UNIVERSIDADE D
COIMBRA

Maria de Melo Cartaxo Correia Martins

Relatórios de Estágio sob a orientação da Dra. Edite Balau e da Dra. Dina Lopes e Monografia intitulada "Drug Repurposing for Skin Cancer Treatment: A Nanotechnological Approach" sob a orientação da Professora Doutora Patrícia Sofia Cabral Pires referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2023

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Eu, Maria de Melo Cartaxo Correia Martins, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2018273725, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Drug Repurposing for Skin Cancer Treatment: A Nanotechnological Approach" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 7 de setembro de 2023.

Maria de Melo Cartaxo Correia Martins

(Maria de Melo Cartaxo Correia Martins)

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PARTE I

Relatório de Estágio em Farmácia Comunitária

Farmácia Nova de Luso

Sob orientação da Dra. Edite Balau

Lista de Abreviaturas

APPACDM - Associação Portuguesa de Pais e Amigos do Cidadão Deficiente Mental

DST - Doença Sexualmente Transmissível

FC - Farmácia Comunitária

FN - Farmácia Nova

INFARMED I.P. - Autoridade Nacional do Medicamento e dos Produtos de Saúde I.P.

MICF - Mestrado Integrado em Ciências Farmacêuticas

MNSRM - Medicamento Não Sujeito a Receita Médica

PIM - Preparação Individual da Medicação

PVP - Preço de Venda ao Público

SWOT - do inglês *Strengths, Weaknesses, Opportunities, and Threats*

1. Introdução

A Farmácia Comunitária (FC) é uma das portas de entrada no Sistema de Saúde dada a sua acessibilidade a toda a população. Segundo a Ordem dos Farmacêuticos, este local de prestação de serviços de saúde é "a face mais visível da profissão", o primeiro local a que a população recorre para resolver questões de saúde. Ainda assim, muitas vezes, o seu papel na sociedade passa despercebido e é apenas associado a um local de dispensa de medicamentos. No entanto, a farmácia desempenha um papel preponderante no que diz respeito à prestação de cuidados de saúde com elevada diferenciação técnico-científica, à promoção da saúde, ao uso responsável dos medicamentos e ao aconselhamento confiável com base científica[1].

O farmacêutico comunitário é o agente de saúde especialista do medicamento e é, habitualmente, o primeiro profissional a quem o indivíduo doente recorre em caso de afeções de saúde menores, visto que, em muitas zonas do território nacional, as farmácias são a única estrutura de saúde disponível capaz de prestar cuidados de proximidade. A atual posição do farmacêutico em Portugal torna-o essencial para poder contribuir em áreas como a gestão e adesão à terapêutica, revisão da medicação, administração de medicamentos, promoção de estilos de vida mais saudáveis, determinação de parâmetros e identificação de pessoas em risco, entre outras[1].

O lema "Há luzes que nunca se apagam" reflete o papel ativo do farmacêutico na sociedade, visto estar sempre presente nas mais diversas situações relacionadas com a saúde. Além disso, estes profissionais estão em constante aprendizagem para conseguirem oferecer a melhor prestação de cuidados de saúde possível.

Ao fim de quatro anos e meio de formação em várias unidades curriculares, os alunos do Mestrado Integrado em Ciências Farmacêuticas têm a oportunidade de realizar um estágio em FC de forma a pôr em prática, em contexto real, todo o conhecimento teórico adquirido ao longo do curso[2].

Para realizar o meu estágio curricular escolhi, com enorme orgulho, a Farmácia Nova (FN) de Luso, uma farmácia que foi inaugurada em 1960 e que acarreta consigo a enorme responsabilidade de um passado de histórias e trabalho de excelência. Tive a oportunidade de integrar a equipa entre os dias 9 de janeiro e 28 de abril de 2023, tendo sido orientada pela Dra. Edite Balau, Diretora Técnica da referida farmácia. O presente relatório pretende descrever detalhadamente o meu estágio com base numa análise SWOT (*Strengths, Weaknesses, Opportunities, and Threats*) e, por fim, apresentar cinco casos práticos que pude experienciar e a respetiva resolução.

2. Análise SWOT

A análise SWOT é uma ferramenta simples, de uso comum, vastamente utilizada, tendo como objetivo executar uma análise crítica e uma gestão estratégica e ponderada. Esta análise é realizada a dois níveis, interno e externo, com a identificação de quatro vertentes: *Strenghts* (Pontos Fortes), *Weaknesses* (Pontos Fracos), *Opportunities* (Oportunidades), *Threats* (Ameaças). Internamente são avaliados os pontos fortes e os pontos fracos, e externamente o foco incide nas oportunidades e nas eventuais ameaças que possam surgir.

Neste relatório foi realizada uma análise SWOT onde foram referidos os conhecimentos adquiridos e as atividades realizadas, durante o Estágio Curricular na Farmácia Nova, e algumas observações importantes. Na **Figura 1** estão esquematizadas as quatro vertentes, mencionadas anteriormente, relativas ao referido estágio.

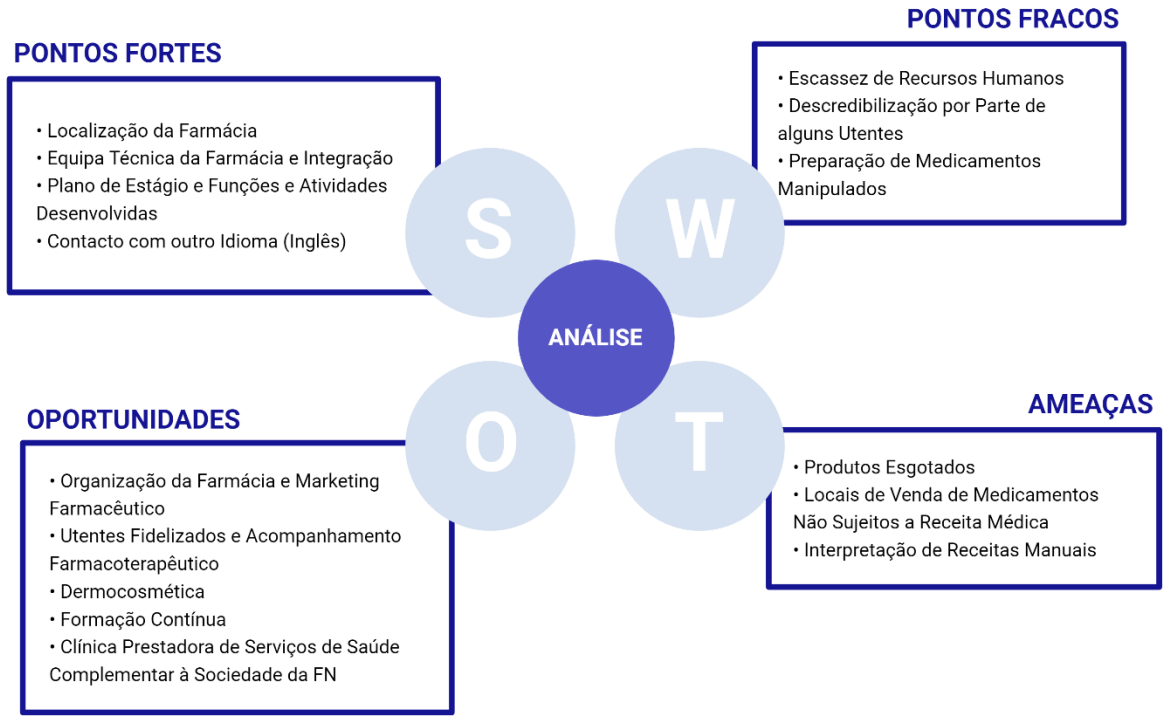

Figura 1. Análise SWOT do Estágio Curricular na FN

2.1. Pontos Fortes

Localização da Farmácia

A FN fica situada na Rua Dr. Francisco António Diniz, no Luso, uma pequena vila termal e bastante turística. Localiza-se no centro desta vila, onde se encontra concentrada a zona comercial, e próxima da Extensão de Saúde e da Clínica Balau e, por isso, num local de destaque e estrategicamente posicionada. Assim, a afluência de utentes é bastante elevada todos os dias, atraindo pessoas de todas as faixas etárias, desde jovens a adultos, moradores

locais e visitantes ocasionais, incluindo turistas estrangeiros. Lidar com esta heterogeneidade de utentes permitiu-me realizar atendimentos bastante diversificados e personalizados, tendo sido para mim, enquanto estagiária, um desafio diário e uma oportunidade de crescimento pessoal e profissional.

Equipa Técnica da Farmácia e Integração

A FN é constituída pela Diretora Técnica, Dra. Edite Balau, por duas farmacêuticas, Dra. Joana Balau e Dra. Rita Neves e duas Técnicas de Farmácia, Dra. Mafalda Balau e Dra. Íris Carvalho, que se encontrava de licença de maternidade aquando da realização do meu estágio curricular. É uma equipa experiente, humana e dedicada, que prima pelo rigor, ética e confiança dos seus utentes, demonstrando, em cada interação, dedicação e preocupação com o seu bem-estar. Além disso, é uma equipa bastante inovadora, criativa e sempre recetiva a novas ideias e iniciativas. Cada colaborador tem algumas tarefas distintas e individualizadas, possibilitando a maximização das habilidades e potenciais de cada um e, por sua vez, a otimização do desempenho coletivo e a rentabilidade das diferentes áreas. É importante destacar que, sempre que determinado colaborador responsável por determinada tarefa não está presente, há sempre outro habilitado para o fazer, demonstrando a versatilidade desta equipa.

O facto de já conhecer a equipa técnica, pois foi nesta farmácia que realizei o meu Estágio de Verão no terceiro ano, demonstrou ser uma mais-valia e facilitou a minha integração. Desde o primeiro dia do presente estágio, todos os elementos da equipa colaboraram ativamente no meu processo de aprendizagem, ajudando-me a aplicar os conhecimentos adquiridos na faculdade e a superar as dificuldades com que me ia deparando, nomeadamente através da resposta empática a todas as minhas questões. Além disso, sempre acreditaram e confiaram em mim e no meu trabalho, compreendendo que, enquanto estagiária, por vezes, enfrentava alguns obstáculos e inseguranças. Esta postura de apoio não só fomentou a minha autoestima e autoconfiança, mas também fortaleceu o meu desenvolvimento profissional.

Plano de Estágio e Funções e Atividades Desenvolvidas

Ao longo do estágio curricular, tive a oportunidade de desempenhar um amplo e variado conjunto de tarefas, que se encontravam predefinidas no meu plano de estágio, colaborando, de forma organizada e estruturada, com toda a equipa. Todo o plano foi pensado de forma a facilitar a minha transição do trabalho de *backoffice* para o atendimento ao público.

Participei ativamente no desenvolvimento de tarefas como Receção e Armazenamento de Encomendas, Atendimento ao Público, Preparação Individualizada da Medicação e

Promoção da Saúde Através de Outros Serviços Farmacêuticos. A diversidade de tarefas desempenhadas enriqueceu a minha experiência de estágio, permitindo-me aplicar os conhecimentos anteriormente adquiridos na faculdade, compreender o circuito completo do medicamento dentro da farmácia, alargar a minha visão sobre o papel do farmacêutico comunitário e adquirir uma variedade de habilidades práticas e outros conhecimentos de grande utilidade para o meu futuro profissional.

No decorrer do estágio e durante a realização das tarefas que desempenhei, fui sentindo que os elementos da equipa me iam dando gradualmente uma maior autonomia e responsabilidade.

Receção e Armazenamento de Encomendas

Uma das primeiras etapas do meu estágio envolveu a responsabilidade de rececionar e, posteriormente, armazenar as encomendas. Desta forma, iniciei o meu processo de familiarização com os diversos produtos e marcas existentes na farmácia, associando os nomes comerciais aos princípios ativos dos medicamentos e relembrando as suas indicações terapêuticas e respetivas dosagens.

A receção de encomendas revelou-se uma tarefa relativamente fácil, pois já tinha sido realizada, de forma autónoma, no Estágio de Verão. Voltei a contactar e a ambientar-me com o programa utilizado na farmácia, o SIFARMA 2000®, para dar entrada dos medicamentos. Tive a oportunidade de verificar na prática a diferença entre preço de venda ao público (PVP) e preço de venda à farmácia e respetivo impacto nas margens de lucro, conhecimentos anteriormente adquiridos na unidade curricular de Comunicação e Marketing Farmacêutico. Compreendi também a necessidade de balancear os níveis de *stock* com as necessidades dos utentes, de maneira a que o investimento fosse lucrativo.

Na FN, após a receção, é necessário proceder ao armazenamento manual dos medicamentos e outros produtos farmacêuticos. Percebi o papel fundamental desta tarefa para que o atendimento ao público se faça de forma célere e eficaz. Durante o armazenamento tive sempre a preocupação, tal como me ensinaram, de organizar os produtos de modo a serem facilmente localizados, seguindo o princípio *"first expire, first out"*. Esta abordagem garante que os produtos com prazos de validade mais curtos sejam escoados primeiro, evitando desperdícios e salvaguardando sempre a segurança dos utentes.

Atendimento ao Público

O atendimento ao público foi uma tarefa extremamente desafiante e enriquecedora em que me socorri dos conhecimentos teóricos lecionados em várias unidades curriculares, principalmente em Farmacologia, Farmacoterapia, Farmácia Clínica e Indicação Farmacêutica,

para conseguir fazer aconselhamentos com uma maior segurança e precisão, garantindo assim uma maior qualidade do serviço prestado aos utentes.

Durante o período em que tive um acompanhamento mais próximo dos elementos da equipa pude familiarizar-me com o módulo "Atendimento" do SIFARMA 2000®, ajudaram-me a interpretar as receitas médicas, fui orientada relativamente às perguntas essenciais a realizar durante o ato do aconselhamento farmacêutico e sensibilizada para a necessidade de uma comunicação eficaz, habilidades essenciais para um atendimento ao público de excelência.

Posteriormente, quando comecei a sentir uma maior confiança e a equipa considerou que já estava preparada, comecei a executar esta tarefa sozinha, tendo sempre em mente que, quando precisasse de ajuda, poderia solicitá-la. Ao realizar o atendimento ao público, respeitei sempre a privacidade dos utentes, mantendo a confidencialidade das informações médicas e pessoais, escutei atentamente as suas preocupações e tentei sempre responder-lhes de forma clara e acessível, demostrando empatia e interesse pelo seu bem-estar, compreendi a importância de manter um espírito crítico perante as receitas médicas, estando sempre atenta a possíveis erros ou omissões, e expliquei a importância da adesão à terapêutica e do uso correto dos medicamentos, alertando para a necessidade de seguir as indicações prestadas pelo médico ou farmacêutico, nomeadamente em relação à dosagem, horários e outras precauções especiais.

Para além da dispensa de receitas médicas, tive a oportunidade de atender utentes que procuravam aconselhamento farmacêutico. Primeiramente, analisava os sinais e sintomas apresentados ou descritos e recolhia informações sobre a pessoa à qual se destinava o aconselhamento, seguidamente, optava pelo aconselhamento de medidas não farmacológicas, dispensava um Medicamento Não Sujeito a Receita Médica (MNSRM) ou fazia o encaminhamento para o médico caso se tratasse de uma situação mais grave e que não pudesse ser resolvida na farmácia.

Preparação Individualizada da Medicação

A Preparação Individual da Medicação (PIM) foi uma tarefa que realizei semanalmente para os utentes da Associação Portuguesa de Pais e Amigos do Cidadão Deficiente Mental (APPACDM) de Casal Comba, no entanto este serviço está disponível a todos os utentes que dele necessitem. Para facilitar esta tarefa, mensalmente, colocava, em sacos identificados com o nome do utente, todos os medicamentos que este tomava. Todas as semanas realizava a PIM, dispensando os medicamentos em caixas e blisters descartáveis, tendo sempre por base uma tabela organizada com o nome do utente, medicação e hora da toma. É importante realçar

que tinha sempre a preocupação de fazer várias verificações ao longo do processo para minimizar qualquer erro.

Os serviços prestados na FN vão muito além da dispensa de medicamentos e outros produtos, abrangem também cuidados ligados à saúde e bem-estar do utente, como medição de parâmetros bioquímicos, medição e avaliação da pressão arterial, determinação do índice de massa corporal, administração de primeiros socorros e administração de medicamentos injetáveis.

Promoção da Saúde Através de Outros Serviços Farmacêuticos

Durante o meu estágio tive, não só, a possibilidade de realizar medições de glicémia, perfil lipídico e tensão arterial, mas também contribuir para a consciencialização dos utentes no sentido da adoção de estilos de vida mais saudáveis.

Contacto com outro Idioma (Inglês)

Como referi anteriormente, o Luso é uma vila bastante turística, sendo visitada por muitos estrangeiros. Neste sentido, consegui colocar em prática a língua inglesa, que domino com alguma fluência, auxiliando os turistas que necessitavam de algum serviço farmacêutico. A oportunidade de usar o inglês em contexto profissional, tendo a necessidade de utilizar alguns termos técnicos, foi uma experiência valiosa e permitiu-me melhorar as minhas habilidades linguísticas, enquanto prestava um serviço útil aos visitantes internacionais.

2.2. Pontos Fracos

Escassez de Recursos Humanos

A falta de recursos humanos foi uma realidade ao longo do estágio curricular e criou alguns constrangimentos ao funcionamento da farmácia. Uma consequência direta dessa escassez de recursos humanos foi a sobrecarga de trabalho imposta aos colaboradores existentes, que tinham de ser responsáveis por mais tarefas do que aquelas que seria suposto. No entanto, o atendimento ao público foi sempre priorizado e a qualidade desse atendimento nunca ficou comprometida, graças ao profissionalismo da equipa e ao respeito pelos seus utentes. Para segundo plano ficaram, algumas vezes, as tarefas de receção das encomendas e de armazenamento dos produtos farmacêuticos.

Os colaboradores da FN demonstraram uma notável capacidade de adaptação, assumindo responsabilidades adicionais e trabalhando com afinco para manter os padrões de qualidade e segurança. Eu, como estagiária, esforcei-me para ser um elemento proativo da equipa, participando em todas as tarefas diárias no sentido de aliviar a carga de trabalho dos

colaboradores, e compreendi a importância da flexibilidade e da capacidade de assumir responsabilidades adicionais quando necessário.

Descredibilização por Parte de alguns Utentes

Ao longo do estágio, principalmente no início, observei que alguns utentes não se sentiam muito confiantes pelo facto de estarem a ser atendidos por uma estagiária e, por vezes, solicitavam ser atendidos por um elemento da equipa técnica ou até mesmo por um colaborador em específico. No entanto, esta situação foi pouco frequente e foi-se dissipando no decorrer do estágio, passando os utentes a confiar mais no meu atendimento e na minha capacidade de prestar um aconselhamento farmacêutico de qualidade, dando-me a oportunidade de evoluir.

Preparação de Medicamentos Manipulados

Com a evolução da indústria farmacêutica, o número de pedidos de medicamentos manipulados tem vindo a diminuir bastante.

Ao longo do meu estágio curricular, apenas tive a oportunidade de fazer a preparação de um manipulado, uma solução de álcool a 70% boricado à saturação, utilizado devido ao seu efeito antissético e desinfetante para tratar otites externas. Durante esta preparação, foi-me explicado todo o seu processo, incluindo o preenchimento da ficha de preparação de manipulados, os dados a inserir no rótulo e o cálculo do PVP, tendo por base a Portaria n.º 594/2004 de 2 de julho e a Portaria n.º 769/2004 de 1 de julho[3; 4].

Considero que teria sido importante ter tido a oportunidade de preparar mais medicamentos manipulados e outras formulações galénicas.

2.3. Oportunidades

Organização da Farmácia e *Marketing* Farmacêutico

A combinação de uma organização eficiente da farmácia com estratégias de *marketing* farmacêutico bem planeadas pode criar uma experiência de compra atraente para os clientes, aumentar as vendas e construir uma sólida base de clientes fidelizados.

Relativamente à organização da FN, esta tem por base um *layout* estratégico, em que os produtos de interesse são dispostos estrategicamente nos lineares e colocados em locais de maior tráfego para atrair a atenção dos clientes, uma setorização inteligente, em que os produtos similares e complementares são agrupados próximos uns dos outros para facilitar a procura por parte dos clientes e incentivar a compras adicionais, e uma sinalização clara, em que são utilizadas etiquetas simples e informativas para ajudar os clientes a encontrar produtos específicos com facilidade e fornecer informações sobre promoções e produtos em destaques.

Para além disso, a montra costuma ser frequentemente renovada, de forma bem delineada e de acordo com determinadas temáticas, e é sempre reservado um linear para expor os produtos a destacar na altura.

No que se refere à política de *marketing* da FN, esta recorre a diversas estratégias e abordagens para promover a própria farmácia, anunciar promoções e envolver os clientes, nomeadamente usando várias plataformas *online*, em especial o Instagram, onde são realizados *giveaways* e criados *reels* e vídeos informativos sobre produtos farmacêuticos, saúde e bem-estar.

Utentes Fidelizados e Acompanhamento Farmacoterapêutico

A FN tem uma grande quantidade de utentes habituais (utentes fidelizados) a frequentar a farmácia para os quais foi criado um cartão de fidelização que é essencial e vantajoso tanto para o utente, que acumula dinheiro no seu cartão que pode ser posteriormente descontado numa compra, como para a farmácia, que mantém um leque fiel de clientes.

Normalmente são estes clientes que têm ficha aberta na farmácia e para os quais é possível fazer um acompanhamento farmacoterapêutico, no qual o farmacêutico desempenha um papel ativo e interveniente. Este acompanhamento é possível, recorrendo ao separador "Histórico" existente na ficha do utente no SIFARMA 2000®, onde ficam registadas todas as compras realizadas pelo utente na farmácia. Estas informações são importantes para um aconselhamento informado e responsável.

