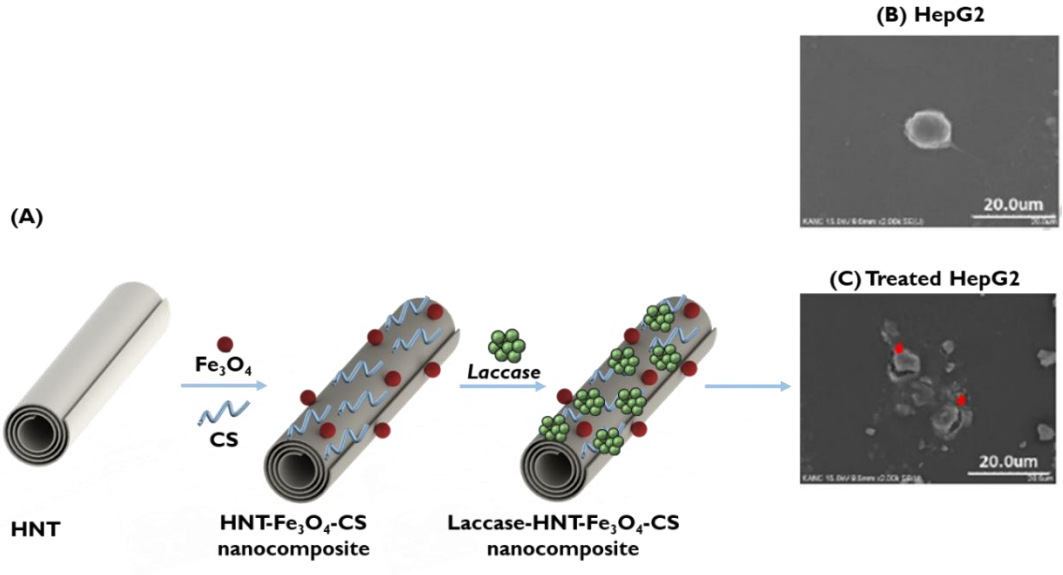

Figure 18: (A) Illustration of formation of CUR-HNT-Au-CS nanocomposite. **(B)** Untreated HepG2 cells (Adapted from [100]). **(C)** Morphological changes induced by laccase release from HNT-Fe_3O_4-CS nanocomposite oh HepG2 cells. Red arrows indicate cell damages such as cell crack or apoptotic core (Adapted from [100]).

Appendix XX

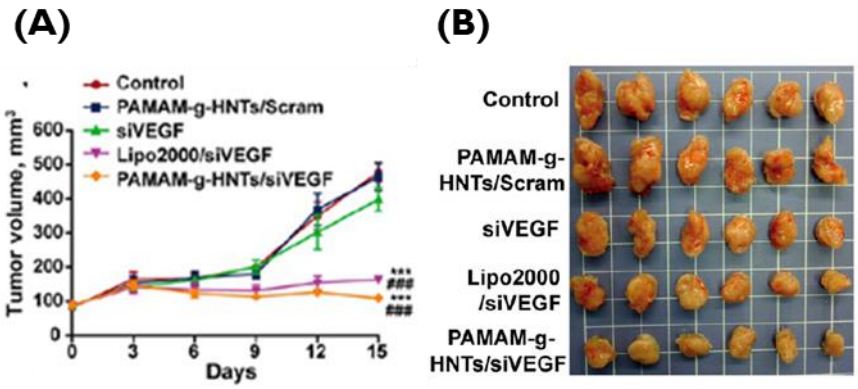

Figure 19: (A) *In vivo* antitumor effect obtained from treatment with siVEGF-HNT-PAMAM in 4T1-bearing mice, expressed as tumor volume (mm^3). **(B)** Typical photograph of excised 4T1 solid tumor (Adapted from [105]).

Appendix XXI

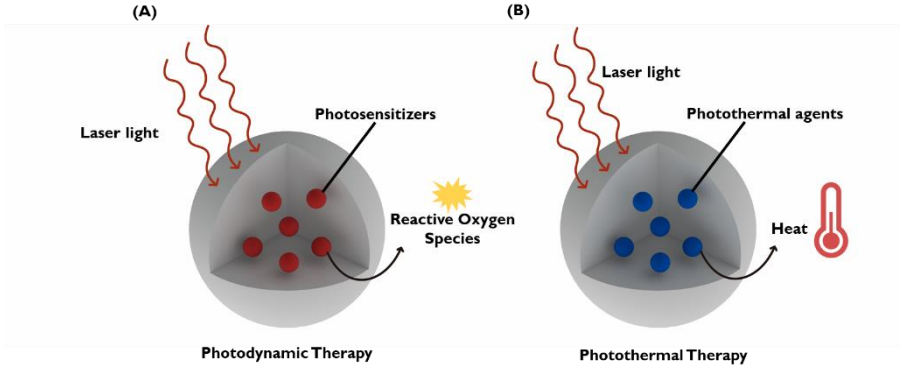

Figure 20: Representation of the mechanism of action of photodynamic therapy **(A)** and photothermal therapy **(B)**.