Dermocosmética

Durante o meu estágio curricular tive oportunidade de consolidar conhecimentos, adquiridos na unidade curricular de Dermofarmácia e Cosmética, e adquirir outros na área da Dermocosmética, uma área de grande interesse pessoal e essencial na promoção da saúde e do bem-estar dos clientes. A FN possui grandes lineares desta área, com diversas marcas e produtos, que atraem muitos clientes à farmácia. Tive, portanto, a oportunidade de fazer aconselhamentos personalizados de acordo com as necessidades específicas de cada pessoa.

Formação Contínua

A evolução terapêutica é uma realidade dos nossos dias que implica uma constante atualização dos conhecimentos técnicos e científicos do farmacêutico. Neste sentido, tive interesse e oportunidade de participar em várias formações, que decorreram na FN ou por videoconferência, e que me permitiram manter informada e atualizada relativamente a diversos

produtos farmacêuticos. Além disso, também contribuíram para melhorar o meu desempenho no aconselhamento farmacêutico.

Destaco, assim, algumas formações realizadas ao longo do presente estágio: *Frontline*®, *Aquilea*®, *Depuralina*®, *Curaprox*®, *Bayer*® (*Gino-Canesten*® e *Bephanthene*®), *Caudalie*®, *Vichy*®, *CeraVe*® e *La Roche-Posay*®,

Clínica Prestadora de Serviços de Saúde Complementar à Sociedade da Farmácia Nova

A Sociedade da FN tem, desde junho de 2022, uma clínica médica prestadora de serviços de saúde, a Clínica Balau, que tem ao dispor da população diversas especialidades médicas e a possibilidade de realização de exames complementares de diagnóstico.

A proximidade desta clínica médica à farmácia não tem beneficiado apenas a população local, mas também tem contribuído para o crescimento da farmácia, atraindo novos clientes e fidelizando outros.

2.4. Ameaças

Produtos Esgotados

Durante o estágio deparei-me diversas vezes com a problemática dos medicamentos esgotados e sem previsão de retorno, situação que prejudica não só a farmácia, mas principalmente os utentes que ficam, muitas vezes, sem os medicamentos necessários para controlar as suas doenças crónicas. Esta situação prejudica financeiramente a farmácia, mas também acrescenta trabalho que, em situações normais, não haveria, nomeadamente a criação e atualização de listas de medicamentos esgotados e o contacto constante com os armazenistas. No entanto, a tarefa mais difícil para um farmacêutico, numa situação como a referida, é mesmo ter de informar o utente sobre a impossibilidade de lhe conseguir dispensar o medicamento e presenciar, muitas vezes, o desespero daqueles que necessitam destes para viver.

Infelizmente, pude presenciar a falta de alguns medicamentos como o *Inderal*®, utilizado em alguns problemas cardíacos e sem genéricos, o *Ozempic*®, indicado para diabetes *mellitus* tipo II e também sem genéricos, que chegou a ser procurado por doentes do norte do país, e o *Lasix*®, usado no tratamento de edemas e problemas de hipertensão arterial, que apesar de ter genéricos, não era bem aceites pelos utentes.

Locais de Venda de Medicamentos Não Sujeitos a Receita Médica

O Decreto-Lei n.º 134/2005 de 16 de agosto veio legitimar a venda de MNSRM fora das farmácias, em locais de venda, também eles, fiscalizados pelo INFARMED I.P. - Autoridade

Nacional do Medicamento e dos Produtos de Saúde I.P., sendo que o Governo considerou que este alargamento proporcionava benefícios aos consumidores em termos de acessibilidade e de preço[5].

Por um lado, estes locais são uma verdadeira ameaça para as farmácias, pois, ao efetuarem grandes volumes de compras, conseguem PVP significativamente inferiores, retirando, desta forma, clientes às farmácias, prejudicando-as ao nível económico e financeiro, e, por outro, são uma ameaça para os consumidores, pois promovem a automedicação e a utilização inconsciente do medicamento, pelo facto de não terem colaboradores suficientemente qualificados para fazerem um aconselhamento seguro e responsável.

Interpretação de Receitas Manuais

Apesar da grande maioria das receitas médicas serem prescritas eletronicamente, ainda aparecem na farmácia algumas receitas manuais. Este método tradicional de prescrição pode levar a erros de dispensa por dificuldades na interpretação devido a caligrafias pouco legíveis e aumenta significativamente o tempo de atendimento do utente, nomeadamente devido à necessidade de digitar manualmente as informações da receita. É também importante realçar que este método de prescrição torna-se menos prático e menos seguro para as farmácias, na concretização da comparticipação por parte do Serviço Nacional de Saúde.

3. Casos Práticos

Caso Prático I

Utente A, do sexo feminino, adolescente de cerca de 15 anos, dirigiu-se à farmácia, acompanhada pela mãe, queixando-se que lhe estavam a surgir algumas borbulhas no rosto e que não se andava a sentir muito bem com a sua pele, visto estar constantemente oleosa. Afirmei que era muito normal nestas idades e que com os produtos adequados, iria melhorar. Pela observação que fiz no momento, a situação não era grave, podendo ser tratada e amenizada com produtos dermocosméticos existentes na farmácia.

Comecei por perguntar sobre os produtos utilizados para limpeza e hidratação, à qual me respondeu que apenas lavava a cara com água de manhã e à noite e que utilizava um creme que a mãe tinha lá em casa, que concluí ser bastante comedogénico devido à sua constituição.

Perante esta situação e, de acordo com os conhecimentos anteriormente adquiridos, considerei que uma das melhores opções seria a utilização da gama *Effaclar* da *La Roche-Posay*®, visto ser a gama n.º 1 na Europa para o cuidado da pele oleosa propensa a acne e que garante uma elevada eficácia e ótima tolerabilidade[6]. Optei por aconselhar, para limpeza da pele, o *Effaclar Purifying Foaming Gel Cleanser* que purifica suavemente a pele oleosa, removendo o excesso de sebo e as impurezas sem secar, deixando-a limpa e fresca[7]. Aconselhei o uso de água morna inicialmente para desobstruir os poros e a lavagem do rosto com movimentos circulares suaves, retirando o produto com água fria, no final, para voltar a fechar os poros. Adverti de que deveria fazer a lavagem apenas duas vezes por dia, pois uma lavagem excessiva poderia irritar e secar a pele. Para hidratação do rosto, escolhi o *Effaclar Mat*, hidratante específico para pele oleosa com tendência a acneica com poros visíveis e dilatados. Este hidratante tem textura *oil-free* e tem um efeito matificante, pois reduz o excesso de sebo[8]. Alertei que o deveria usar diariamente, após o gel lavante. Para pequenas imperfeições que pudessem surgir, ainda aconselhei o *Effaclar A.I.*, específico para o cuidado localizado da pele oleosa com tendência a acneica[9]. Ainda relembrei a importância da utilização diária de protetor solar e aconselhei o *Anthelios Uvmune Oil Control Gel-Creme Fps50+*, pois tem uma textura mais leve com efeito matificante[10].

Por fim, chamei à atenção para não tocar nas borbulhas, de forma a evitar processos inflamatórios e consequente cicatriz.

Caso Prático II

Utente B, do sexo feminino, com cerca de 30 anos, dirigiu-se à farmácia com queixas de mamilos bastante doridos e gretados, situação que estava a dificultar a amamentação do seu bebé recém-nascido e, por isso, estava a ponderar a ida ao pediatra para o questionar sobre a possibilidade de começar a dar-lhe uma fórmula infantil.

Posto isto, e como se tratava do seu primeiro filho, falei um pouco dos benefícios da amamentação, para ela, como mãe, particularmente da prevenção de hemorragias no período pós-parto, da promoção da involução uterina e da recuperação do seu peso habitual. Referi ainda que o leite materno era o melhor e mais completo alimento para o bebé e que, embora já existissem muitas fórmulas infantis semelhantes ao leite materno, este continuava a ser insubstituível, contribuindo para o seu desenvolvimento saudável, nomeadamente, prevenindo infeções e alergias e facilitando a digestão. Para além disso, mencionei que era uma situação frequente no início da amamentação e que, com os cuidados que lhe iria sugerir, sentir-se-ia mais confortável. No entanto acrescentei que o mais importante era o seu bem-estar e que, caso não conseguisse, havia outras soluções.

Dispensei, então, o *Purelan*™, creme que contém lanolina, uma substância natural, hidratante e 100% segura para o bebé, podendo ser usada sem necessidade de remoção antes da amamentação. Ainda aconselhei o uso de protetores de silicone, apenas enquanto não melhorasse, pois, o uso prolongado pode causar habituação ao bebé, levando-o a rejeitar a mama. Também recomendei que deveria verificar sempre se o bebé pegava bem na mama, massajar os mamilos com o próprio leite pois ajudava no processo de cicatrização e lavar os mamilos apenas com água uma vez por dia, não friccionando ao limpar[11]. Sugeri ainda a compra de uma bomba tira-leite que, para além de poder ser usada naquele período de maior desconforto, também seria muito útil para outras situações, nomeadamente, para deixar o leite quando precisasse de se ausentar.

Caso Prático III

Utente C, do sexo feminino, com cerca de 40 anos, dirigiu-se à farmácia queixando-se de ardor, prurido, irritação e inchaço na zona vulvar e vaginal. De forma a averiguar uma possível candidíase vulvovaginal, questionei a utente sobre a existência de um corrimento anormal branco e pastoso, à qual a resposta foi positiva, acrescentando que não tinha tido nenhum comportamento de risco. Por fim, perguntei se tomava alguma medicação, ao que me respondeu estar a tomar *Betamox Plus*® 875mg + 125mg para o tratamento de uma otite.

Com base nas informações cedidas, pude concluir que se tratava de uma candidíase vulvovaginal, uma vez que tinha os sintomas associados, o aspeto do corrimento que era

comum nesta infeção, não tinha tido qualquer comportamento de risco que pudesse estar associado a uma Doença Sexualmente Transmissível (DST) e estava a fazer antibioterapia de largo espetro com o *Betamox Plus*® 875mg + 125mg que tem como um dos possíveis efeitos secundários a candidíase, pois pode provocar um desequilíbrio na flora vaginal, promovendo o desenvolvimento de fungos.

Para a utente ficar mais descansada, expliquei que a candidíase vulvovaginal era uma situação bastante comum, causada por fungos do género *Candida*, mais vulgarmente *Candida albicans* (microrganismos que vivem no nosso organismo) que, em condições normais, não provocavam qualquer infeção. Acrescentei ainda que havia circunstâncias que podiam levar à sua proliferação e causar infeção, mas que, com o uso de medicação adequada, seria rapidamente tratada.

Visto que os sintomas eram tanto externos, como internos, aconselhei o *Gino-Canesten*® creme vaginal a 1% de Clotrimazol, princípio ativo que é um derivado imidazólico com largo espectro de ação antimicótica e que atua contra os fungos, inibindo a síntese do ergosterol. Alertei para o facto da necessidade de lavar muito bem as mãos antes da utilização do creme, expliquei o seu modo de aplicação e a posologia, referindo que este deveria ser aplicado à noite durante 6 dias, mesmo que os sintomas passassem antes de terminar o tratamento. Avisei também que, caso os sintomas não passassem ou se houvesse mais irritação, deveria dirigir-se a um médico especialista[12].

Para além disso, aconselhei ainda um gel de lavagem íntima, o *Lactacyd*® *Pharma Suavizante*, visto ajudar a aliviar sintomas de infeções vaginais, como a irritação e prurido, alertando para a importância de uma higiene íntima diária e adequada. O *Lactacyd*® *Pharma Suavizante* tem na sua constituição Aloé Vera e extrato de Margarida Azul, conhecidos pelas suas propriedades calmantes, importantes nesta situação. Com um pH de 3,5, este gel lavante restabelece o equilíbrio da flora vaginal e protege-a[13]. Alertei-a ainda para a necessidade de enxaguar bem após a utilização e para interromper o uso em caso de irritação.

Por fim, aconselhei a adoção de medidas não farmacológicas, tais como, após o banho, secar bem a zona íntima para evitar humidade, limpar a área genital e anal sempre da frente para trás, usar roupa interior de algodão e evitar lavar a roupa íntima com produtos irritantes.

Caso Prático IV

Utente D, do sexo masculino, com cerca de 50 anos, dirigiu-se à farmácia com queixas de prurido, edema, dor e desconforto na região anal. Questionei então sobre a duração desses sintomas, a existência de hemorragia e a toma de alguma medicação para os sintomas descritos. Após esta conversa, pude concluir que se tratava de uma situação inicial de

hemorroida externa, sem sangue, explicando que era uma condição clínica que ocorria quando as veias hemorroidárias estavam danificadas e inflamadas e que era frequente e benigna, podendo ser facilmente resolvida com medicação adequada e alguns cuidados.

Passei então ao aconselhamento farmacológico, recomendando um creme retal *Procto-Glyvenol*® 50 mg/g + 20 mg/g que tem na sua constituição Tribenosido, com capacidade de reduzir a permeabilidade do tónus vascular e propriedades anti-inflamatórias com ação antagonista em substâncias endógenas mediadoras do processo inflamatório e na indução da dor, e Cloridrato de Lidocaína, um anestésico local que alivia o prurido, ardor e dor. Alertei para o modo de utilização e também para a posologia deste creme (começar por aplicar 2 vezes por dia, de manhã e à noite, até os sintomas agudos diminuírem, e ir reduzindo a aplicação passando a 1 vez por dia). Para além disso, avisei sobre possíveis reações alérgicas devido à presença de parahidroxibenzoato de metilo e parahidroxibenzoato de propilo e de latéx na embalagem[14; 15].

Sugeri também o começo da toma de *Daflon*® 500mg que é constituído por dois bioflavonoides, 90% de diosmina e 10% de flavonoides, expressos em hesperidina. O *Daflon*® atua ao nível das veias, reduzindo a distensibilidade venosa e a estase venosa, e ao nível da microcirculação, normalizando a permeabilidade capilar e reforçando a resistência capilar. Para a situação descrita, recomendei a toma de 2 comprimidos 3 vezes ao dia, nos 4 primeiros dias, 2 comprimidos 2 vezes ao dia, nos 3 dias seguintes e 2 comprimidos 2 vezes ao dia, até ao desaparecimento dos sintomas. Também recomendei que, se não se sentisse melhor ou se piorasse após 7 dias da toma do medicamento, deveria consultar o médico[16].

Relativamente às medidas não farmacológicas, recomendei, nomeadamente, a limpeza diária eficaz com água fria, o aumento da ingestão diária de água, a ingestão de alimentos com fibras, a diminuição do consumo de substâncias irritantes para a mucosa (especiarias, álcool e café), a manutenção de bons hábitos defecatórios e que evitasse estar sentado durante períodos prolongados.

Caso Prático V

Utente E, do sexo masculino, com cerca de 70 anos, chegou à farmácia e trazia consigo um papel com o nome de um medicamento, no qual estava escrito "*Codipront*®". Referiu ainda que estava com muita tosse com expetoração, o que o incomodava bastante, desde o dia anterior, e que a mulher lhe tinha dito que aquele medicamento "fazia maravilhas e parava a tosse rapidamente". Avisei com prontidão que o *Codipront*® era um antitússico indicado somente para alguns casos de tosse seca aguda e que, para além de ser um MSRM, que a farmácia não poderia dispensar sem a respetiva receita médica, era muito perigosa a sua toma

com o tipo de tosse descrita, pois ao inibi-la, iria acumular expetoração dentro dos pulmões e poderia provocar infeções graves.

No seguimento da explicação, perguntei ao utente se tinha algum outro tipo de sintomatologia associada (dificuldade respiratória, dor torácica, febre) ou doença crónica como úlcera péptica ou asma brônquica, ao qual me respondeu que tomava apenas "uns medicamentos para o colesterol e para a próstata". Perante a sua resposta, decidi, então, aconselhar o *Fluimucil*® 600 mg, comprimidos efervescentes, 1 vez por dia de preferência à noite, durante cerca de 1 semana. O *Fluimucil*® é um adjuvante mucolítico para a terapêutica relacionada com hipersecreção e muco viscoso e tem na sua constituição Acetilcisteína que tem uma ação mucolíticafluidificante sobre as secreções mucosas e mucopurulentas[17].

Alertei o utente que o comprimido era para colocar num copo de água e, após dissolvido, ingerir. Referi ainda que a expetoração não iria parar de imediato e poderia até aumentar durante os primeiros dias da toma do *Fluimucil*®. Como medidas não farmacológicas, aconselhei a ingestão de muita água, para facilitar a expulsão das secreções, e de bebidas quentes, como a infusão de mel e limão, para acalmar as vias respiratórias, e a elevação, se possível, da cabeceira da cama para aliviar a tosse durante a noite. Acrescentei ainda que se a tosse não parasse deveria dirigir-se ao médico ou novamente à farmácia pois esta poderia ter uma componente alérgica.

4. Considerações Finais

O estágio curricular em FC foi uma experiência extremamente gratificante e enriquecedora que contribuiu significativamente para o meu desenvolvimento profissional e pessoal, não só por ter tido a oportunidade de consolidar e aperfeiçoar os meus conhecimentos técnicos e científicos, mas também por ter feito parte de uma equipa comprometida com excelência nos cuidados de saúde.

Durante o meu estágio, pude confirmar a importância insubstituível do farmacêutico comunitário na prestação de serviços de saúde de qualidade à população e admirar ainda mais o seu papel na sociedade. Pude confirmar que o trabalho de um farmacêutico é multidisciplinar, abrangendo um leque imenso de competências e responsabilidades, e que exige uma constante atualização e investimento na sua formação.

Realço a importância deste estágio curricular no plano de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF), que me permitiu pôr em prática todos os conhecimentos adquiridos na faculdade ao longo de quatro anos e meio, e o acréscimo que terá, certamente, no meu futuro profissional.

Aproveito para deixar uma palavra de profundo agradecimento a todos os colaboradores da FN por me terem acolhido e orientado ao longo do estágio e por me terem dado autonomia e confiado em mim. Sinto-me, sem dúvida, mais preparada para iniciar o meu caminho como farmacêutica e profissional de saúde.

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16. INFARMED I.P. – **Daflon 500mg - Resumo das Características do Medicamento**

17. INFARMED I.P. - **Fluimucil 600 mg - Resumo das Características do Medicamento**

PARTE II

Relatório de Estágio em Assuntos Regulamentares do Medicamento

INFARMED I.P.

Infarmed
Autoridade Nacional do Medicamento
e Produtos de Saúde, I.P.

Sob orientação da Dra. Dina Lopes

Lista de Abreviaturas

AIM - Autorização de Introdução no Mercado

CAM - Comissão de Avaliação de Medicamentos

DAM - Departamento de Avaliação do Medicamento

FFUC - Faculdade de Farmácia da Universidade de Coimbra

INFARMED - Instituto Nacional da Farmácia e do Medicamento

INFARMED I.P. - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

MICF - Mestrado Integrado em Ciências Farmacêuticas

SWOT - do inglês *Strengths, Weaknesses, Opportunities, and Threats*

1. Introdução

O plano curricular do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) contempla, para além do estágio curricular obrigatório em Farmácia Comunitária, a realização de um estágio suplementar numa outra área de interesse relativa ao medicamento. A possibilidade de realizar a componente formativa em áreas diversas permite o desenvolvimento de um conjunto mais amplo de habilidades e proporciona uma visão mais completa e abrangente do mercado de trabalho[1].

Os farmacêuticos, sendo por excelência os profissionais do medicamento, estão envolvidos em todas as suas etapas, desde a sua investigação e desenvolvimento, à sua dispensa e utilização. Uma das etapas fundamentais é a Autorização de Introdução no Mercado (AIM), uma vez que para que o medicamento seja comercializado e disponibilizado para prescrição e dispensa é necessário haver um processo de avaliação rigoroso que, no caso de Portugal, é feito pelo INFARMED I.P. - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.[2]. De acordo com a Agência Europeia de Medicamentos: "Tendo em vista proteger a saúde pública e assegurar a disponibilidade de medicamentos eficazes, seguros e de elevada qualidade para os cidadãos europeus, todos os medicamentos carecem de uma autorização antes de serem colocados no mercado da União Europeia"[3].

Os assuntos regulamentares são então uma área de extrema importância no setor farmacêutico e os profissionais que trabalham nesta área têm presença fundamental em diversas atividades: processos de desenvolvimento, registo, acesso ao mercado, informação e apoio aos profissionais de saúde, monitorização da utilização dos medicamentos e dispositivos médicos, entre outras. Para além disto, esta área ainda exige um conhecimento aprofundado e detalhado de padrões exigentes de qualidade, segurança e eficácia impostos pelas autoridades competentes nacionais e comunitárias[4; 5].

Após compreender a relevância e exigência da área de assuntos regulamentares no ciclo de vida do medicamento, decidi ponderar a realização de parte do meu estágio curricular nesta área. Como a FFUC celebra acordo com o INFARMED I.P., aproveitei esta oportunidade única de aprendizagem para estagiar neste instituto público e colocar em prática todas as competências e conhecimentos técnico-científicos adquiridos ao longo do curso.

O presente relatório incide sobre o estágio curricular realizado no INFARMED I.P., mais concretamente no Departamento de Avaliação do Medicamento (DAM). O objetivo deste relatório visa sintetizar as atividades e os conhecimentos adquiridos ao longo do estágio através de uma reflexão crítica e pessoal, tendo por base uma análise SWOT (*Strengths, Weaknesses, Opportunities, and Threats*), nomeando, internamente, os pontos fortes e fracos e,

externamente, as oportunidades e ameaças sentidas (**Figura 2**). O estágio teve a orientação da Dra. Dina Lopes e decorreu ao longo de três meses, entre os dias 2 de maio e 28 de julho de 2023.

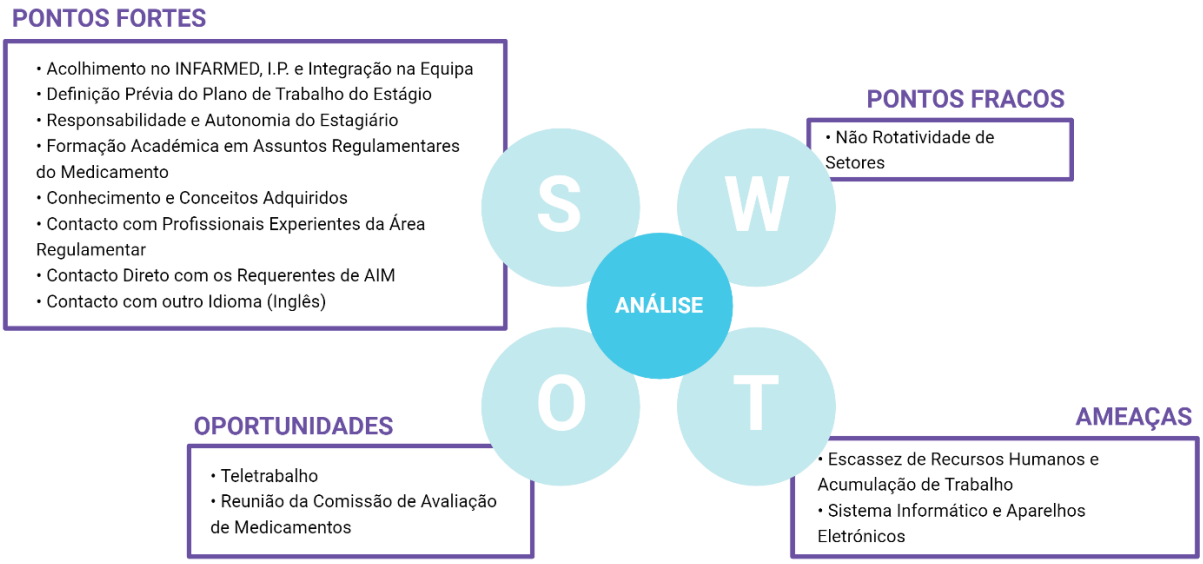

Figura 2. Análise SWOT do Estágio Curricular no INFARMED I.P.

2. Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

Em 1993 foi fundado o INFARMED - Instituto Nacional da Farmácia e do Medicamento, assim denominado na época. O INFARMED destacava-se como uma das únicas autoridades europeias que integrava todas as valências relativas ao medicamento, tendo sido criado com o objetivo de desempenhar um papel ativo no novo Sistema Europeu do Medicamento. Atualmente designado por INFARMED I.P. - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P., é um instituto público de regime especial integrado na administração indireta do Estado, com autonomia administrativa, financeira e património próprio, sediado em Lisboa, sob tutela e superintendência do Ministério da Saúde. Tem como objetivo "regular e supervisionar os sectores dos medicamentos de uso humano e produtos de saúde, segundo os mais elevados padrões de proteção da saúde pública, e garantir o acesso dos profissionais da saúde e dos cidadãos a medicamentos e produtos de saúde de qualidade, eficazes e seguros"[4; 6].

O INFARMED I.P. está organizado hierarquicamente de forma estruturada e criteriosa, sendo constituído por cinco órgãos e catorze unidades orgânicas. Como é possível ver no organograma representado no **Anexo I** - Organograma do INFARMED I.P., são órgãos do INFARMED I.P. o Conselho Diretivo, o Fiscal Único, o Conselho Consultivo, as Comissões

Técnicas Especializadas e o Conselho Nacional de Publicidade de Medicamentos e Produtos de Saúde. Por sua vez, as unidades orgânicas são subdivididas em nove com função de negócio (Direção de Avaliação de Medicamentos, Direção de Gestão do Risco de Medicamentos, Direção de Produtos de Saúde, Direção de Inspeção e Licenciamentos, Direção de Comprovação da Qualidade, Direção de Avaliação das Tecnologias de Saúde, Direção de Informação e Planeamento Estratégico, Gabinete de Relações Internacionais e Desenvolvimento, Unidade de Projetos Interinstitucionais e para o Sistema de Saúde) e cinco com função de suporte (Direção de Sistemas e Tecnologias de Informação, Direção de Recursos Humanos, Financeiros e Patrimoniais, Direção de Gestão de Informação e Comunicação, Gabinete de Planeamento e Qualidade, Gabinete Jurídico e de Contencioso)[7].

2.1. Direção de Avaliação de Medicamentos

A DAM, dirigida pela Dra. Marta Marcelino, é uma das unidades orgânicas do INFARMED I.P. e tem diferentes subunidades orgânicas[8]. No meu caso, fui integrada na equipa das AIM.

Nos primeiros dias de estágio, tive a oportunidade de visualizar alguns vídeos explicativos e relembrar algumas temáticas abordadas na unidade curricular de Assuntos Regulamentares do Medicamento (bases legais, tipos de AIM, tipos de procedimentos, entre outras). Além disso, foi ministrada uma formação sobre as plataformas que seriam usadas ao longo dos três meses, proporcionando assim a obtenção das bases necessárias para a realização correta de todas as tarefas solicitadas.

Posteriormente, integrei a equipa da Dra. Maria Susana Matos e fiquei a colaborar na finalização dos processos descentralizados e de reconhecimento mútuo de AIM. A finalização dos processos foi acompanhada desde o momento em que estes estavam em fase nacional, ou seja, após o fim do processo a nível europeu (dia 210 para o processo descentralizado e dia 60/90 para o de reconhecimento mútuo) até à emissão do Certificado de AIM e envio do Resumo das Características do Medicamento, Folheto Informativo e Rótulos para publicação no Infomed.

3. Análise SWOT

3.1. Pontos Fortes

Acolhimento no INFARMED, I.P. e Integração na Equipa

A participação ativa da equipa que recebe um estagiário desempenha um papel fundamental para o seu sucesso e aprendizagem. A dedicação e disponibilidade demonstrada

pelos profissionais do INFARMED I.P. proporcionaram-me uma experiência enriquecedora, possibilitando-me um crescimento quer a nível profissional, com a aquisição de variados conhecimentos, quer a nível pessoal, com o estabelecimento e fortalecimento de relações interpessoais.

Para uma melhor integração dos estagiários na equipa a comunicação foi a chave e todos aprendemos uns com os outros. As gestoras que nos acompanharam partilharam as suas experiências enquanto farmacêuticas na área regulamentar, dando-nos uma visão geral desta profissão. Para além disso, forneceram-nos todas as informações e bases necessárias para o desenvolvimento autónomo e eficaz das tarefas e estiveram sempre disponíveis para esclarecer as dúvidas que surgiram no decorrer do estágio, permitindo-me crescer como gestora de processos de AIM.

Definição Prévia do Plano de Trabalho do Estágio

Ter um plano de estágio definido permite a compreensão das responsabilidades dos estagiários e proporciona clareza e direção para a realização das tarefas propostas. Deste modo, como estagiária, tive uma visão geral do que iriam ser as metas e objetivos ao longo de todo o programa. As atividades foram apresentadas com uma estrutura bem definida, possibilitando a realização das tarefas de maneira eficaz, contínua e fluída.

Responsabilidade e Autonomia do Estagiário

No INFARMED I.P. consideram que a valorização do trabalho do estagiário permite o enriquecimento de todo o trabalho deste instituto. Desde o início do estágio foram-me entregues tarefas de grande responsabilidade e fui encorajada pelos colaboradores a desenvolver um trabalho autónomo.

As primeiras semanas representaram o período mais desafiador do estágio, visto que me foram entregues tarefas que nunca tinha realizado e, por este motivo, tive de recorrer com maior frequência às gestoras que estavam a supervisionar os meus processos. Após este período de adaptação, passei a realizar as tarefas de forma mais autónoma e com muito mais facilidade. Embora houvesse sempre alguém disponível da parte do INFARMED I.P. para esclarecer dúvidas e fornecer orientações quando necessário, eu e os meus colegas estagiários tentámos, em trabalho colaborativo, apoiar-nos na resolução das questões que foram surgindo durante a realização das tarefas. De facto, a autonomia e a responsabilidade, que nos foram incutidas, ajudaram-me a fortalecer as capacidades de tomada de decisões e de resolução de problemas, duas habilidades cruciais no desempenho e sucesso profissionais.

Formação Académica em Assuntos Regulamentares do Medicamento

A unidade curricular de Assuntos Regulamentares do Medicamento, lecionada pelo Professor Doutor João José Sousa, e que integra, com caracter obrigatório, o plano de estudos de MICF, foi uma mais-valia durante o estágio no INFARMED I.P., visto que algumas temáticas abordadas nas aulas tiveram aplicação prática e revelaram-se de grande utilidade na finalização dos processos de AIM que me foram entregues. Também possibilitou uma adaptação e domínio mais rápidos dos termos técnicos que tive de dominar para a execução do meu trabalho.

Conhecimento e Conceitos Adquiridos

A experiência de estágio no INFARMED I.P. foi extremamente valiosa a nível profissional. Durante os três meses de estágio tive a oportunidade de me familiarizar com diversos novos conceitos essenciais para construir uma base sólida na minha futura carreira profissional. É importante destacar que a aprendizagem foi contínua, uma vez que surgiam frequentemente processos com situações diferentes e desafiantes, que contribuíram para alargar o meu conhecimento na área de Assuntos Regulamentares.

Contacto com Profissionais Experientes da Área Regulamentar

A minha experiência de estágio no INFARMED I.P. proporcionou-me a oportunidade de contactar com profissionais extremamente competentes na área regulamentar. A elevada experiência destes colaboradores contribuiu significativamente para o meu crescimento e desenvolvimento enquanto estagiária no âmbito da gestão de processos de AIM. Ao longo deste período, tive a oportunidade de ampliar de forma exponencial o meu conhecimento relativamente a todos os procedimentos relacionados com AIM. Além disso, consegui explorar a minha capacidade de trabalho e superação e aperfeiçoar métodos de trabalho que contribuíram para o meu crescimento enquanto futura profissional.

Contacto Direto com os Requerentes de AIM

Na finalização dos processos, o contacto com os requerentes de AIM foi recorrente e fundamental. Este contacto ocorria para solicitar o envio de documentos em falta para conseguir avançar com o processo ou para esclarecer dúvidas inerentes ao estado em que determinado processo se encontrava.

A comunicação frequente com os requerentes de AIM proporcionou-me uma nova visão da dinâmica da indústria farmacêutica na área regulamentar. Através desta interação direta, tive a oportunidade de compreender melhor os desafios enfrentados pelas indústrias farmacêuticas, aquando da comercialização de um novo medicamento.

Contacto com outro Idioma (Inglês)

Considerando que todos os processos que finalizei eram descentralizados e de reconhecimento mútuo, na grande maioria dos casos a comunicação era feita em inglês. Para além da comunicação com o requerente de AIM, em muitos processos era necessário verificar traduções, pondo-me em contacto constante com esta língua, o que me ajudou bastante no aperfeiçoamento da minha comunicação escrita em inglês e enriqueceu o meu domínio técnico dos temas abordados neste idioma.

3.2. Pontos Fracos

Não Rotatividade de Setores

Como referido anteriormente, a DAM aloja diferentes subunidades orgânicas. Durante os três meses no INFARMED I.P., os estagiários ficaram responsáveis apenas por funções inerentes a uma dessas subunidades. A rotatividade por outras subunidades e a realização de novas tarefas teria permitido um conhecimento mais amplo e abrangente do trabalho realizado pelos colaboradores do INFARMED I.P. e enriquecido ainda mais a minha formação e aprendizagem. No entanto, viabilizar esta rotatividade nos estágios curriculares, permitindo aos estagiários a compreensão do trabalho desenvolvido em cada subunidade, seria muito difícil durante o curto espaço de tempo em que estes decorrem.

3.3. Oportunidades

Teletrabalho

No decorrer do estágio, foi-nos dada a possibilidade de adotar um modelo de trabalho híbrido. O cumprimento do horário previamente estabelecido foi uma realidade, tanto em regime de trabalho presencial, como em regime de teletrabalho. Para comunicação *online* foi utilizada a plataforma Webex que permitiu o contacto fácil e rápido com as gestoras de processos e a colaboração entre colegas. O teletrabalho também reduziu a necessidade de deslocamentos diários, permitindo economizar tempo e recursos financeiros.

Reunião da Comissão de Avaliação de Medicamentos

A Comissão de Avaliação de Medicamentos (CAM) é um órgão consultivo do INFARMED I.P. responsável pela análise técnico-científica dos novos medicamentos e é constituída por avaliadores de áreas distintas, nomeadamente médica, farmacêutica e toxicológica. O objetivo destas reuniões, que acontecem periodicamente, centra-se na análise de pareceres relacionados com alterações ou renovações aos termos de AIM emitidos pelos

peritos. A opinião bem fundamentada destes pareceres garante a qualidade, segurança e eficácia de todos os medicamentos analisados[9].

Na última semana de estágio, todos os estagiários da DAM tiveram a oportunidade de assistir a esta reunião por videoconferência e, mais uma vez, foi um momento de aprendizagem pois os processos aí discutidos foram anteriormente avaliados pela equipa de AIM, onde eu estava inserida.

3.4. Ameaças

Escassez de Recursos Humanos e Acumulação de Trabalho

Durante o período de estágio, foi bastante notória a discrepância entre os recursos humanos existentes e a real necessidade desses mesmos recursos. Como pude constatar ao longo do estágio, o INFARMED I.P. está a funcionar bastante abaixo da sua capacidade devido à escassez de recursos humanos aliada ao crescente volume de trabalho. Apesar desta situação, os colaboradores continuam motivados e a trabalhar ativamente para que a qualidade do seu trabalho se mantenha e para que possam cumprir com os seus objetivos. Além disso, o auxílio que me foi prestado enquanto estagiária foi sempre efetivado, tendo recebido todo o apoio no esclarecimento das minhas dúvidas, principalmente nas primeiras semanas e, posteriormente, sempre que necessitei.

Sistema Informático e Aparelhos Eletrónicos

O sistema informático do INFARMED I.P. é muito complexo e, pontualmente, foi necessário recorrer ao departamento de informática para resolver algumas questões técnicas mais difíceis de solucionar. No decorrer do estágio, deparei-me com alguns problemas no *software* utilizado que, por vezes, provocaram a perda de algumas tarefas que estavam a ser realizadas, obrigando à sua repetição.

Relativamente aos aparelhos eletrónicos, verifiquei que, apesar de já existirem alguns computadores mais recentes que suportam perfeitamente a complexidade dos programas utilizados, ainda há bastantes computadores antigos que não permitem um trabalho tão rápido e eficiente como seria desejável.

4. Considerações Finais

A realização de parte do estágio curricular no INFARMED I.P., mais especificamente na subunidade orgânica DAM, revelou-se uma experiência muito enriquecedora e desafiante, quer a nível pessoal, quer a nível profissional.

Durante este período tive a oportunidade de mergulhar no mundo dos Assuntos Regulamentares do Medicamento, aprofundando os conhecimentos adquiridos na faculdade e apropriando-me de outros que serão, com toda a certeza, essenciais na minha vida como futura farmacêutica.

Esta experiência permitiu-me a colaboração com uma equipa altamente competente e comprometida que me fez compreender a importância do INFARMED I.P., que em cooperação com a Indústria Farmacêutica, trabalha em benefício da saúde pública. Os colaboradores com quem contactei mais diretamente estiveram sempre dispostos a partilhar com os estagiários todo o seu saber e apoiá-los nas suas dúvidas, não descurando o incentivo à autonomia e responsabilidade.

Considero que o presente estágio superou as minhas expetativas e contribuiu para o meu crescimento profissional na medida em que me proporcionou um primeiro contacto prático com as complexas questões regulatórias da indústria farmacêutica, permitindo-me adquirir competências valiosas, colaborar com uma equipa experiente e participar ativamente na finalização de processos AIM. Esta experiência ampliou de forma significativa a minha compreensão sobre o setor farmacêutico.

Aproveito para agradecer à Dra. Dina Lopes, minha orientadora, à Dra. Maria Susana Matos e à Dra. Carla Almeida, que me acompanharam de perto na finalização dos processos de AIM.

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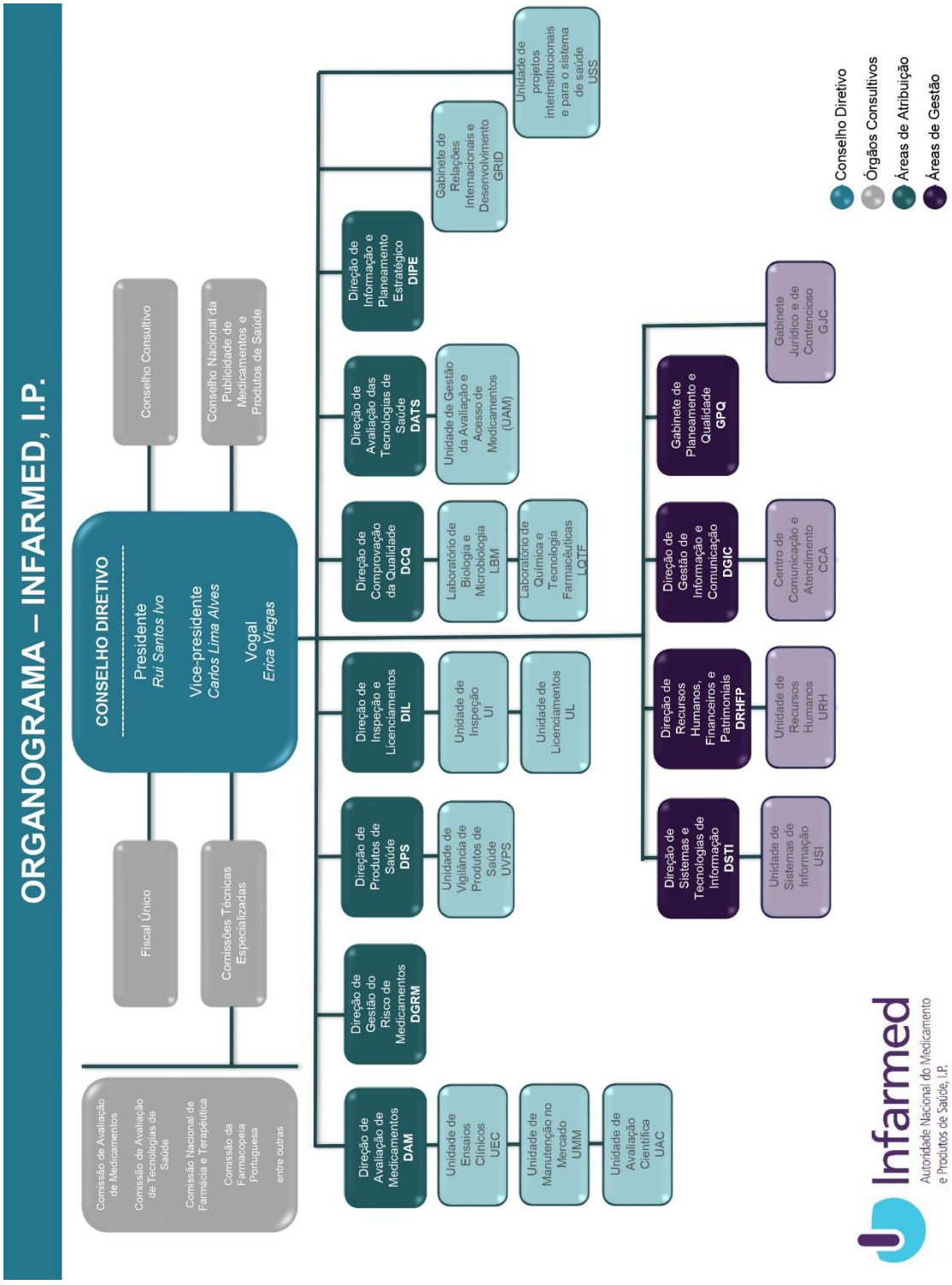
Anexo

Anexo I - Organograma do INFARMED I.P.



PARTE III

Monografia

"Drug Repurposing for Skin Cancer Treatment: A Nanotechnological Approach"

Sob a orientação da Professora Doutora Patrícia Sofia Cabral Pires

Abstract

Skin cancer represents a major health concern due to its rising incidence and limited treatment options. Excessive sun exposure, among other risk factors, is mostly responsible for this cancer's prevalence. Current treatments (surgery, chemotherapy, radiotherapy, immunotherapy, targeted therapy) often entail high costs, significant adverse effects, and patient inconvenience. The search for novel treatment options is also marked by the high capital investment and extensive development involved in the drug discovery process. In response to these challenges, a more cost-effective and patient-centered approach lies on the repurposing of existing drugs for topical application and optimizing their delivery through nanotechnology incorporation. This innovative strategy aims to take advantage of the known pharmacological background of commonly used drugs to expedite therapeutic development, direct application on the cancer tissue for localized action and diminished systemic side effects, and ultimately nanoparticle enhanced drug delivery and therapeutic efficacy. The present review dissects the different concepts involved in this approach and various works that demonstrate the remarkable impact of numerous lipid-based, polymeric, and inorganic nanoparticle systems in topical repurposed drug products being investigated as potential skin cancer therapy options. More specifically, when comparing to free-drug simple formulations, the nano-based systems provide an improvement of skin permeation, bioavailability, system stability and essentially therapeutic efficacy and efficiency.