Appendix XXII

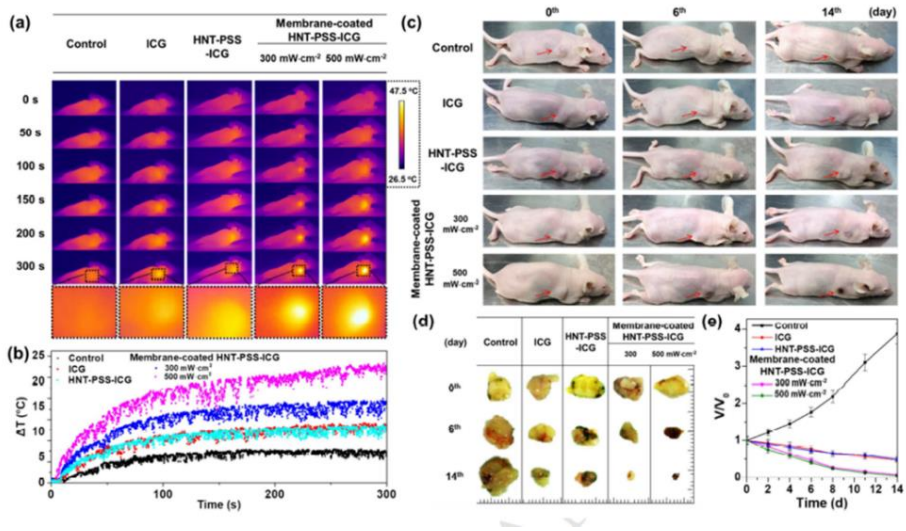

Figure 21: (a) Photothermal imaging and **(b)** temperature variations of mice from groups control, ICG, HNT-PSS-ICG and membrane-coated HNT-PSS-ICG at 808 laser irradiation (300 mW/cm^2) for 300 s. **(c)** Photographs of representative mice from groups control, ICG, HNT-PSS-ICG and membrane-coated HNT-PSS-ICG on the 0th, 6th and 14th days. **(d)** Photographs of excised tumors from the mice at 24 hours after administration of ICG, HNT-PSS-ICG and membrane-coated HNT-PSS-ICG on the 0th, 6th and 14th days. **(e)** *In vivo* antitumor effect expressed as the average values of the relative tumor volume V/V_o, where V is the tumor volume at the test time points and V_o is the initial tumor volume at the beginning of the treatment (Adapted from [113]).

Appendix XXIII

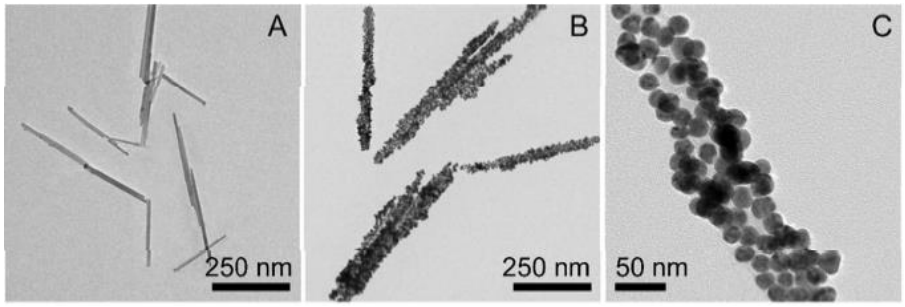

Figure 22: TEM images of pristine PAL **(A)** and Au-Pal nanocomposites **(B, C)** (Adapted from [118]).