Keywords: drug delivery, drug repurposing, nanotechnology, topical formulation, skin cancer

Resumo

O cancro da pele representa um enorme problema de saúde devido à sua crescente incidência e às limitações dos tratamentos disponíveis. A exposição excessiva ao sol, entre outros fatores, é o principal responsável pela prevalência deste cancro. Os atuais tratamentos (cirurgia, quimioterapia, radioterapia, imunoterapia, terapia dirigida) implicam custos elevados, efeitos adversos significativos e outros inconvenientes para o paciente. Para além disso, a descoberta e desenvolvimento de novos fármacos obriga a um elevado investimento económico, acompanhado de um processo moroso e complexo. Em resposta a estes desafios, surge uma nova abordagem, centrada no doente e com um melhor custo-benefício, que assenta no reaproveitamento de fármacos existentes no mercado, potenciados através da incorporação em nanotecnologia para aplicação tópica. Esta estratégia inovadora parte do conhecimento farmacológico já existente, acelerando o desenvolvimento terapêutico, e incide na aplicação direta no tecido cancerígeno, garantindo uma ação localizada e menor efeito sistémico, aliada a uma otimização de entrega de fármacos e eficácia através da nanotecnologia. A presente revisão descreve conceitos, envolvidos nesta nova abordagem terapêutica, e analisa estudos que têm demonstrado um impacto notável, tanto dos diversos sistemas de nanopartículas (lipídicas, poliméricas e inorgânicas), como do reaproveitamento de fármacos, nas formulações tópicas para o tratamento do cancro da pele. Especificamente, quando comparados com formulações simples constituídas por fármacos livres, estes sistemas nanoparticulados proporcionam um aumento na permeação cutânea, biodisponibilidade, estabilidade do sistema e eficácia e eficiência terapêutica.

Palavras-chave: entrega de fármacos, reaproveitamento de fármacos, nanotecnologia, formulação tópica, cancro da pele

Lista de Abreviaturas

AA - Ascorbic Acid

AhR - Aryl Hydrocarbon Receptor

AMPK - Adenosine 5' Monophosphate-Activated Protein Kinase

AP - Ascorbyl Palmitate

API - Active Pharmaceutical Ingredient

BCC - Basal Cell Carcinoma

BCS - Biopharmaceutical Classification System

BLIP - Blank Liposomes

BPLGA - Blank PLGA

CEL - Celecoxib

Ch-NLC - Chitosan-Coated Nanostructured Lipid Carrier

CM - Cutaneous Melanoma

COX-2 - Cyclooxygenase-2

CPE - Chemical Permeation Enhancers

CQA - Critical Quality Attribute

DDAB - Didodecyldimethylammonium Bromide

DHODH - Dihydroorotate Dehydrogenase

DMARD - Disease-Modifying Antirheumatic Drug

DNA - Deoxyribonucleic Acid

DOX - Doxycycline Hyclate

DOXO - Doxorubicin

EE% - Entrapment Efficiency

FDA - Food and Drug Administration

Gel - Gelatin

HA - Hydroxyapatite

Hh - Hedgehog

HLB - Hydrophilic-Lipophilic Balance

HMG-CoA - Hydroxymethylglutaryl-Coenzyme A

HPMC - Hydroxypropyl Methylcellulose

IARC - International Agency for Research on Cancer

IC50 - Half-Maximal Inhibitory Concentration

ITZ - Itraconazole

IV - Intravenous

L - Limonene

LDL - Low-Density Lipoprotein

LFD - Leflunomide

MB - Methylene Blue

MET - Metformin

MMP - Matrix-Metalloproteinase

MN - Microneedle

MO - Monoolein

mTOR - Mammalian Target of Rapamycin

NB - Suppocire NB

NE - Nanoemulsions

NEG - Nanoemulgel

nHA - Hydroxyapatite Nanoparticles

NIC - Niclosamide

NLC - Nanostructured Lipid Carrier

NLIP - Niclosamide-Loaded Liposomes

NMSC - Non-Melanoma Skin Cancer

NP - Nanoparticle

NSAID - Nonsteroidal Anti-Inflammatory Drug

PBS - Phosphate-Buffered Saline

PCL - Poly-ε-Caprolactone

PDI - Polydispersity Index

PDT - Photodynamic Therapy

PEG - Polyethylene Glycol

PG - Propylene Glycol

PhS - Photosensitizer

PLGA - Poly(lactic-co-glycolic Acid)

PS - Particle Size

PS80 - Polysorbate 80

QbD - Quality by Design

ROS - Reactive Oxygen Species

SC - Stratum Corneum

SCC - Squamous Cell Carcinoma

SIM - Simvastatin

SLN - Solid Lipid Nanoparticle

SMO - Smoothened Protein

SPLGA - Simvastatin-Loaded PLGA

STAT3 - Signal Transducer and Activator of Transcription 3

UV - Ultraviolet

ZP - Zeta Potential

1. Introduction

The field of oncology is focused on understanding cancer and the pursuit of novel therapeutic agents to combat it. Much of the research aims attention at skin cancer due to its rising prevalence[1]. However, the discovery and development of new drugs requires significant investments of time and resources, often along with the arduous process of conventional therapy that relies on intravenous (IV) administration[2; 3]. These challenges have prompted a paradigm shift towards exploring alternative approaches that are both cost-effective and patient-centered[4]. One such approach involves harnessing the potential of repurposed drugs with reported antitumor activity delivered via nanocarrier systems in topical formulations for convenient localized application, presenting a promising avenue for alternative strategies to fight skin cancer.

Skin cancer is described as a global public health concern and has seen its number of cases rise steadily over the years[1]. While advancements in targeted therapies have improved treatment outcomes, the cost and time associated with bringing new drugs to market are considerable hurdles[4]. Moreover, conventional treatment options are often associated to high costs, complex procedures, systemic toxicity, and unwanted side effects, as it is observed for chemotherapy[5]. These limitations have prompted a reevaluation of existing drugs, setting off the concept of drug repurposing.

Drug repurposing offers a unique advantage by capitalizing on pre-existing knowledge of drug safety profiles, pharmacokinetics, and mechanisms of action. This strategy not only accelerates the development process of novel therapeutics into clinical applications but also addresses the financial burden associated with novel drug discovery. Repurposed drugs can target pathways involved in skin cancer, offering a potential solution to complement existing therapies[6].

A pivotal development in drug delivery that complements the repurposing approach involves the utilization of nanocarrier systems that can facilitate the encapsulation and controlled release of drugs, enabling targeted and localized delivery. In the context of skin cancer, nanocarrier-based topical formulations offer several distinct advantages over traditional systemic approaches. The topical route allows for direct application to the affected area, minimizing systemic exposure. Additionally, the use of nanocarriers enhances drug stability, permeation through the skin and cellular uptake, all of which contribute to an improved therapeutic effect[7].

This review aims to explore the synergistic potential of combining drug repurposing with nanocarrier-mediated topical delivery in the context of skin cancer therapy. By examining

and providing a comprehensive overview of the current landscape, I have highlighted the potential of topical formulations featuring repurposed drugs in nanocarrier systems as a promising approach in advancing the battle against skin cancer.

2. Skin Anatomy, Physiology, and Function

The skin is the largest body organ covering about 15% of body mass and all of the body's external surface[1; 8]. This continuous self-renewing tissue serves as the barrier between the "inside" and the "outside" of the organism, which, for example, blocks the entry of pathogens, protects from chemical assaults, heat, infections, radiation and regulates loss of water and solutes that are essential for human body function[1; 8].

Normal skin with an effective barrier consists of 3 layers: the epidermis, the dermis, and the hypodermis (**Figure 1**)[1]. The two primary layers encompass the epidermis or epithelial component coating on the surface and the dermis or connective component of nutrition[8]. They are both composed of various elements, such as epithelial, mesenchymal, glandular, and neurovascular tissue that make up the basement membrane, a highly specialized matrix structure that works like a physical separation of the two sections and grants a stabilized and dynamic interface[1; 8; 9; 10].

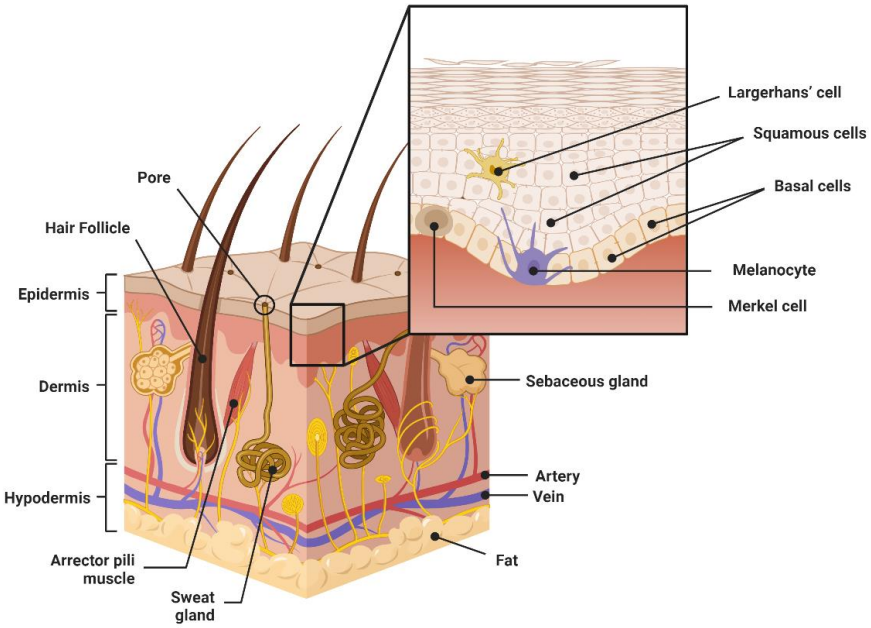


Figure 1. Schematic representation of human skin (produced with BioRender)

2.1. Epidermis

The epidermis, of ectodermal origin, is the peripheral layer of skin, which contacts with the environment and has a major role as a barrier against pathogens, chemicals, and ultraviolet (UV) radiation[11]. The thickness of the epidermis can change due to many factors[12]. Any

modifications in epidermal differentiation and lipid composition can lead to a modified barrier function, thus alterations and diseases[1; 8; 11]. Although the whole skin structure actively participates in the host defense, epidermis works like an inside-outside barrier when it prevents loss of water and other body components to the environment and like an outside-inside barrier when it protects the body from the environment adversities[8; 11].

Despite being the thinnest layer of all three, the epidermis itself is composed of five sublayers (**Figure 2**) responsible for continuous regeneration of the surface of the skin[13]. These sublayers represent different stages of maturation of the actively dividing cells, keratinocytes, which occur over a 30-day period[9]. Keratinocytes are the most abundant cells in the epidermis, and they are tightly connected to each other by desmosomes and tight junctions, forming an effective physicochemical barrier[11]. The epidermis also contains the melanocytes (the cells in which melanoma develops), the Largerhans' cells (involved in the immune system in the skin), Merkel cells and sensory nerves[9; 14].

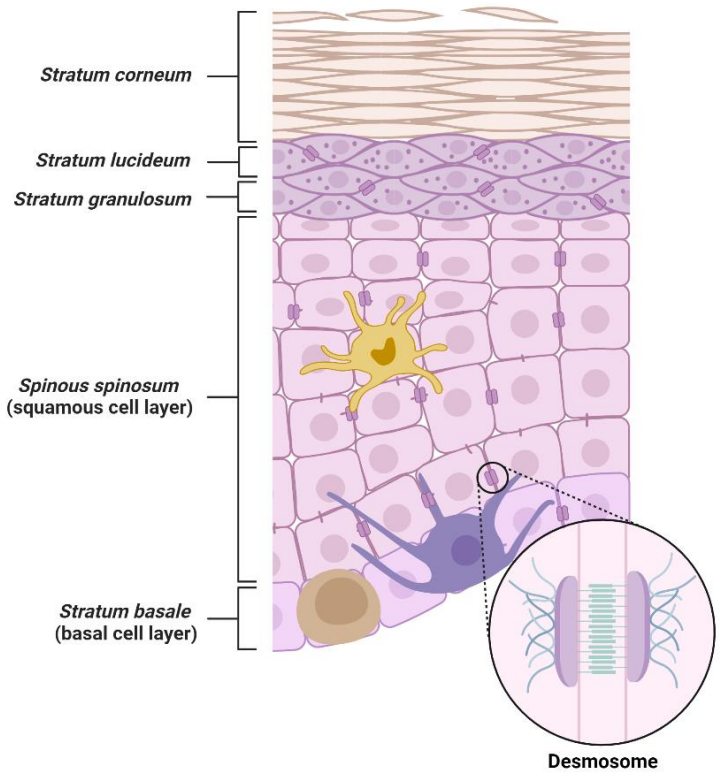


Figure 2. Schematic representation of epidermal layers (produced with BioRender)

The *stratum basale* (basal cell layer) or *stratum germinativum* is the innermost sublayer of the epidermis and it contains small round basal cells that are continually dividing and pushing existing and old cells to a higher layer, where they eventually shed[13; 14]. This sublayer also contains melanocytes that are responsible for producing the dark skin pigment, melanin[9]. It is stored within specialized organelles called melanosomes[14]. These melanosomes are

transported along the dendritic processes of melanocytes to adjacent keratinocytes, where they form a shielding barrier, an umbrella-like cap over the nucleus[14]. Melanin production is triggered by UV radiation exposure as a response to increase its function of protecting deeper skin layers, resulting in the darkening of the skin, also known as a suntan[11; 13].

The *stratum spinosum* (squamous cell layer), the next sublayer and the thickest one, consists of cells held together by adhesive intercellular junctions, the desmosomes[15; 16]. The basal cells that were pushed upward into this sublayer have become more mature and flatter and are now called keratinocytes or squamous cells[13; 17]. Keratinocytes produce precursors of the skin barrier, such as keratin, a protective protein essential to the structure of the skin, hair, and nails, filaggrin and lipids that will eventually "seal" the skin surface[13].

Right above is the *stratum granulosum* and *stratum lucideum*, respectively[9]. Here, the cells become bigger, less round with a flatter shape, start to lose their nuclei, have microscope-visible granules, and secrete lipid into the intercellular spaces[9; 14; 15]. Eventually, they become dehydrated and die[13].

The most superficial sublayer, the visible, thin, and heterogeneous one is the *stratum corneum* (SC), which is composed of several laminated, fully differentiated, nonnucleated and loosely attached keratinocytes called corneocytes[9]. Corneocytes are stronger, tightly linked dead but undamaged cells that protect from abrasions, light, heat, and pathogens and are the principal barrier of epidermal coating[11]. This sublayer is composed of those corneocytes entrenched in a lipoprotein matrix that has been compared to a "brick and mortar system", where the "bricks" resemble corneocytes and the "mortar" is the lipid environment surrounding them[18; 19]. The SC gives an essential protection to the underlying layers, thus being the principal barrier for penetration of most drugs[20; 21].

2.2. Dermis

The dermis, located underneath the epidermis, has a thickness of 3-5 mm, making it the major component of human skin[9; 21]; this layer encompasses a network of capillaries that connect to the systemic circulation[21]. It has origin from mesoderm and anchors cutaneous structures like hair follicles, nerves, sebaceous, apocrine, and eccrine glands and blood and lymph vessels[9]. Blood vessels and nerves can allow sensations of touch, temperature, and pain[9]. This layer also contains abundant fibroblasts, producers of collagen and elastin, macrophages and immune cells that participate in many physiological responses in the skin[9; 11]. The main functions are temperature regulation, to supply the epidermis with nutrient-saturated blood and to store most of the body's water[13].

This layer is hydrophilic due to its composition, a property that does not significantly block the transport of substances[20]; however, it can be a barrier for highly lipophilic molecules[20]. The cutaneous structures which have their origin in dermis can also originate a "shunt" pathway for some permeants[20]. These appendages provide additional opportunities for drug delivery through the skin and can contribute to the overall permeation process[20; 21].

The dermis layer is made up of two sublayers, the upper papillary dermis, and the lower reticular dermis[9]. The papillary layer is the one that contacts with the epidermis and contains collagen fibers[13]. This sublayer supplies nutrients to select layers of the epidermis and regulates temperature, due to the extensive vascular system[13]. The reticular layer is thicker because the collagen fibers are arranged in parallel[13]. Furthermore, it exhibits greater density than the papillary dermis, enhancing skin integrity by providing structure and elasticity [13].

2.3. Hypodermis

The hypodermis, subcutaneous layer or subcutis is the deepest layer of the skin and lies below the dermis and above the underlying muscle[14]. Hypodermis contributes to fat storage as an energy reserve of the body, temperature regulation, shock absorption, structural support and protecting the inner organs[13; 14]. The structure of subcutaneous tissue varies across individuals and its thickness exhibits differences depending on body location, gender and age and can be an indicator of an individual's nutritional status[14].

This layer consists of two distinct types of connective tissue: loose connective tissue, also referred to as areolar tissue, and adipose tissue[22]. The main cells are adipocytes that are arranged in lobules and separated by connective tissue septa[14; 22]. Most of the interstitial space is situated within these septa[22].

Subcutaneous administration uses the subcutaneous layer and is a common route used to administer various types of drugs given its high bioavailability and fast onset of action[23]. This route allows for efficient drug absorption into the bloodstream[23]; however, one potential drawback is the sensation of pain or discomfort at the injection site that can negatively impact patient adherence[23].

2.4. Accessory Structures

Apart from skin layers' complexity described above, this organ also contains various accessory structures that contribute to its functionality: hair, sebaceous glands, sweat glands (both eccrine and apocrine) and nails[24; 25]. Drug molecules often take advantage of these structures as a bypass into the deeper layers of the skin, circumventing the SC barrier[20].

Externally, hair is composed of thin and flexible tubes of keratinized epithelial cells[26]. Beneath the skin's surface, the hair follicles (cylindrical extensions of epithelial tissue)

correspond to invaginations of keratinocytes and melanocytes that have extended into the dermis and even the hypodermis[26]. The primary roles of hair follicles include protection of the skin against mechanical harm, to facilitate thermoregulation and contribute to sensory function, by enhancing the perception of its surface to *tactile stimuli*[26].

Sebaceous glands are microscopic exocrine glands that produce an oily or waxy substance called sebum, which serves to moisturize both the skin and hair and grants permeability to the epidermis[24]. These glands are derived from epidermal cells and often connected to hair follicles[24; 25].

Sweat glands are subcategorized in two main types of sweat glands: apocrine glands and eccrine glands[24]. Apocrine glands are found primarily in the axillae and genital regions and their ducts are positioned within the hair follicle, just above the sebaceous gland openings[25]. Eccrine glands are distributed throughout the body and are crucial for regulating body temperature through the production of sweat and its evaporation from the skin surface helps dissipate excess heat[25].

Nails, composed of densely packed keratinized layers with approximately 0.3 to 0.5 millimeters thick, serve as protective covers for the fingers and facilitate grasping and tactile sensitivity[24].

3. Skin Cancer: Incidence, Types and Conventional Therapy

3.1. Incidence of Skin Cancer

Skin Cancer, or Cutaneous Carcinoma is a pre-eminent global public health problem, representing the most common type of malignancy in humans, particularly in the Caucasian population[1]. Over recent decades, the cases of all forms of skin cancers have been increasing across the world. Individuals with fair skin are affected the most as the biggest risk factor is chronic UV exposure[27]. According to the International Agency for Research on Cancer (IARC), the number of new melanoma and non-melanoma cases, in 2020, both sexes and all ages, was, respectively, 324 635 and 1 198 073[28].

This type of cancer really represents a particular challenge for estimating incidence, so it is very important to have caution while interpreting[28; 29]. Nowadays, we still have a limited quality and coverage of cancer data worldwide mostly in low- and middle-income countries, where the lack of resources lead to poor data sets[28]. Moreover, there are several sub-types of skin cancer that can present problems when collating data[29]. For instance, non-melanoma skin cancer is often not tracked by cancer registries, or sometimes the registrations of this cancer are often incomplete because most early cases are successfully treated via surgery or

ablation[29]. Due to these factors, it is very likely that the global numbers reported by IARC in 2020 are underestimated[29].

3.2. Types of Skin Cancer

Although genetic factors have a lot of influence on skin cancer risk, nearly all skin cancers are related to UV exposure, which is the most common modifiable risk factor[30]. UV radiation causes skin cancers without any additional initiators or promoters, thus is designated as a "complete carcinogen". The mutagenic and carcinogenic effects of this radiation are related to the induction of DNA damage and errors in repair and replication. Long-term and continual exposure to UV radiation leads to a progressive deterioration of cutaneous structure and function. Actinic damage has the potential to initiate a multistep process in which mutations are induced and not intervened by immune surveillance. These aberrant mutations lead to fast multiplication of skin cells which in turn gives rise to several forms of malignant tumors. The type of skin cancer that can affect a person is determined by where the cancer begins and its clinical behavior[31].

Skin cancers are classified into two main categories, non-melanoma skin cancer (NMSC) and cutaneous melanoma (CM)[32]. While CM begins with the transformation of melanocytes, NMSC occurs from other epidermal cells[33]. NMSC can be subdivided into two main types, depending on the originating epidermal layer: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (**Figure 3**)[33]. BCC and SCC are the most common types of skin cancer, often related to dangerous sun exposure[32]. However, there are a lot of types of NMSC, for example, rare tumors involving Merkel cells (Merkel Cell Carcinoma) and sebaceous glands (Sebaceous Carcinoma)[32].

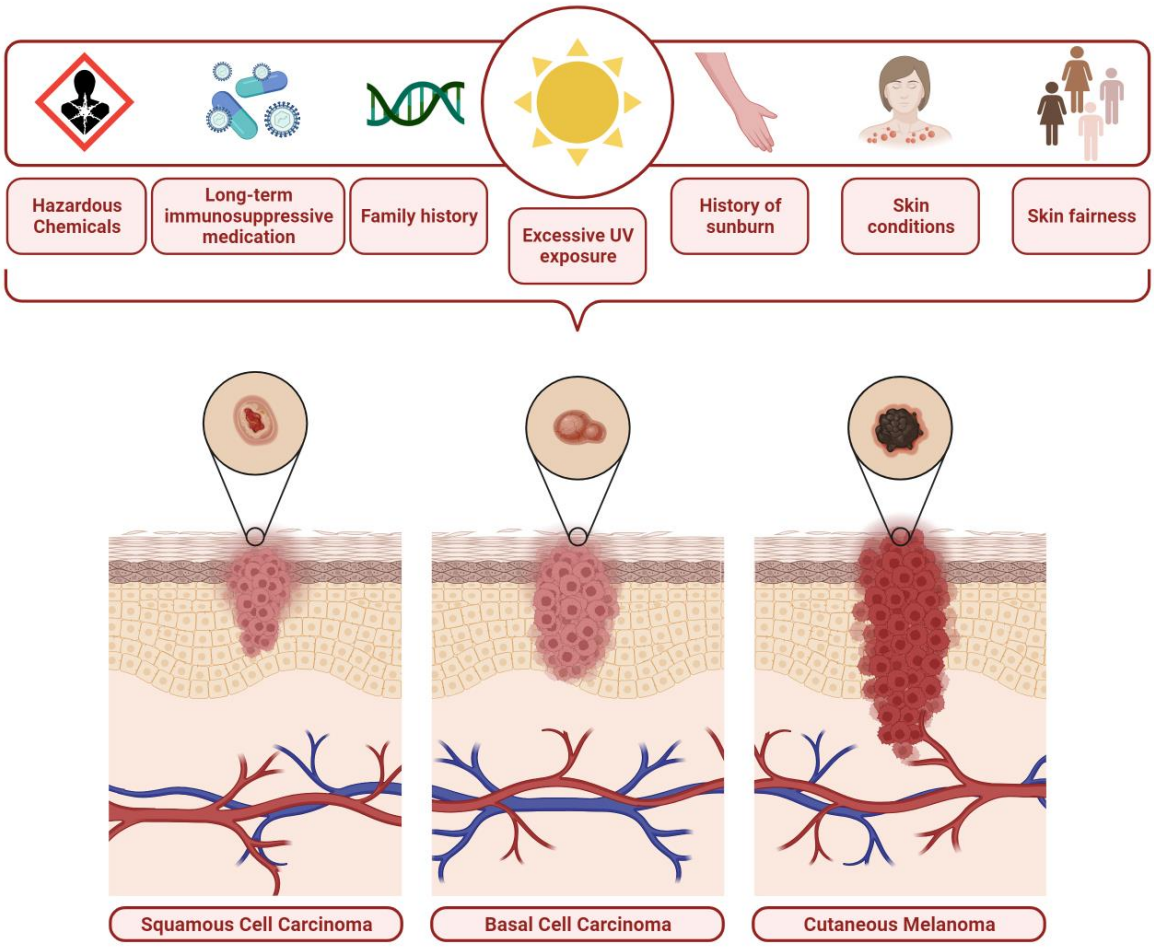


Figure 3. Schematic Representation of Skin Cancer Risk Factors and Different Types of Skin Cancer (produced with BioRender)

3.2.1. Basal Cell Carcinoma

Basal cell carcinoma starts in the basal cells and is the most common form of skin cancer, making up about 80% of all cases. Rates of BCC have been increasing and the experts believe this is due to more sun exposure, longer life expectancy, and better skin cancer detection methods[32; 34].

These cancers usually develop in areas more exposed to the sun, such as the face, head, ears, shoulders, and neck. BCC tends to grow slowly; it is mostly curable and causes minimal damage when caught and treated early. It is very uncommon for a BCC to spread to other parts of the body. However, if it is left untreated, can become locally invasive, growing wide into nearby areas, namely bone or other tissues beneath the skin. In rarer occasions, this variety of BCC can pose a life-threatening risk. Furthermore, people who have had this type of cancer are more prone to reexperiencing it in other places[32; 34].

3.2.2. Squamous Cell Carcinoma

Squamous cell carcinoma, as the name indicates, originates in squamous cells and is the second most common form of skin cancer, consisting of about 20% of all skin cancers[34]. This type of cancer also usually appears on sun-exposed areas of the body where the skin often reveals signs of sun damage (wrinkles and age spots) and can develop in scars or chronic skin sores elsewhere. SCC can sometimes grow very fast and metastasize if not detected and treated early and it can develop from precancerous skin spots, known as actinic keratosis. It is also more likely than BCC to invade deeper skin layers and metastasize[32; 34; 35].

3.2.3. Cutaneous Melanoma

Despite being the less common, CM is the most dangerous of the three types of skin cancer as it tends to spread to other parts of the body. Melanoma encompasses only about 1% of all cases but is responsible for the large majority of skin cancer mortality[34]. Nevertheless, if caught and treated early, it can be curable[32]. CM develops when melanocytes undergo malignant transformation, triggered by intense, prolonged sun exposure, and can emerge on any region of the skin, even in areas that are not usually exposed to the sun[36]. Moreover, when there is advancement into the skin's deeper layers, melanoma reaches the bloodstream, subsequently causing metastasis to healthy organs [37].

3.3. Skin Cancer Conventional Therapy

Surgery is the primary treatment for most skin cancers at an early stage; this may be the only treatment option necessary if it successfully removes the cancer in its entirety[13]. While effective, surgical excision is many times undesired for cosmetic purpose since it can compromise the skin integrity. When the cancer starts to grow into deeper parts of the skin, or even metastasize, more extensive treatments are required, such as chemotherapy, immunotherapy, radiation therapy and targeted therapy[38]. These treatment strategies require careful consideration of the risks and challenges involved (**Figure 4**). Adverse side effects such as, diarrhea, nausea and vomiting, alopecia, anemia, thrombocytopenia, fertility issues, delirium, sleep problems, infections, pain, organ inflammation, urinary and bladder problems, and even more, are most feared from the patient's point of view as it can completely disrupt their standard of living. In addition, the multidrug resistance is another obstacle in the management of tumors, which leads to an escape from the effects of chemotherapeutic drugs[5].

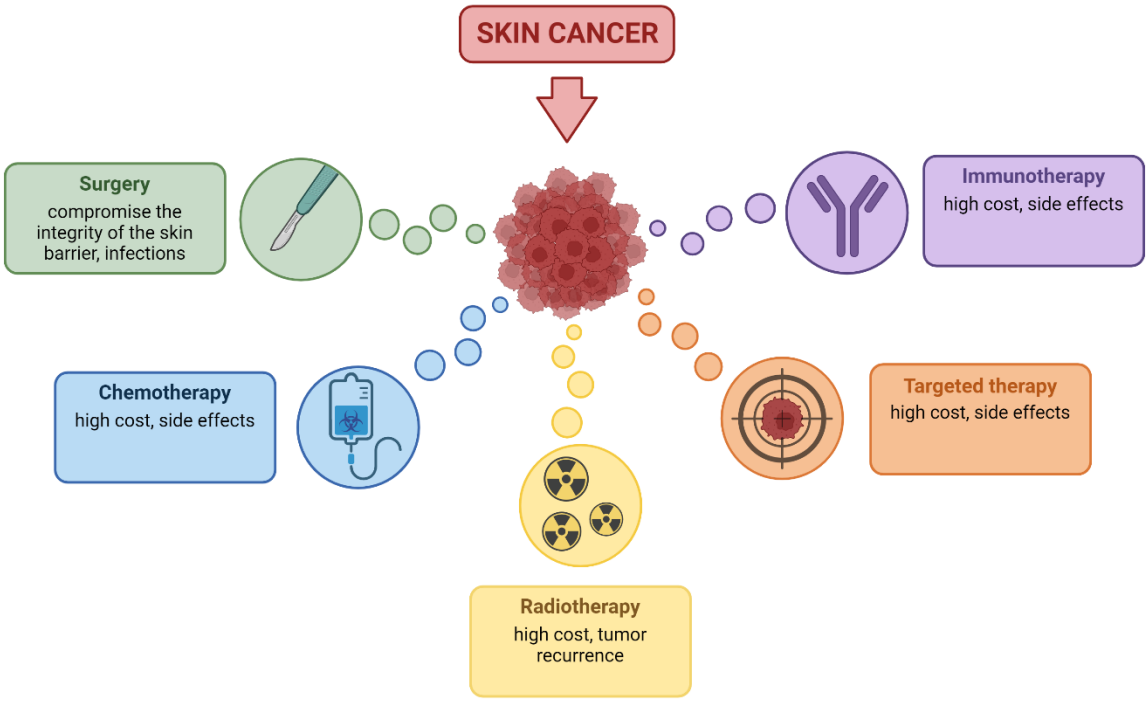


Figure 4. Diagrammatic representation of conventional treatments for skin cancer (produced with BioRender)

Furthermore, the great majority of these therapies are administered via IV route, which despite having a lot of advantages including rapid-acting directly into the general circulation, 100% of bioavailability, more predictable absorption, being the emergency route and a safe way to administer multiple medications to a patient, it also has its disadvantages: in older people IV route can be a challenge due to comorbidities and age-changes related to the skin and veins; it is usually painful at the injection site; it can cause irritation; there is a high risk of bacterial contamination, so aseptic conditions are a priority; self-medication is not possible due to the inexperience of the patient; the drug cannot be removed when injected, meaning that a high drug concentration can be fatal; only aqueous solutions of the drug can be administered so preparations like oily solutions and aqueous suspensions cannot be given through this route as they may cause an embolism; if extravasation is accessory to the drug administration, thrombophlebitis and venous thrombosis of the vein in which drug is injected and tissue necrosis around that particular vein can occur; it may induce hemolysis if the drug is administered too rapidly[2; 39].

4. Topical and Transdermal Administration

Due to the many drawbacks associated with the IV route, alternative options have emerged and are a constant source of interest, with a goal to avoid unwanted secondary effects and increase the localized therapeutic effect[18]. In particular, the topical and transdermal routes (**Figure 5**) have been proposed and shown as promising alternatives for skin cancer therapy admnistration[40; 41].

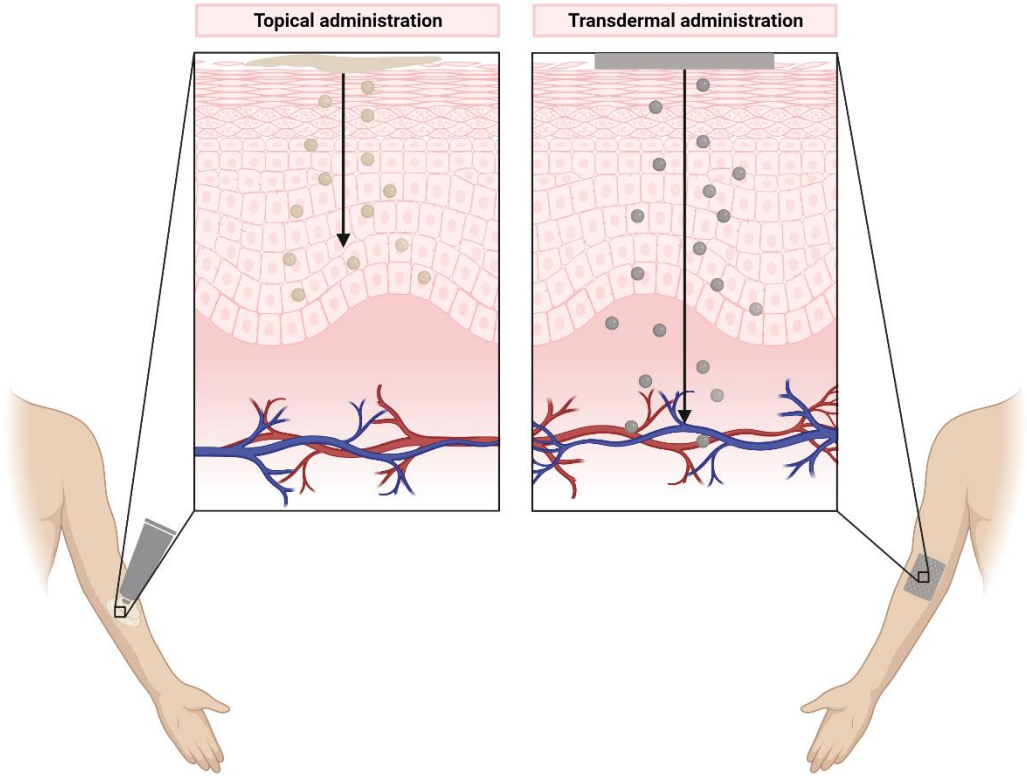


Figure 5. Illustrated difference between topical and transdermal administration (produced with BioRender)

Drugs can be applied directly onto the skin to generate either a local (topical delivery) or a systemic effect (transdermal delivery). Topical delivery systems are typically formulated to target the precise local tissue, whereas transdermal delivery systems are specifically designed to achieve systemic absorption of the drug. There are a lot of clinical benefits using these routes, over conventional routes of drug administration, including:[42]

- Provide effectiveness in low and continuous doses, reducing dosing frequency[43].
- Self-administration is a possibility and can facilitate in cases of age limitations, being a poor surgical candidate and individuals with cosmetically sensitive areas who wish to prevent scarring[43].
- Avoid drug degradation due to the first pass metabolism and interactions in the intestine and liver[20].

- Avoid variables that could affect drug absorption like pH changes, presence of enzymes and drug-food interaction in gastrointestinal tract[20; 44].
- Good patient compliance and acceptance (no needle phobia, no need to swallow pills)[20].
- Ease of use and convenience of application[44].
- Non-invasive methods of drug delivery[44].
- Formulation can be easily removed in the event of acute toxicity[20].

4.1. Drug Permeation of the Skin

As mentioned before, SC is the skin permeability barrier to most substances, and it is considered the rate-determining step for drug diffusion in topical and transdermal administration. This layer's lipidic composition and distinctive structural organization result in low permeability of most drug molecules[20; 21].

Drug transport from topical formulation into the skin entails both partitioning and diffusion processes. In the case of transdermal formulations, there is an additional convective transport which facilitates the delivery of solutes from the dermis into the systemic circulation. The initial step is the partitioning of the drug molecules in contact with the skin as they will permeate into the epidermis and diffuse deeper into the subsequent layers, while the remaining drug molecules in the formulation will redistribute to take their place. This will be favorable for some specific cases like lipophilic molecules. Therefore, there are some requirements for drugs to be applied through the skin that include the following[20; 42].

- High potency (dose <10 mg/day)[20].
- Moderate lipophilicity (log P = 1 – 5) so that the molecules can pass both lipophilic and hydrophilic layers of skin[20; 21].
- Low molecular weight (less than 500 Da) to be easier to penetrate through the dense membrane via passive diffusion. Smaller molecules are more likely to diffuse at higher rates compared to larger molecules[21].
- Low melting point (<250°C) because it reflects the noncovalent interactions between drug molecules that are related to drug solubility in the SC[21].
- Uncharged molecules since the ionized ones can only pass through appendageal route, which means that transportation amount would be small compared to other pathways. Ionized molecules partition less than uncharged molecules[21; 42].

It is believed that drug molecules are conveyed through the SC by passive diffusion via three possible routes, paracellular (intercellular), transcellular, and appendageal (transfollicular) (**Figure 6**).

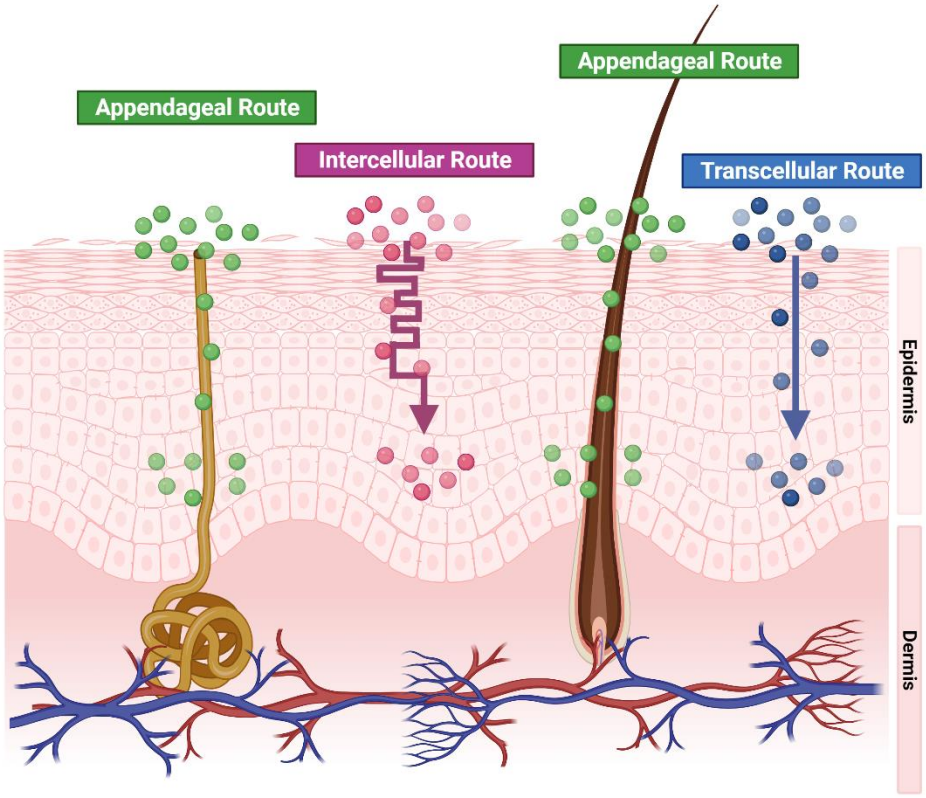


Figure 6. Schematic representation of skin permeation routes (produced with BioRender)

4.1.1. Paracellular/Intercellular Route

The paracellular route leads around corneocytes through the lipid matrix, although not homogeneous, it is the only continuous phase in the SC. This lipid route is the principal pathway for drug molecules that can pass through the gaps between cells (small molecules). Nonetheless, the pathlength is significantly greater than the SC thickness[20; 21].

4.1.2. Transcellular Route

The transcellular route is another possible route, and it results in thorough crossing of the SC involving transport of molecules through keratinocytes with hydrated keratin-filled cell interiors and the lipid matrix, mainly composed of fatty acids, ceramides, and cholesterol. This pathway, comprised of sequential partitioning of molecules between the hydrophilic and lipophilic portions of SC, facilitates penetration for hydrophilic drugs[21; 45].

4.1.3. Appendageal Route

In the appendageal route, drug molecules may bypass the SC by taking the "shunt" route through skin appendages, such as sweat ducts and hair follicles. Besides being the shortest route, the area of these appendages is significantly small, representing 0.1% – 1% of skin absorption. This is a limitation for the use of this pathway for most drugs; however, it can serve as a promising pathway since the hair follicle can be considered a route for systemic

drug delivery. These appendages also can be used as a repository of micro and nanoparticles (NPs), which facilitate the gradual and continuous release. The most critical parameters that impact follicular targeting are particle size and fluidity of the nano- or micro-carriers[20; 21; 41; 45].

4.2. Topical and Transdermal Formulations

The great majority of topical products exist in the form of semi-solid formulations, such as creams, ointments, gels, and pastes, liquid dispersions including solutions, suspensions, emulsions, and lotions or solid powders in certain cases. Transdermal products are mainly formulated as transdermal patches, which allow for controlled release of the drug[42].

The design of a topical formulation must consider its material composition and how it affects critical parameters for effective excipient functionality. These parameters include drug stability and homogeneity, skin affinity, controlled release of the active pharmaceutical ingredients (APIs), maximized local delivery and minimized systemic delivery, and low toxicity. Topical formulation excipients play crucial functionalities as solvents, emollient agents, emulsifiers and surfactants, thickening/gelling agents, permeation enhancers, humectants and more (**Table 1**). Some excipients used in topical drug products may have multiple functionalities[46].

Table 1. Excipient Functionalities in Topical Formulations

Function	Description	Example	References
Solvent	Facilitate the dispersion and/or dissolution of APIs.	Purified water, Propylene glycol, Mineral oil	46
Emollient	Main structure-forming materials for semi-solid formulations. Enhance skin pliability and softness, usually not compromising the skin's protective barrier.	Paraffin, Stearic acid, Carnauba wax, White wax, Yellow wax, Cetyl alcohol, Lanolin-derivates	46; 47; 48
Emulsifier/Surfactant	Reduce surface or interfacial tension, serving as stabilizers for emulsions and suspensions. Facilitate the dispersion of a liquid within a vehicle that is immiscible. Improve the solubility of hydrophobic substances.	Lecithin, Polyethylene glycol, Polysorbates, Poloxamers, Propylene glycol monostearate, Diethylene glycol monoethyl ether	46; 47; 49; 50
Humectant	Promote water retention within the system.	Glycerin, Propylene glycol, Sorbitol	46
Thickening/gelling agent	Increase viscosity. Main structure-forming materials for gels.	Carbomers, Hydroxypropyl methyl cellulose, Methyl cellulose, Hydroxypropyl cellulose, Gelatin, Sodium	46; 47; 51

	Enhance stability of the formulation, to achieve optimal cutaneous delivery of the drug.	carboxyl methyl cellulose, Carrageenan, Colloidal silicon dioxide, Guar gum, Gellan gum, Xantham gum, Sodium alginate, Chitosan,	
Permeation enhancer	Enhance permeability by promoting drug diffusion, partitioning, or solubility across the SC. Decrease the barrier resistance, reversibly to facilitate skin penetration.	Propylene glycol, Ethanol, Isopropyl Alcohol, Oleic acid, Polyethylene glycol	46; 51
Chelating agent	Forms complexes with metal ions to decrease degradation catalyzed by metals and to amplify the preservative effect.	Ethylene diamine tetraacetate	46
Antioxidant	Protect the components of the formulation from oxidative degradation.	Butylated hydroxyanisole, Ascorbyl palmitate	46; 50
Preservatives	Increase product's shelf life, protecting it against microbial attack.	Methyl paraben, Propyl paraben, Benzoic acid, Sorbic acid	47; 50
Buffering agent	Adjust and stabilize the pH of the formulation.	Citric acid, Sodium hydroxide, Trolamine	46

For this work it is helpful to better understand specific formulations and the excipients that constitute them. Further formulations discussed in later sections will be based on emulsions, gels, creams and bioadhesive films.