Appendix XXIV

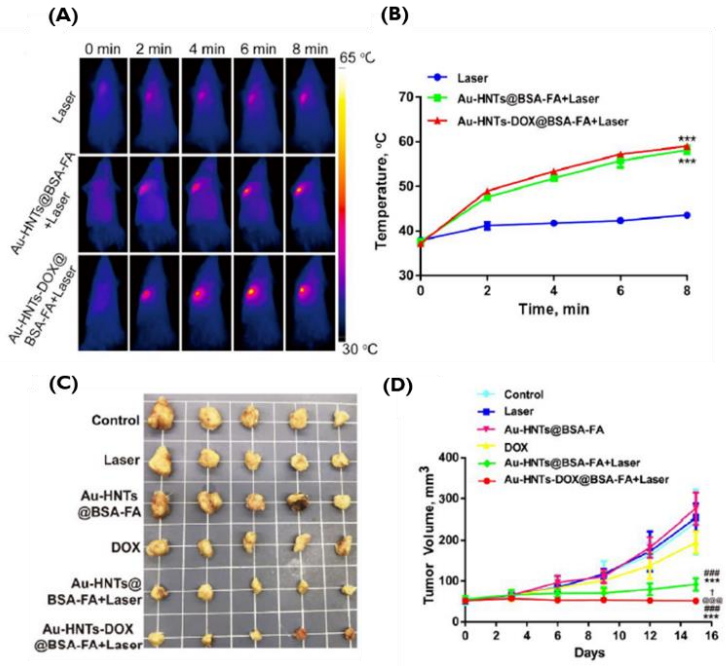

Figure 23: (A) Infrared thermal images of Au-DOX-HNTs-BSA-FA nanocomposite in 4T1-bearing mice. **(B)** Temperature variation in tumor of mice during the laser irradiation. **(C)** Photographs of excised 4T1solid tumor on the 16th day after administration of Au-HNTs-BSA-FA, DOX, Au-HNTs-BSA-FA with laser irradiation and Au-DOX-HNTs-BSA-FA nanocomposite with laser irradiation. **(D)** In vivo antitumor effect obtained from treatment with Au-HNTs-BSA-FA, DOX, Au-HNTs-BSA-FA with laser irradiation and Au-DOX-HNTs-BSA-FA nanocomposite with laser irradiation in mice, expressed as tumor volume (mm3) (Adapted from [120]).

Appendix XXV

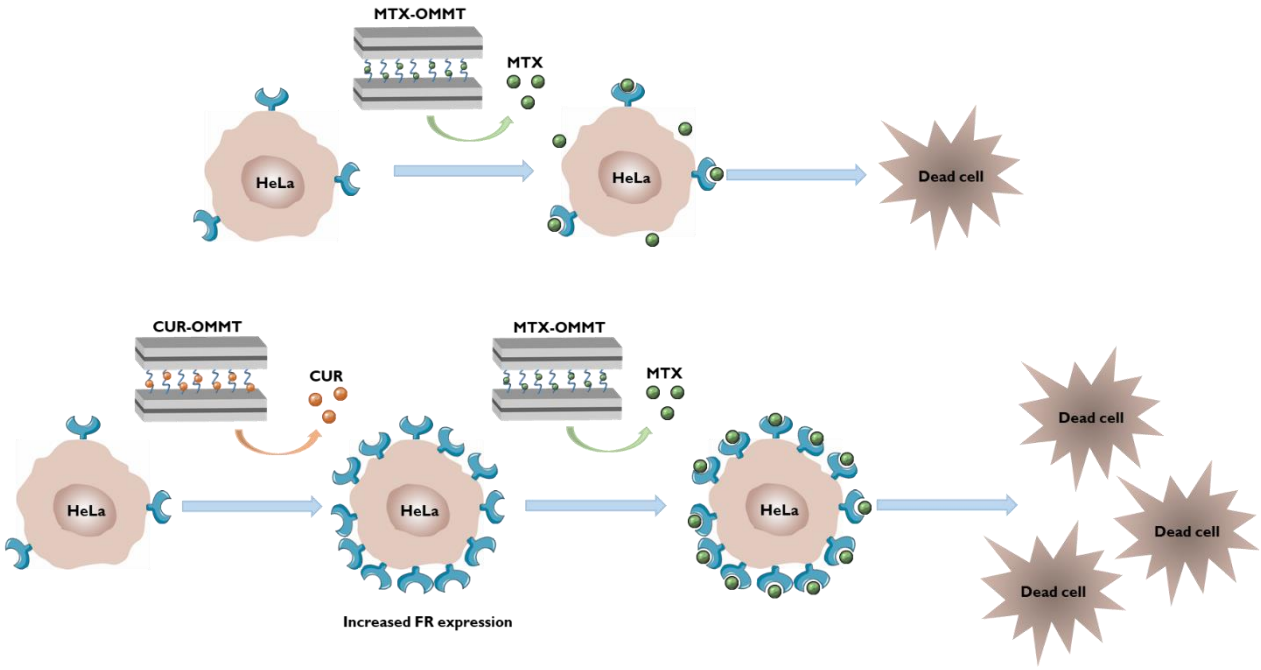

Figure 24: Schematic illustration of MMT organically modified with CTAB loaded with MTX **(A)** and CUR **(B)**. CUR induced enhanced expression of FR on the surface of HeLa cells **(C)**, which increases the uptake and deposition of a high concentration of MTX **(D)**.