4.2.1. Emulsions

Emulsions are colloidal systems that form a biphasic dispersion of two immiscible liquids stabilized by an interfacial layer of emulsifiers like amphiphilic surfactants, and a co-surfactant that improve surfactant's performance[52; 53; 54]. Emulsions form when the two immiscible phases are exposed to a shearing mechanical force. The methods to induce shear force for emulsion creation can be categorized as high and low energy approaches. High energy methods involve using a device to apply shear force, while low energy methods leverage the alteration of physical properties of each liquid phase and the chosen surfactants through thermal or chemical energy in the system[55]. Emulsions can be described as water in oil (W/O) where the preferable principal surfactant is oil soluble (hydrophilic-lipophilic balance (HLB) less than 10) or oil in water (O/W) that is favored if the principal surfactant is water soluble (HLB more than 10)[54; 56; 57]. Moreover, they can arrange in more complex systems, the multiple emulsions like water in oil in water (W/O/W), which the inner water phase is dispersed within an oil phase, which is then distributed inside a single system's bulk aqueous phase[56].

4.2.2. Gels (Hydrogels and Thermogels) and Creams

Another important formulation are gels which are soft semi-solid materials composed in great majority of a solvent and gelling agent[58]. Relevant to this work, hydrogels consist of a series of relatively hydrophilic polymers that form a 3-dimensional cross-linked network that swells when exposed to aqueous liquids, because they absorb and retain a large amount of those liquids[59; 60]. Hence, they have a water-rich composition and are biocompatible[61]. This network enables superior drug dissolution and facilitates drug release compared to creams or ointments due to its higher aqueous content. However, hydrogels are limited when incorporating hydrophobic molecules[50]. A polymer solution can be formulated and permitted to undergo through gelation. The gelation process can be achieved through photopolymerization, chemical crosslinking, ionic crosslinking, or in response to an environmental stimulus like alterations in temperature, pH, or ionic strength of the surrounding medium[62]. Thermo-responsive gels or thermogels are a type of hydrogels made of polymers that react to changes of temperature, altering the interactions between hydrophilic and hydrophobic components, resulting in a phase transition from a solution to a gel state[59].

Creams are semi-solid emulsions that, similarly to gels, feature a thickener to increase its viscosity and offer better spreadability[63]. The oil-phase present in the formulation means that creams are more emollient and can incorporate hydrophobic drugs better than gels.

4.2.3. Bioadhesive films

Bioadhesive drug delivery systems are becoming very prevalent on the market[64]. Bioadhesive films are film platforms with the unique ability to adhere themselves to biological tissue surfaces and are composed of natural polymeric materials with mucoadhesive and rigid properties. They are biocompatible and biodegradable and facilitate controlled drug release[65]. When compared with other formulations, bioadhesive films represent an improvement because they can remain at the pathological site longer, have more dosage flexibility, do not leave a greasy sensation upon application[65; 66].

5. Nanotechnology

Nanotechnology harnesses the potential of manipulating matter at the size range of 1 to 1000 nm[7]. At this scale, the structures, materials, and particles employed in nanotechnology possess distinctive physical, chemical, and biological attributes. These unique characteristics serve as invaluable resources in the realm of nanomedicine, enabling the development of novel

components for engineering systems used in diagnosing and treating diseases such as skin cancer[67].

As outlined above, the complex structure of human skin poses a challenge to the delivery of the drug directly to tumor cells. The skin barrier may significantly compromise the expected therapeutic effect[18; 40]. Since many molecules do not have the ideal physicochemical criteria, the therapeutic quantity that penetrates the skin is not enough. The topical and transdermal routes are significantly hindered by this phenomenon; however, due to the great effort to investigate and develop innovative strategies, new technologies have been discovered, thus maximizing the quantity that can cross the skin barrier[68; 69]; for instance, nanocarriers have been highly considered as a permeation enhancing strategy[41]. NPs offer significant advantages over conventional delivery systems because they can:

- Prospect a continuous, direct, and controlled drug release to the target cells with facilitated cellular uptake[70].
- Enhance skin barrier penetration and permeation[69].
- Increase the solubility of highly hydrophobic drugs[70].
- Improve colloidal stability with an ideal repulsive behavior to prevent aggregation[70].
- Reduce off-target toxicity[69].
- Improve bioavailability of drugs[69].
- Enhance *in vitro*/*in vivo* stability of therapeutic substances by chemical or physical methods, increasing the efficacy of the therapeutic[70].

5.1. Nanoparticle Systems

The design and preparation of the NP systems rely on having definitive properties to better execute their function as effective drug carriers. Researchers must optimize the formulation material composition and fabrication process to achieve particles with ideal properties. Particle size (PS), polydispersity index (PDI), zeta potential (ZP) and entrapment efficiency (EE%) are usually determined to evaluate the potential of the NP systems[37; 59].

Nanocarrier particle size is a critical parameter to be considered for successive passive targeting to the skin, via potentiating skin penetration and deposition. A nano-size range smaller than 200 nm is necessary for effective function. Moreover, the EE% or encapsulation efficiency is utilized to assess the efficacy of the NP in vehiculation/vesiculation of the drug molecule. It is defined by the concentration of the incorporated drug detected in the formulation over the initial concentration used to make the formulation. Higher EE% represents superior potential of drug delivery systems or better production procedures[37; 59; 71].

In relation to the systems' dispersion stability, PDI is used to describe the degree of heterogeneity of a size distribution of particles. As PDI value becomes closer to zero, the particles became more homogeneous, hence a PDI smaller than 0.3 will evidence that there is uniform distribution and homogeneity in the system. ZP is a physical property which is exhibited by any particle in suspension. This translates into the electrical charge on the surface of the particles which can be related to their ability to stay suspended without aggregating or sediment. Essentially, the higher the absolute value of the ZP, the greater the repulsion between the suspended particles[72]. For effective stabilization of a nanodispersion, an absolute ZP value of 30 mV should be targeted[73]. The large ZP value ensures the system's dispersion stability and reduces the possibility of aggregates forming or an increase in PS, but the ZP value is not an absolute measurement of NP stability[74]. Also, high absolute value of ZP is important for stabilization of emulsions, to avoid coalescence[57].

Relevant to this research, it is worth focusing on the three main groups of NPs that have distinguishing characteristics for pharmaceutical applications: lipid-based, polymer-based and inorganic NPs (**Figure 7**). Additionally, nanoemulsions and nanoemulgels correspond to nano-based topical formulations.

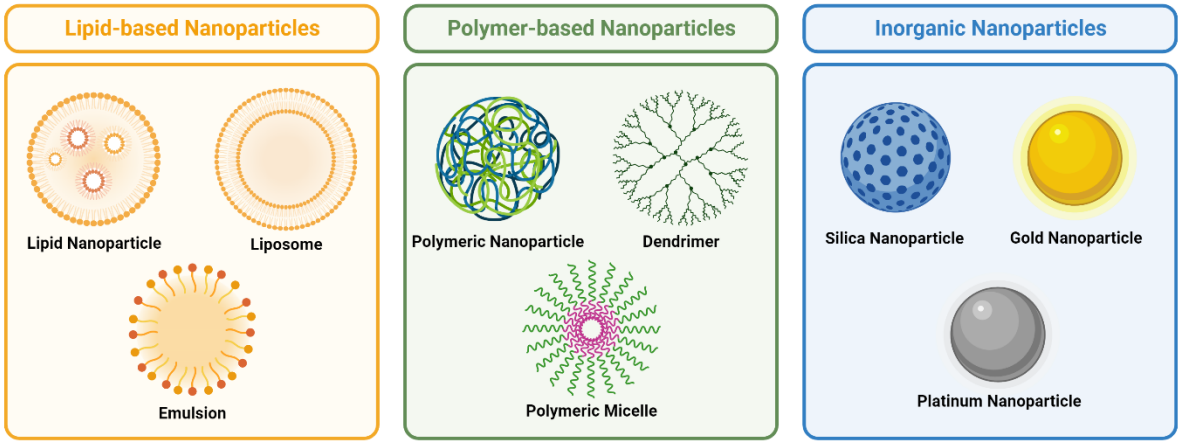


Figure 7. Schematic representation of different types of nanoparticle systems (produced with BioRender)

5.1.1. Nanoemulsions and Nanoemulgels

Nanoemulsions (NEs) are a type of NP system involving finely dispersed droplets spanning from 10 to 1000 nm, having great promise as topical formulations, allowing for effective drug delivery. The production of NEs requires a two-step procedure: initially generating coarse emulsions, followed by the utilization of high-pressure homogenization or ultrasonication to break down larger droplets into nano-scale dimensions[55].

Compared to traditional emulsions, NEs offer a lot of benefits, such as greater interfacial area, quicker absorption, unique rheological characteristics (reduced viscosity), the

potential to enhance drug solubility, thus amplifying bioavailability[56; 72]. NEs also exhibit a safety toxicological profile, high content of lipid phase and the possibility to be produced in a large-scale[53]. These formulations are inherently thermodynamically unstable systems that tend to phase separate over time[55]. However, when stabilized by surfactants, this separation process is significantly delayed, resulting in emulsions that are kinetically stable[55]. This characteristic holds particular significance in the realm of emulsion nanomedicine, where any alterations to a formulation over time could pose serious consequences for patient well-being[55].

To help mitigate these concerns and facilitate skin application, it is common to introduce cross-linking agent to transform the formulation into a gel-like consistency, resulting in nanoemulgels (NEGs). This NE-based topical gel formulation can be W/O or O/W type, that has been incorporated into a gel base[51; 75]. The preparation of the gel base involves the use of a gelling agent that modify the NE into a non-greasy, more stable, and with a higher viscosity formulation[51].

The fusion of the NE-system with the gel base overcome the drawbacks inherent in both NE and hydrogel[50]. The incorporation of the drug within the oil phase of NE followed by its incorporation into the gel base make it possible to integrate hydrophobic molecules into a hydrogel, while enhancing the NE's viscosity at the same time[50; 76]. Thus, the preparation of NEGs emerges as a solution to the solubility-related issues of the drug and acts as a system that ensures controlled release of drugs, enhancing the pharmacological effect at the administration site and reducing adverse reactions[51]. Moreover, due to its greater adhesive characteristics on the skin surface and high capacity to dissolve drugs, NEG formulation causes a more pronounced concentration gradient towards the skin, providing a better skin penetration[50]. The drug entrapment capacity of NEG is also elevated because of the extensive network of the gel[50]. Nonetheless, NEGs have limitations, such as entrapment of bubbles during the incorporation of the NE into the gel matrix and lack of stability of its NE phase for a longer period[50; 51].

5.1.2. Lipid-based nanoparticles

Lipid-based NPs represent unique carrier systems that contain two primary structural configurations: those featuring a lipid monolayer (solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs)) and those comprising a lipid bilayer (liposomes, niosomes, ethosomes) [7]. These lipid-based systems further incorporate distinct cores: solid lipids for SLNs, liquid and solid lipids for NLCs, and aqueous core for liposomes, niosomes, ethosomes, wherein the drugs are either dissolved or dispersed[7; 77; 78].

All these nanoparticles emerged as the optimal choice for carrier systems because of their lipidic structure derived from physiologic and biodegradable lipids[7; 77]. The advantages of lipid-based NPs include controlled release mechanisms, easy formulation processes, compatibility with various substances, high drug loading efficiency (both hydrophilic and hydrophobic), and a notably safety profile[7].

5.1.2.1. Liposomes

Liposomes are lipid based shaped vesicular systems with one or more concentric phospholipid bilayer enclosing a hydrophilic aqueous core[37; 79; 80]. They are characterized as colloidal spherical structures formed by the self-arrangement of amphiphilic lipid molecules in solution, such as phospholipids, composed of a hydrophilic head group orientated towards both internal and external aqueous phase and a hydrophobic tail in between[80; 81]. This unique organized structure offers the capacity to encapsulate and transport molecules with different solubilities: hydrophilic molecules in the internal aqueous core, hydrophobic molecules incorporate into the lipid bilayer, and amphiphilic molecules at the interface between the water and the lipid bilayer[80].

Different phospholipids have been used in this system[82]. The careful choice of phospholipid type and concentration for the formulation holds significance in the development as these factors will impact the PS, EE%, ZP, stability, and penetration properties of the vesicles[82].

Cholesterol is a rigid steroid molecule that can also be incorporated into liposomes[83]. This incorporation may be important since it enhances the stability and EE% of drugs[82]. Also, it has an important role in preventing leakage and reducing vesicular permeability and vesicular fusion of drugs from vesicles, avoiding the premature release of therapeutics[7; 82; 83].

Liposomes stands out as powerful drug delivery systems because of their versatility structure, biocompatibility, biodegradability, non-toxic and non-immunogenicity nature, and protection of drug against enzymes degradation[78; 80]. They are considered to be the safest payload carriers due to the amphiphilic nature of phospholipids in solution that mimics natural cell membranes and allows great interactions between liposomes and mammalian cell membranes[37; 80]. Nonetheless, liposomes require complex and high-cost production methods, demonstrate low efficiency at entrapping drugs, pose challenges to perform production on large scales and have short shelf life and poor stability[78; 84].

5.1.2.2. Ethosomes

Ethosomes are spherical structures that feature soft and malleable lipid nanovesicles[85]. They are a modification of classical liposomes containing water, phospholipids and, in addition,

alcohol (ethanol, isopropyl alcohol, or polyol) in relatively high concentrations (20%-45%)[82; 85]. Their unique composition increases their drug-delivery potential and the capability to overcome the skin barrier, enhancing the permeability of drugs across the SC barrier[85].

There are three different types of ethosomes: classical ethosomes, binary ethosomes and transethosomes. Classical ethosomes (water, phospholipids and ethanol) were reported to be superior to classical liposomes, showing a smaller PS, a more negative ZP and a higher EE%[82]. Binary ethosomes were developed by adding isopropyl alcohol or other alcohol to the classical ethosomes[82]. Transethosomes contains the basic components of classical ethosomes but a penetration enhancer or a surfactant (responsible for disorganizing the intercellular lipids of the skin, improving penetration) is added to the structure[52; 82].

Ethanol acts as both permeation enhancer and stabilizer, contributes to flexibility of the vesicles and gives them unique characteristics in terms of PS, ZP, stability and EE%[82; 83; 85]. Ethanol plays a pivotal role in enhancing the drug's permeation rate, accomplished through its interaction with the polar head group of SC lipid molecules, lowering the melting point of the SC lipids[83]. Thus, it fluidizes the lipid bilayer of SC by intercalating into intercellular lipids, reducing the density of the lipid layer. As a result, the ethosomal vesicles gain the advantage of being soft and malleable, enabling them to efficiently penetrate the disorganized lipid bilayer of the SC[85]. The optimal concentration for ethosomes preparation has been reported to be around 40% because it produced a high EE% and permeation[83]. Nonetheless, increasing the ethanol concentration beyond the optimum level can result in a leaky bilayer, resulting in a severe decrease in the EE% and slight increase in the vesicle size. Also, increasing even more the concentration can cause vesicle solubilization[82].

Additionally, isopropyl alcohol also serves as a skin penetration enhancer, increases EE% and ZP and decreases PS. In a binary ethosome, the high EE% of the formulation can be attributed to the addition of isopropyl alcohol with ethanol[83].

5.1.2.3. Solid Lipid Nanoparticles

Solid lipid nanoparticles are an additional lipid-based carrier system to liposomes and NEs[78]. SLN have a spherical morphology and represent a colloidal dispersion containing non-polar lipids like steroids, mono-, di- or triglycerides, waxes and fatty acid that remain solid at room and body temperature, one or more surfactants which are usually non-ionic and/or cationic to enhance stability, and water[78; 86; 87; 88]. The major advantage of SLN lies in the use of solid lipid, which drastically reduces the mobility of incorporated drugs within lipid matrix, thus preventing coalescence of particles and minimizing drug migration to the emulsifier film[86].

The choice of the lipid and the surfactant as well as their concentration influence factors such as PS, long-term stability during storage, drug loading, and release behavior[78; 86]. The method employed in their preparation also holds a great significance, as it determines whether SLN can accommodate, hydrophilic or lipophilic drugs[7; 78].

Relative to other systems, SLN offer many benefits: ease of preparation, economical production, capacity for large-scale manufacturing, excellent physical stability, good release profile, chemical versatility, non-toxic lipid carrier systems, biodegradable lipid components[78]. Compared to liposomes, they have drug stability and prolonged release and are safer than polymeric NPs because its preparation is organic solvent free[78].

However, SLN exhibit some problems, including relatively low drug capacity for drug loading[89]. On long-term storage, SLN can go through crystallization that results in not only in a denser and more organized matrix but also in a shape modification, which creates an unfavorable localization for many drug molecules and can lead to the expulsion of encapsulated drug into the surrounding media[84; 87]. These challenges prompted the development of NLC[89].

5.1.2.4. Nanostructured Lipid Carriers

NLCs emerge as the second generation of lipid NPs, aiming to address the disadvantages of the first generation SLN[90]. NLCs are modified SLN with a lipid phase that incorporates solid (fat) and liquid (oil) lipids at room temperature and an aqueous phase containing surfactant[78; 87]. The mixture between solid and liquid lipids in a ratio of up to 70:30, respectively, forms an imperfect matrix with more spaces available for the drug and less ordered crystalline arrangement, which permits a more efficient drug loading and incorporation[87; 90]. The reduced crystallinity of NLCs avoids the potential expulsion of the drug during storage[84; 87].

5.1.3. Polymeric Nanoparticles

Polymeric NPs are simple and non-complex carrier systems that have the capacity to dissolve, disperse and encapsulate drugs, exhibiting a versatile drug delivery capacity[7]. Therapeutic agents can be encapsulated within the NP's core, entrapped within the polymer matrix, chemically linked to the polymer chains, or attached to the NP's surface[91]. This diverse range of approaches enables the transportation of various payloads, including both hydrophobic and hydrophilic drugs[91]. They can permit a good adhesion with a sustained release, a target action in the tumor and the protection of therapeutic moieties[7; 52]. All these parameters plus the biocompatibility, biodegradability, good shelf life, water solubility and cost-effectiveness, enable polymeric NPs to be good delivery vehicles[7; 92].

Polymeric NPs can be produced using either natural or synthetic materials, from monomers or preformed polymers[91]. This versatility enables the production of an extensive range of potential structures and characteristics[91].

5.1.3.1. Natural Polymeric Nanoparticles

Natural polymers are derived from animals or plants, so they do not contain any synthetic chemical, being biodegradable, biocompatible, and less toxic[93]. These include protein-based polymers like gelatin and polysaccharides such as chitosan[94].

One of the great advantages of the NPs based on naturally occurring polymers is their biocompatibility and cell-recognition[94; 95]. However, it is challenging to create these NPs with reproducible characteristics and drug delivery profiles due to the lack of batch-to-batch consistency and uncontrollable formulation characteristics[70; 93]. Natural polymers also face instability that leads to changes during storage and when compared with synthetic polymers, they have a shorter shelf life[93].

Chitosan is a linear polysaccharide derived from chitin, which can be found in crustaceans' exoskeleton and is one of the most used natural polymers in the synthesis of NPs[70]. Chitosan's biocompatibility, nontoxicity, noncarcinogenic, mucoadhesive and antibacterial properties have led to its utilization as a drug carrier[92; 94]. Among the advantages of this natural polymer, the NPs have a quick uptake by cells, facilitated by its positive surface amine groups, leading to an enhanced drug bioavailability[70; 94]. Chitosan-based nanoparticles have been the most extensively studied for topical drug delivery[70].

5.1.3.2. Synthetic Polymeric Nanoparticles

On the other hand, as the name indicates, synthetic polymers are synthetized in the lab through a lot of chemical reactions, either from natural polymers or only from synthetic monomers[93]. Compared to natural polymers, they have higher purity (uniformity of composition), stability and batch-to-batch reproducible[52; 93]. They are predominantly used to deliver lipophilic, small drug molecules[70].

One of the most used biocompatible and biodegradable synthetic polymers in polymeric NPs synthesis for drug delivery is Poly(lactic-co-glycolic acid) (PLGA) [96]. PLGA includes a family of linear copolymers that can be synthetized with different ratios of its constituent monomers: glycolic acids and lactic acid[97]. This versatile polymer has gained widespread popularity in pharmaceutical research due to its exceptional properties[59]. PLGA is known for its ability to form NPs with controlled and sustained drug release profiles, making it an ideal candidate for designing efficient drug delivery systems[59].

5.1.4. Inorganic Nanoparticles

Inorganic NPs are derived from diverse sources, such as metals, metal oxides, carbon, ceramics, silica, and more[7]. They are very stable with wide-range functionality and have grasp significant attention in the realm of oncology[7; 52]. The distinctive physicochemical characteristics inherent to them, marked by their small size, big surface area, bioactivity, biocompatibility, and capacity for functionalization, position them as optimal candidates for addressing skin cancer[7]. Researchers have also explored an incredible characteristic: inorganic NPs have inherent therapeutic potential, which makes them capable to combat cancer cells by themselves[7]. It was also studied that these NPs have a possibility of aggregation when applied on the skin, which would decrease penetration[52]. However, their surface can be altered by physical adsorption, covalent bonding, layer-by-layer assembly, ligand exchange, and in situ polymerization, which can enhance their potential to penetrate the skin[52].

6. Drug Repurposing

Classic drug discovery and development encompasses various stages that span an extensive timeline, from the initial identification of a potential drug to obtaining commercialization marketing approval[3]. This approach involves several sequential steps over many years, including, target identification, high-throughput screening, lead optimization, pre-clinical development, clinical trials, regulatory approval[98]. Furthermore, the pursuit of novel drugs demands huge capital investments and carries tremendous risk regarding failure to reach the market and business competition. The cost of failure impacts the industry's ability to bring to market innovative drug therapies for rare or chronic diseases, as companies struggle to overcome these challenges and meet their financial obligations. A valuable option in the field of new drug research is drug repurposing[4; 99].

Drug repurposing is an unconventional drug discovery approach that gives new applications for existing drugs. This concept is currently emerging to overcome the bottleneck constraints faced during traditional drug discovery and development in grounds of financial support, timeline, and resources. When compared to traditional pathway, drug repurposing is efficient, cost-effective, and less risky, as employing repurposed drugs that have undergone the research and development pipeline provides the current understanding of the pharmacokinetics, pharmacodynamics, dose, metabolic profiles, molecular pathways of the drugs, the mode of actions of the drug, different target interactions. Hence, less effort will be needed to characterize the drug's pharmacokinetic and toxicity profiles[6].

Successful instances of drug repurposing originally emerged based on anecdotal reports, *in vitro* and *in vivo* screening, fortuitous discoveries of positive effects during clinical trials or post-market analysis of patient health records[6]. A relevant example of this occurrence is for Minoxidil;[100] oral minoxidil was originally developed as a potassium channel opener, which caused hyperpolarization of cell membranes leading to vasodilatation. It was approved by the United States Food and Drug Administration (FDA) for arterial hypertension in 1979. In result of an observance of unwanted hair growth during the clinical trials, a topical formulation was developed and evaluated in to treat hair loss; in 1988, the FDA approved topical minoxidil for androgenetic alopecia[100; 101].

The prevailing goal for drug repurposing technologies is to make this process systematic, independent from anecdotal prognosis. Despite the accelerated timeline and available clinical and toxicological data, this process demands key investigative work for the new application of the drug. This research focuses on optimizing the formulation to consider a new dosing regimen, route of administration and drug molecule stability and reprofiling pharmacodynamic, pharmacokinetics and toxicity profiles in its novel indication[4].

7. Objective

The purpose of this review is to summarize and provide a critical analysis of recent literature evaluating repurposed drugs incorporated in nanosystems for topical delivery as skin cancer therapy alternatives.

8. Topical Drug Repurposing and Nanosystem Integration for Cancer Therapy

This section focuses on the review and analysis of publications from 2015 to 2023 that have investigated topical formulations featuring repurposed drugs with proven antitumor effect incorporated in nanotechnology-enhanced drug delivery systems as a targeted skin cancer therapy approach. Various drug therapeutic classes examined include antifungals (itraconazole), dyslipidemics (simvastatin), anthelmintics (niclosamide), antibacterials (doxycycline), disease-modifying antirheumatic drugs (DMARDs) (leflunomide), antidiabetic agents (metformin), and nonsteroidal anti-inflammatory drugs (NSAIDs) (celecoxib).

From the drugs listed, researchers have investigated their integration into the nanoparticle systems described before with the goal of improving topical formulations by enhancing drug delivery and its therapeutic effect against skin cancer. The analysis of the articles provides an overview of the designed formulation and nanoparticle system, followed

by the description of the drug release and skin permeation and retention profiles evidenced by *in vitro or ex vivo* experiments. It also summarizes the cytotoxic effect and cell viability by *in vitro* or *in vivo* assessment that record relevant targeted antitumor activity for skin cancer.

8.1. Itraconazole

Itraconazole (ITZ), a Biopharmaceutical Classification System (BCS) class II drug, is a broad-spectrum antifungal drug, belonging to the triazole class and has been clinically used for over 30 years[71; 85; 102]. This class of antifungal drugs inhibits lanosterol 14-demethylase, the enzyme that converts lanosterol to ergosterol[103]. This compromised ergosterol synthesis leads to inhibition of fungal growth and membrane disruption that increase permeability and anomaly fungal cell membrane integrity, changing membrane-bound enzyme activity[103; 104].

In recent years, scholars have demonstrated with a plethora of *in vitro*, *in vivo*, and clinical phase I/II studies that ITZ has an anticancer and cytotoxic effect, acting on the Hedgehog (Hh) pathway, for instance[71; 105].

Hh stands out as one of the primary signaling pathways frequently used during cellular development for intercellular communication[106]. This pathway holds an important role in orchestrating the organogenesis of almost all organs in mammals, as well as in regeneration and homeostasis, very recurrent processes in such a dynamic organ as the skin.[106] The disruption of Hh pathway has been observed in various types of cancer. Mutations in Smoothened protein (SMO) gene can result in abnormal activation of Hh pathway, leading to uncontrolled cell proliferation and tumor formation[106]. Notably, its crucial involvement in the development of BCC has been convincingly demonstrated by genetic mutations, mouse models of BCCs, and successful clinical trials of BCCs using Hh signaling inhibitors[106; 107]. Furthermore, the Hh pathway activity has also been reported to be involved in the pathogenesis of other skin cancers such as SCC and CM[107].

Regardless of the emergence of drug-resistant SMO mutations, ITZ has the capability to target this essential component and act as an Hh inhibitor[108]. Moreover, ITZ demonstrates potent suppression of neovascularization and angiogenesis, leading to reduced proliferation of endothelial cells and tumor vascularity. It is also capable of inducing auto-phagocytosis. Jointly, these motifs may grant repurposing of ITZ the potential to prompt targeted cutaneous apoptosis and tumor necrosis[71]. Despite its potential as an anticancer agent, ITZ does have certain limitations due to its poor physicochemical attributes, like large molecular weight (705.64 g/mol) and low aqueous solubility (1 ng/mL; at pH 7). These characteristics can hinder its effective delivery and distribution in the body when administered conventionally[71].

To overcome these challenges and enhance its efficacy in skin cancer therapy, topical formulations incorporating ITZ in nanocarriers have demonstrated favorable results. Through local application and nanovehiculation of the drug, ITZ concentration can be maximized in the affected areas, avoiding systemic side effects[71].

ITZ lipophilicity (log P = 5.66) is an important advantage in this context. Its lipophilic nature allows for easier incorporation within lipid carriers and facilitates penetration through cell membranes, potentially enhancing its ability to reach tumor cells effectively[71].

Carbone *et al.* (2018)[103] demonstrated the aforementioned ITZ anticancer potential with a topical SLN formulation. This innovative approach combined ITZ encapsulation within a Suppocire NB (NB) lipid matrix featuring a coating layer of didodecyldimethylammonium bromide (DDAB), a cationic lipid known for its pharmaceutical applications as coating material. DDAB has been recently reported to be effective against human glioblastoma cancer cells lines, thus enhancing the potential anticancer ITZ activity by synergistic effect. The coating layer of DDAB also contributed to increasing the stability without compromising the homogeneity and size of the NPs, which can enhance cell uptake.

The DDAB coating altered the NPs surface charge (ZP = +30.20 mV) enhancing their interaction with the cell membrane. This strong interaction, especially with the negatively charged phospholipids of the plasmatic membranes, facilitated the fusion of NPs with the cancer cells.

The choice to nanoencapsulate ITZ in SLN was driven by the challenge of its poor water-solubility. By using NB, a solid biocompatible lipid matrix with a unique chemical structure characterized by the presence of a longer chain fatty alcohol (C10–C18), greater drug solubility was obtained. In regard to the ideal parameters for an effective topical formulation, the selected nanoencapsulation approach achieved a small particle size (PS = 59.20 nm), low particle size distribution (PDI = 0.290) and extremely favorable entrapment efficiency (EE% = 98.4%); it also exhibited a clear appearance indicating a stable dispersion with no aggregation, corroborated by the recorded mean size of the NPs and high absolute value of ZP.

From dialysis-mediated i*n vitro* drug release profile evaluation, sustained and prolonged (>24h) release of the drug from both uncoated and coated SLN formulations was observed. However, the presence of the coating layer of DDAB led to the initial release of a greater amount (30%) of ITZ compared to the uncoated SLN, thus confirming the important role that the coating layer plays on the nanoparticle's features. The coating generates an irregular layer on the surface of the SLN with the formation of protrusions able to capture the drug, thereby promoting its prompt release within the first hours.

In vitro study to investigate the DDAB-coated ITZ-loaded NB SLN formulation's cytotoxicity on skin cancer cell cultures, namely SCC (A-431 cell line) and CM (SK-MEL-5 cell line), demonstrated a significant reduction in the viability of these cancer cells compared to the uncoated NB-SLN. Notably, the coated SLN achieved this effect at a lower concentration of ITZ, indicating enhanced anticancer activity through the synergistic effect of the coating layer and the encapsulated drug. Moreover, the coated SLN not only exhibited a remarkable ability to reduce the viability of tumoral cells but also preserved the viability of normal skin cells (HaCaT cell line, control). This suggests a selective cytotoxic effect on cancer cells, potentially minimizing adverse effects on healthy cells.

The researchers successfully designed an innovative topical formulation displaying enhanced cytotoxic effect particular to skin tumor cells. The coated SLN system offered an improvement to the drug's delivery profile allowing the drug to have a greater effect at a lower dose. The synergistic action from the ITZ and the NP system encapsulating it highlights the potential of this strategy for novel therapies against skin carcinoma.

Saraf & Gupta (2018)[85], studied the topical application of ITZ-loaded ethosomes, offering a promising treatment for BCC. The preparation of this topical formulation, an ethosomal gel, was achieved using Carbopol as a gelling agent.

Carbomers (Carbopol) are high molecular mass polymers derived from polymerization of acrylic acid and crosslinking with alkenyl ethers of sugars or polyalcohols[109]. Their hydrophilic nature exhibits an important characteristic – when they are exposed to water in a pH range of 4.0 to 6.0, their volume expands significantly due to the large amount of carboxyl groups on the polymer backbone ionize, resulting in repulsion between the negative charges, which leads to polymer swelling[85]. Carbomers molecules undergo a configuration change, thus increasing the viscosity of the liquid in the presence of water and forming a gel[85]. This unique property makes them an ideal and versatile ingredient in various pharmaceutical and cosmetic formulations to achieve desired product textures, controlled drug release and excellent thermal stability[85; 110]. Moreover, these gelling agents have low potential for skin irritation and sensitization, being highly suitable for aqueous formulations on the topical dosage forms[111]. With the high molecular weight, they act as excellent vehicles for drug delivery, ensuring the drug's effectiveness without penetrating the skin or interfering with its activity (high compatibility with other drugs)[85; 110].

To this effect, the prepared gel recorded a pH of 6.8 and achieved a viscosity of 1600-1740 cp. The ethosomal vesicles featured a small PS of 169.0 ± 49.0 nm, a low PDI of 0.384 ± 0.037 and good EE% of 82.00 ± 1.78%.

In vivo skin study utilizing rabbit specimens showed that ethosomal formulations exhibited no significant erythema, when compared with a control saline solution (0.9% w/w NaCl), indicating that the high concentration of ethanol employed did not induce skin redness.

From *ex vivo* drug release profile evaluation in Franz diffusion cells, it was observed that the formulated ITZ-loaded ethosomes exhibited greater penetration depth compared to the free drug. Furthermore, compared with an ITZ suspension, the ITZ-loaded ethosomal gel permeated faster through the dialysis membranes. The ethanol present in the ethosomal system acts as both permeation enhancer and stabilizer. As a result, the ethosomal vesicles become soft and malleable, enabling them to efficiently penetrate the SC. Once within the skin's layers, ethanol fuses with the skin lipids and releases ITZ into the deeper layers, at the tumor site. This further emphasizes the potential of this gel formulation as a highly efficient drug delivery system, capable of reaching deeper layers and delivering therapeutic agents more effectively.

An *in vitro* study was conducted to assess the efficacy of the ethosomal formulation against the BCC1/KMC cell line (BCC), revealing a 34% greater reduction of cell viability when compared to the free drug form. Additionally, the cytotoxicity of this formulation on the tumorous cells was approximately 4.6 times higher than the free drug form.

In this study, the designed formulation for topical application demonstrated significant cytotoxic effect against BCC. The approach to encapsulate ITZ in ethosomes dispersed in a gel resulted in better drug permeation and release profiles when compared to the free drug demonstrating the promise of this strategy in achieving greater therapeutic effect of ITZ against a type of skin cancer.

Lamie *et al.* (2022)[71] explored the nanovehiculation of repositioned ITZ by harnessing the potential of ascorbyl palmitate (AP) as aspasomes. These innovative aspasomes were skillfully incorporated into a cream, thereby creating a formulation that opens up a new therapeutic window for ITZ and offers a localized approach to manage cutaneous malignancies.

At the core of this approach lies AP, a hydrophobic derivative of ascorbic acid (AA), a well-explored vesicle bilayer-forming component that holds FDA approval and is widely popular in skin care products as an inactive ingredient. AP capacity to form multilamellar vesicles (aspasomal dispersions) were the root to design ITZ aspasomes in conjunction with their compatibility of their lipophilic nature. Lipid based vesicles like aspasomes have made significant advantages in drug delivery.

Moreover, given the antioxidant potency of AA, AP possesses the capacity to reduce cellular levels of reactive oxygen species (ROS) after in contact with UV irradiation, making it a potent active oxygen scavenger against dermal oxidative damages. AP is also well-

documented to retain the anticancer potential of AA, acting as an inhibitor of DNA synthesis and proliferation in various cancer cells, including those found in the skin. This synergistic strategy of employing ITZ and aspasomes can be a promising approach in skin cancer therapy.

In order to extend retention of the optimized aspasomes for improved topical application, an O/W (stearic acid: potassium hydroxide, glycerin, and propylene glycol) cream was chosen as the ultimate product. The formulated cream featured aspasomes with small size of 67.83 ± 6.16 nm, negative surface charge (ZP = -79.40 ± 2.23 mV), great ITZ entrapment (>95%) and high colloidal stability (evidenced by low PDI = 0.321 ± 0.14 to 0.493 ± 0.52, suggesting monodispersity, and high absolute value of the ZP, indicating strong repulsion and good dispersibility).

Ex vivo quantitative evaluation of skin deposition utilizing mice skin layers demonstrated the superior performance of the ITZ aspasomal cream, achieving a maximum deposition in the SC and epidermis up to 3.33- and 2.24-fold greater than an ITZ aspasomal dispersion, respectively. Similarly, in comparison to an ITZ conventional cream, the ITZ aspasomal cream exhibited a 2.19- and 4-fold increase of deposition in the SC and in the epidermis, respectively. The quantitative findings were confirmed via qualitative assessment of skin deposition of fluorescently labeled aspasomal dispersion and aspasomal cream.

In vitro cytotoxicity assessment of both pure ITZ and the optimized ITZ aspasomes on BCC cells (A431 cell line) was conducted. The comparison of IC50 values revealed a clear distinction, indicating that ITZ loaded in aspasomes exhibited approximately 2.5-fold higher toxicity against A431 cells compared to free drug. These results indicated that ITZ can induce greater cytotoxicity when loaded in the aspasomes.

The researchers also explored the anticancer potential of ITZ aspasomes, using the Ehrlich Carcinoma Model. ITZ aspasomal cream showed a significant reduction in tumor weight by over 50% (specifically 62.82%) when compared to the untreated control group.

According to the findings of this research, encapsulating ITZ in aspassomes allowed for a synergistic effect capable of achieving greater cytotoxicity against BCC. The nanovesiculation approach demonstrated an impressive skin deposition profile, highly valuable for topical administration.

In summary, these three works have demonstrated the value and applicability of nanosystem integration for enhancement of the therapeutic effect of ITZ against skin cancer. The researchers were able to achieve promising topical formulations with encapsulation of ITZ in three distinct types of nanosystems (SLN, ethosomes and aspasomes) that delivered exceptional performance in enhanced drug delivery. Compared to the free drug, the

nanovehiculated ITZ demonstrated improved cytotoxic effect on skin carcinoma cell lines. These preliminary findings truly evidence the potential of this therapeutic strategy.

8.2. Simvastatin

Simvastatin (SIM), classified as a BCS class II drug, is a well-recognized cholesterol synthesis inhibitor, utilized for dyslipidemia management[59]. It has the potent activity in reducing circulating lipids, specifically the cholesterol present in low-density lipoprotein (LDL)[112; 113]. The mechanism behind this process stems from the competitive inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, an essential enzyme to the synthesis of cholesterol[113].

HMG-CoA reductase inhibitors, more known as statins, have been found to possess antitumor properties in various types of cancer, including melanoma[112]. These effects are observed when statins are used alone or in combination with conventional treatments. Studies have shown how they can effectively block the G1-S phase of melanoma cycle[112]. SIM, more specifically, has demonstrated antitumor potential by inducing apoptosis in different types of cancer[59]. It can promote apoptosis due to the induction of caspases 3, 8 and 9 and to the DNA fragmentation of many human melanoma cells, when exposed to the drug's action at least for 48 h[59].

SIM is a water-insoluble hydrophobic drug as evidenced by its high log P value of 4.46[59]. This water-insolubility poses a significant challenge in its delivery, pharmacokinetics, and pharmacological activities[59]. For overcoming these limitations, researchers aim to unlock a new strategy to SIM delivery.

Barone *et al.* (2019)[112] explored the potential of repurposing SIM to support conventional therapies against melanoma treatment. The researchers developed a topical bioadhesive film containing SIM encapsulated within chitosan-coated nanostructured lipid carrier (Ch-NLC), which were used as the drug vehicle to achieve SIM incorporation and controlled release. Ch-NLC, a particular type of hybrid lipid-polymer (HLP) nanoparticles, are composed of a lipid matrix formed by the combination of solid and liquid lipids, which remain solid at room and body temperatures. This lipid matrix is then electrostatically surrounded by chitosan, as polymer. This hybrid nanosystem has both advantages of lipid and polymer materials, improving the interaction with the skin, which is attributed to the cationic nature and intrinsic bioadhesive properties of chitosan. Squalene, a natural lipid, was employed as the nanostructuring agent of the lipid NP matrix as well as a permeation enhancer, facilitating skin permeation and uptake into melanoma cells.

The formulation approach for this system included a predetermined ideal concentration of chitosan of 1% w/v, where increased chitosan concentration improved the system's stability due to the formation of complexes with the coating mechanism; however, it was observed that higher concentrations of chitosan also resulted in a significant increase in the average PS of the NPs. Further design of the NLC matrix encompassed optimizing critical formulation attributes, squalene concentration, solid:liquid (glyceryl palmitostearate:squalene) lipid ratio, polysorbate 80 (PS80) concentration, to achieve an optimal PS, PDI and ZP. The analysis suggested that an increase in lipid concentration led to an increase in PS. Additionally, when the surfactant (PS80) concentration was increased, a reduction in the PS was observed. This decrease in the PS is a direct consequence of the surfactant's inherent role as an interface component. Finally, a 25:75 solid:liquid lipid ratio results in a smaller PS compared to a 50:50 ratio. This behavior can be attributed to the increased dispersion viscosity when the solid lipid content is present at higher proportion, leading to an elevated surface tension and consequently larger PS. With the selection of the optimal attributes of 5% w/w lipid concentration, 2.5% w/w PS80 concentration, and 25:75 solid:liquid lipid ratio, the NLC system was able to obtain a PS of 108 ± 1 nm, a PDI of 0.226, indicating a relatively narrow size distribution and the ZP was recorded to be 17.0 ± 0.6 mV, suggesting a stable formulation. The EE% was high at 99.86 ± 0.08%, indicating a very large percentage of SIM incorporated into the NPs.

Additional formulation strategy covered the study of the effect of chemical permeation enhancers (CPEs) on the drug release, skin permeation, skin irritation, and cytotoxicity profile of the optimized SIM-loaded and unloaded Ch-NLC systems embedded in a mixture of sorbitol, polyethylene glycol (PEG) 400, and hydroxypropyl methylcellulose (HPMC) to form an adhesive film. Propylene glycol (PG) and limonene (L) were the relevant CPEs prepared into the films and compared with the Ch-NLC (reference) film.

In vitro release and permeation studies were performed using Franz diffusion cells to comprehend the release behavior of drug from the formulated films. This release was dependent on the partitioning of the drug within the Ch-NLC lipid matrix, followed by the rate of diffusion through the adhesive layer. The results indicate a sustained release throughout the 48 h of the test. The lowest release rate was observed for the PG-film (54 ± 2%), whereas for the reference-film and L-film, 98 ± 4% and 99 ± 7% of SIM was released, respectively. The presence of PG in the formulation had an inhibiting effect on the release profile of SIM. As for skin permeation profiling, newborn pig epidermis (resembles the human skin anatomically, physiologically, and biochemically) was implemented as a barrier model. The study demonstrated that the presence of enhancers (PG and L) did not promote a significantly higher

flux, when compared to the reference film. In particular, the film without CPEs presents the highest permeation through the SC, highlighting the permeation enhancing properties of squalene in the NP matrix, originating from its ability to disrupt the ordered lipid bilayer structure of cells.

In vivo tolerability study of the drug-free PG-, L- and reference-films was conducted on human subjects to evaluate the potential skin irritation of the Ch-NLC topical films and the respective influence of the CPEs. The films did not cause any skin irritation or alterations in the SC. Additionally, the topical films showed no signs of structural damage after 72 h, indicating their stability and suitability for topical use.

In vitro evaluation of cytotoxicity activity of SIM in the free form and encapsulated within the proposed Ch-NLC film determined that no cytotoxic effect was observed for both free SIM and the reference film (cell viability around 100% after 48 h) on HaCaT and COS-7 cell lines, representing healthy/normal human keratinocytes and fibroblasts, respectively. The cytotoxic effect on melanoma cell lines (COLO-38 and SK-MEL-28) was subsequently investigated and dose- and time-dependent response was observed. Comparable performance of the proposed hybrid system and free drug was reported. In particular, at 48 h of exposure, loaded nanocarriers demonstrated a higher cytotoxic effect in comparison with free drug in the COLO-38 cell line. A synergistic effect with SIM was observed from squalene's intrinsic characteristic of destabilizing the cellular membrane, promoting the delivery of drug inside the cell. Further evaluation of the cytotoxicity of the formulated films spiked with CPEs on COLO-38 cell confirmed that films including SIM encapsulated into Ch-NLC provided an increased cytotoxic activity. Distinct cytotoxicity levels were observed depending on the enhancer used, and films containing L- demonstrated the most potent cytotoxic effect among the tested enhancers.

This publication has successfully demonstrated the application of an NLC system for topical application via an adhesive film featuring repurposed SIM with reportable cytotoxic effect against melanoma cell lines. The hybrid polymer-lipid (chitosan-glyceryl palmitostearate:squalene) NP matrix featured ideal properties for topical application and recorded a better permeation profile without the CPE spiking, while also demonstrating no decrease of cell viability for healthy cell lines, implying a safe toxicological profile, high tolerability and biocompatible formulation.

Famta *et al.* (2022)[59] focused on repurposing SIM, loading it into a PLGA nanoparticle matrix, which was subsequently integrated in a poloxamer-based thermogels for topical application and targeted therapeutic effect against skin cancer. This strategy demonstrated that

the incorporation of these NPs into the thermogel facilitated the formation of a depot in the upper dermal layers, achieving targeted drug delivery, reducing systemic exposure to the drug.

For this formulation approach, the desired *in situ* gelation temperature that would impart a thermos-responsive depot behavior to the formulation was achieved by adjusting the balance ratio of poloxamers in the formulation.

Poloxamers are a class of water-soluble nonionic and synthetic triblock copolymers formed by a central hydrophobic chains of poly(propylene oxide) sandwiched between two hydrophilic chains of poly(ethylene oxide) that give them the amphiphilic character[59; 114]. They show an important inherent thermo-reversible behavior and possess solid-to-gel phase transition near body temperature, being excellent candidates to take part in thermogels[59].

Poloxamer 407 and 188 stand out as the most widely used grades of poloxamers used in the development of various drug delivery systems[59; 114]. When concentrated aqueous solutions of these poloxamers are exposed to body temperature (near 37°C), they form a reversible gel that returns to a solution state at room temperature (25°C)[59].

Poloxamer 407 has the ability to toughen the gel matrix and enhance bio-adhesion, but the phase transition temperature is higher than the required gelation temperature. Poloxamer 188 is more hydrophilic and has a lower molecular weight than Poloxamer 407, which regulates sol-gel transition temperature and shear-thinning behavior of Poloxamer 407[59]. So, the addition of Poloxamer 188 to Poloxamer 407 causes a reduction of the phase transition temperature[59]. Furthermore, it was observed that the optimal ratio of Poloxamer 407 and Poloxamer 188 was 9:1 because it showed the optimal gelation temperature (34 °C) for thermogel synthesis.

Using a Quality by Design (QbD) approach, the formulation was optimized, taking into consideration the NPs' PS, PDI and EE% as Critical Quality Attributes (CQAs). The optimized formulation achieved a ZP of -28.1 ± 5.64mV, a small PS (177 nm) and a low PDI (0.147) and the EE% confidently assumed to be around 90%, based on preliminary model screening.

In vitro drug release studies of free drug and SIM-loaded NPs were performed using the dialysis sac method. The results revealed a sustained release pattern for SIM-loaded NPs, with approximately 50.79 ± 5.56% of the drug being released over a period of 72h. In contrast, the free drug showed a fast release, where it was completely released within 6h, leading to the conclusion that there was a halt in liberation of the drug from the PLGA NPs.

To evaluate the thermo-responsiveness of the thermogel, *ex vivo* diffusion studies were performed using Franz diffusion method, maintaining two different temperatures in the receiver compartment, 25 ± 2°C and 37 ± 2°C. Notably, at 37 ± 2°C, the nanoparticulate thermogel demonstrated the ability to sustain drug release, which was significantly different

compared to the other temperature. This restricted drug release at higher temperature can be attributed to the sol-to-gel transition that occurs, where the observed increase in viscosity in turn restricts loaded NPs diffusion within the formulation, creating the desired drug depot.

In vitro studies were performed to measure the cell viability of melanoma cells (SK-MEL-28 cell line) upon exposure to various treatments: free SIM, blank PLGA (BPLGA) NPs, and SIM-loaded PLGA (SPLGA) NPs. SPLGA NPs' cytotoxic activity was 9.38-fold higher than free SIM. The entrapment of SIM in PLGA NPs increased its accumulation in the SK-MEL-28 cells (CM cell line) and reduced its precipitation in the aqueous cell culture media, which is a common obstacle faced with the administration of the free drug. As a result, the increased intracellular accumulation of SIM led to enhanced anticancer activity. BPLGA NPs exhibited low cytotoxicity, as evidenced by high cell viability rate of 97.55 ± 1.29%, indicating that NPs themselves are a biocompatible option for drug delivery. Furthermore, the sustained release of SIM from the PLGA NPs exposed the cancer cells to the drug over a longer period leading to increased cytotoxicity.

In this study, researchers repurposed SIM in polymeric (PLGA) NPs for topical application and formulated a thermogel to achieve drug depot formation for enhanced cytotoxicity in melanoma cell lines by localized prolonged exposure. The cytotoxic effect of the designed system was significantly increased when compared with the free drug. Once again, the developed blank formulation was proven to be biocompatible.

The analyzed works have highlighted the benefit of encapsulating SIM for enhanced drug delivery in topical application and increased anticancer activity against melanoma. Incorporation of nanosystems (hybrid polymer-NLC and polymeric NPs) in these formulations have substantially aided in achieving those two goals. The two systems have unlocked the potential of SIM as a repurposed drug targeting skin cancer therapy by topical delivery.

8.3. Niclosamide

Niclosamide (NIC), a BCS class II drug, is an FDA-approved anthelmintic drug used to treat parasitic infections[115; 116]. Despite of this widespread usage, NIC's mechanism of action remains only partially comprehended[117]. Early studies associated NIC's activity to the uncoupling of oxidative phosphorylation[117].

Over the past few years, studies have shown that NIC possesses a wide range of clinical applications, encompassing its viability in addressing cancer and other diseases[115; 116]. Alternative targets of NIC have emerged, including the Wnt/β-catenin, mTOR, AKT- PI3K, Hh and JAK/STAT3 signaling pathways, which inevitably linked NIC's therapeutic potential to many diseases involving these critical signaling cascades;[37; 117]

In melanoma, signal transducer and activator of transcription 3 (STAT3), a transcription factor, plays a crucial role in various cellular processes, such as, tumor cell growth and proliferation, survival, migration, invasion, immune evasion, and metastasis[37; 118]. NIC is a potent inhibitor of STAT3 and has also demonstrated remarkable promise in inducing apoptosis within some cancer types linked to breast, lung, colon, and others[37].

NIC has exhibited potent *in vitro* and *in vivo* activity in melanoma and metastasis. Nonetheless, local non-invasive topical dermal delivery targeting the bioactive site, without systemic exposure had not been explored[37]. Its poor water solubility (log P = 4.48) and poor penetration across skin layers are discouraging factors when considering conventional topical formulations[37; 115].

Shah *et al.* (2022)[37], followed the same methods as the previous article of SIM study with the goal to formulate a thermogel with poloxamers that could incorporate NIC loaded liposomes for melanoma treatment. This thermogel was intended to form a local depot for targeted topical administration, specifically to the outermost layers of skin, in order to control the drug's delivery to the bioactive site and minimize its entry into the systemic circulation.

Poloxamer 407 and 188 were also used and the ratio between them was 9:1 because it showed the desired gelation temperature (33°C) that was optimum for thermogel synthesis.

Employing the principles of QbD, an optimization was made considering the same CQAs. The results of the optimized formulation exhibited a PS of 131 nm, EE% of 89% and a ZP measuring -13 ± 9.71 mV, suggesting an effective electrostatic stabilization, contributing to improved stability.

The investigation into the stability of liposomes and liposomal thermogel over a one-month period at temperatures of 2-8°C and room temperature revealed that refrigeration is the recommended storage method. This comes based on the observation of a slight rise in PS and a reduction in EE% at room temperature.

As done previously, the dialysis sac method was used to evaluate *in vitro* drug release studies of NIC plain drug suspensions and loaded liposomes. These studies revealed a sustained release profile of about 83.26 ± 4.55% was observed up to 48 h for NIC loaded liposomes. In contrast, the free drug exhibited a complete release within 4h. Furthermore, using Franz diffusion cells, at a higher temperature of 37 ± 2°C, the liposomal thermogel effectively prolonged the drug release when compared to the temperature of 25 ± 2°C. This effect can be attributed to solid to gel transition responsible for heightened viscosity.

Ex vivo studies were conducted in rat skin, using Franz diffusion cells, to evaluate the depth of skin penetration of the NIC-loaded liposomal thermo-responsive gel. It was found that the liposomes exhibited penetration into the skin's deeper layers, whereas the liposomal

thermogel exhibited a different behavior by localizing the liposomes, forming a visible band on the uppermost skin layers, verifying depot formation at the bioactive site.

In vitro studies were performed to estimate the cell viability of melanoma cells exposed to free NIC, blank liposomes (BLIP) and NIC-loaded liposomes (NLIP). There was an evident accumulation of NIC in SK-MEL 28 (CM cell line) with the liposomal encapsulation compared to free drug. The entrapment of NIC within liposomes elevated its cytotoxicity by 1.756-fold. BLIP demonstrated low cytotoxicity with a cell viability of 91.83 ± 2.23%.

Based on the data from this study, the NIC-loaded liposomal thermogel was demonstrated as a safe formulation approach that enhanced the cytotoxicity of NIC against melanoma. The deposition behavior that can offer a localized delivery with prolonged exposure was also evidenced. This research highlights the potential for the liposomal encapsulation of NIC in a topical formulation as a possible therapeutic strategy for helping against melanoma.

8.4. Doxycycline

Doxycycline (DOX), a BCS class I drug, is a second-generation tetracycline widely recognized as an antibiotic with a broad-spectrum efficacy[95; 119]. The bacteriostatic effect of tetracyclines inhibits bacterial protein synthesis, preventing the binding of the aminoacyl t-RNA to the 30S ribosomal subunit of both gram-negative and gram-positive bacteria[120; 121].

Moreover, studies have shown that DOX has many significant nonantibiotic properties, such as anti-inflammatory, antiproliferative, antiangiogenic, anti-collagenolytic, osteoclast-inhibitory, fibroblaststimulatory, immunosuppressive and anticancer effects[95; 119]. Thus, these extra biological actions have prompted a lot of research into alternative applications of this drug[95; 119]. For instance, the antiproliferative activity of DOX is mainly attributed to its ability to inhibit the matrix-metalloproteinases (MMPs), enzymes involved in the degradation and remodulation of the extracellular matrix[95].

Current studies have demonstrated that DOX, as a versatile drug, has the ability to control the invasive and metastatic cancer cells, inhibiting their adhesion and migration, as well as affecting their grown and proliferation and inducing apoptosis[119; 121]. *In vivo* investigations have solidified the anti-cancer properties of this drug against different carcinoma cells such as melanoma[95].

Ramírez-Agudelo *et al.* (2017)[95] devised a controlled release system where hybrid electrospun nanofibrous scaffolds composed of poly-ε-caprolactone (PCL) and gelatin (Gel) able to facilitate the controlled release of DOX and hydroxyapatite NPs (nHA). The

researchers investigated this DOX/nHA/PCL-Gel composite nanofiber system's potential antitumor activity.

Hydroxyapatite (HA) is known for being the most widely accepted inorganic nanomaterial in clinical use due to the exceptional biocompatibility and acceptable biodegradation. Leveraging its high surface area to volume ratio, high surface activity and strong ability to absorb diverse chemical species, hydroxyapatite nanoparticles (nHA) have proven to be a potential candidate to serve as delivery carrier for antibiotics, nucleic acids, proteins, and anticancer drugs. Additionally, nHA themselves have demonstrated anticancer properties in multiple cancer cell types like melanoma, through intracellular accumulation of ROS, which are associated with the apoptosis regulation by elevating oxidative stress. Hence, the DOX/nHA combination therapy induces a possible breakthrough, yielding synergistic agents with potential antitumoral effect through different mechanisms of action for cancer combat.

PCL is a hydrophobic, bioresorbable, and biocompatible synthetic polyester that holds the approval of FDA for its utilization in biomedical devices in applications related to drug delivery and tissue repair. One of the greatest disadvantages of synthetic polymers is the lack of cell-recognition signals, so the combination of natural and synthetic polymers can provide the advantage to finetune the desired properties of the electrospun scaffolds. For that matter, Gel is a natural biopolymer derived from the partial hydrolysis of collagens. It can offer different several attributes such as cost-effectiveness, natural abundance and notable biodegradability and biocompatibility in physiological environments. The PCL/Gel composite scaffolds represent a versatile synthetic-natural polymer blending for tissue engineering applications. This synergistic blending permits cell attachment, biocompatibility, and bioactivity of the scaffolds, due to the presence of the natural polymer, Gel. The polymeric blend nanofibers also provide many opportunities to design and adjust release kinetics of DOX as an antitumor agent.

The incorporation of the NPs into the polymer PCL/Gel nanofiber via electrospinning resulted in their selective entrapment preferentially in the Gel phase associated with the hydrophilic nature of nHA and DOX. The integration into the polymer was also made in response to one of the limitations of HA. HA when used as a carrier shows an initial rapid burst release, thereby imposing constraints on its effectiveness within the drug delivery system. By synergistically leveraging all the components, nHA, DOX, PCL, and Gel, a controlled release mechanism for DOX is established, thereby circumventing the issue of fast initial release associated with HA carriers.

The synthesized nHA demonstrated excellent dispersion with uniform morphology exhibiting NPs with a rod-like shape, showing an average diameter of 27 ± 7 nm and a length of 40 ± 17 nm.

Under *in vitro* conditions, using a phosphate-buffered saline (PBS) buffer medium, the DOX release profile exhibited a two-stage pattern. In the first phase, all scaffolds showed a burst release effect, releasing approximately 60% of the loaded drug at the first hour. In the second phase, the remaining drug content was released progressively over 55h. It is worth mentioning that the release rate was not increased with increasing the content of Gel in the fibers. As referred above, the drug has the tendency to localize primarily within the Gel phase, regardless of the ratio of biopolymer present in the blend. Thus, the phenomenon of the initial burst release can be attributed not only to the high solubility of Gel in the PBS release medium but also due to the phase segregation between Gel and PCL that occurred during the electrospinning process, that causes the accumulation of Gel on the nanofibers surface.

The antitumor activity of DOX/nHA/PCL-Gel nanofibers was assessed *in vitro* using three distinct tumor cell lines. Relevant to this work, one of them was A-431 cells (SCC cell line) where this system had the best performance. Study showed insignificant cytotoxicity with the free nHA treatment; similarly, the application of free DOX led to a small decrease of cancer cell viability. In comparison to these single administrations of nHA and DOX, the formulated system enhanced the *in vitro* antitumoral efficacy.

In summary, owing to the notable properties of nHA as a drug carrier with an antiproliferative drug such as DOX, this topical system stands as an efficient approach that addresses challenges linked to ordinary anticancer treatments. It holds promise for targeted and localized drug delivery applications in the biomedical field, namely as a treatment strategy for skin cancer.

8.5. Leflunomide

Leflunomide (LFD) is an isoxazole derived and a BCS class II drug[57]. It functions as a DMARD and has demonstrated effectiveness in managing and treating rheumatoid arthritis[57; 122]. LFD is a prodrug involved in multiple signaling pathways and cellular processes[122]. LFD undergoes swift conversion into an active metabolite known as Teriflunomide (A771726) via isoxazole ring cleavage[57]. A771726 inhibits dihydroorotate dehydrogenase (DHODH), a key enzyme in the *de novo* pyrimidine synthesis in lymphocytes and thus modulates DNA synthesis[57; 123].

Over the years, LFD was used in numerous preclinical studies as a potential cancer treatment[122]. Simultaneously, more mechanisms underlying the anticancer effect of LFD were

identified[122]. In melanoma, the proliferation of human melanoma cells experiences a dose-dependent decrease due to the effects of A771726[57]. Also, LFD, acting as an agonist, strongly activates transcriptional activity of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that regulates a wide range of biological activities[57; 122]. These findings imply the potential clinical application of LFD for addressing melanoma[57].

Despite its beneficial pharmacological effects in the treatment of rheumatoid arthritis and melanoma, the oral administration of LFD is linked to side effects like nausea, vomiting, diarrhea, alopecia, elevated liver enzymes, and hepatic dysfunction[57; 123]. To address these issues, topical administration of LFD could be pertinent; due to its low molecular weight, poor aqueous solubility (less than 40 μg/mL) and lipophilicity (log P = 2.52) it is worth exploring its incorporation in nanosystems for topical application.

Pund *et al.* (2015)[57] created a thermodynamically stable nanoemulsion-based carrier system for LFD to evaluate its anticancer effect against melanoma. The thermodynamic stability derives from the self-emulsifying technique, utilizing Capryol 90, Cremophor EL, Transcutol HP as nanoemulsifying components and finally incorporating Pluronic F127 (Poloxamer 407) as a gelling agent, to achieve the desired nanoemulgel.

Capryol 90 is a propylene glycol monocaprylate with a HLB of 5, denoting its lipophilicity. From a screening of oils and modified oils, Capryol 90 showed the highest solubility for LFD. Due to its low polar surface area, LFD exhibits enhanced solubility in modified oils with small/medium molar volume oil, like medium chain glycerides.

Cremophor EL, a non-ionic surfactant has HLB more than 12, is comparatively safe, biocompatible, less toxic and form micelles at lower concentration than other screened surfactants. All the selected surfactants had the same characteristics; however, Cremophor EL showed the highest LFD solubility, making it the chosen candidate for subsequent developmental stages. Additionally, it has better emulsification ability for Capryol 90.

Transcutol HP is a diethylene glycol monoethyl ether that has an additional effect of enhancing the skin penetration of lipophilic drugs. Moreover, this co-solvent showed maximum solubility of LFD.

The optimized nanoemulsion recorded a remarkable PS of 102.3 nm and PDI of 0.278. As for the ZP, it was found to be -7.8 mV. This value was expected since the excipients used in the formulation were nonionic. The slight negative charge observed may be attributed to the ionization of trace amounts of free fatty acids present in the chosen excipients. Nonetheless, in the current scenario, the magnitude of the ZP is of lesser significance because the formulation was intended to be gelled.

The formulated nanoemulgel, achieved by the incorporation of Poloxamer 407 (gelling agent) in the nanoemulsion matrix, exhibited an increase in globule size, transitioning from 102.3 nm to 123.7 nm. This expansion in hydrodynamic size can be attributed to the absorption of the gelling polymer onto the emulsion globules. Remarkably, the PDI remained unchanged, signifying the overall stability of the system.

Ex vivo studies were performed in Franz-type diffusion cells and showed that an ordinary gel showed a slow, inefficient, and incomplete LFD delivery across rat abdominal skin. As previously noted, the limited aqueous solubility of the drug acts as the first limiting step before the permeation could occur. When the nanoemulgel was evaluated, it showed a significant improvement of 5.65 times in flux. Also, a significant increase in the accumulation of LFD was observed in the skin treated with the nanoemulgel, showing a prolonged release into the underlying tissues over an extended duration at the target site.

In vitro cytotoxicity studies were assessed on two melanoma cell lines (A375 and SK-MEL-2) to evaluate the antiproliferative potential of the formulated LFD nanoemulgel. Significant reduction in cell viability and an accelerated cell mortality rate provides compelling evidence for the heightened efficacy of the nanosized LFD emulsion in gel form compared to the free drug when applied topically to the affected area. This improved therapeutic outcome is ascribed to the enhanced permeability of the nanoemulsion.

This research marks another great application of a NP system enhancing the cytotoxic effect of a drug with therapeutic potential for melanoma. The approach was able to evidence LFD's antimelanoma activity with a targeted-delivery topical formulation system (nanoemulgel). This activity was demonstrated to have an enhanced cytotoxic effect against melanoma, that can be attributed to the impact of the nanoemulsion-based formulation.

8.6. Metformin

Metformin (MET) is a BCS class III and biguanide drug used as a first-line therapy for the treatment of type II diabetes[124; 125]. MET stands as an important antidiabetic medication that reduces the glucose level, consequently resulting in a decrease in the blood insulin level[83]. It has been used for over 60 years because of its outstanding ability to decrease plasma glucose levels, well-established safety profile, and relatively low cost[124; 125].

Convincing data places energy metabolism at the center of MET's mechanism of action in diabetes; however, it may also hold significance in the realms of cardiovascular diseases and cancer.[126] Adenosine 5' monophosphate-activated protein kinase (AMPK) functions as an energy central regulator, which is actively involved in the restoration of energy balance within numerous metabolic pathways.[83; 125]

MET directly triggers the activation of AMPK, which leads to the inhibition of mammalian target of rapamycin (mTOR) signaling that contributes to impede the proliferation of cancer stem cells[83]. mTOR is responsible for the regulation of some important physiological functions, such as cell growth, proliferation, metabolism, protein synthesis, and autophagy[127]. Thus, by inhibiting this protein kinase, all these functions will not happen. MET can also inhibit skin cancer progression through alternate pathways: stimulation of the immune system, enhancing autophagy and cell apoptosis by p53 and p21 activation[83].

Mousa *et al.* (2022)[83], developed an ethosomal formulation designed to act as a vehicle for MET. The work focused on developing an optimal ethosomal gel to incorporate MET and evaluating its potential as a topical treatment against induced skin cancer in mice.

The design of the drug loaded ethosomal gel centered on achieving optimal PS, EE%, drug release % and ZP properties which are significantly impacted by the composition. For the preparation of the ethosomes, the critical parameters were described by the amount of lecithin, ethanol, and cholesterol used.

Lecithin forms a phospholipid structure and is responsible for the establishment of the multilamellar membrane structure found in the ethosomes. Higher lecithin concentration led to a reduction of the EE% because of the hydrophilic nature of MET and led to an increase in the ethosome vesicle size because the molecules tend to coalesce and aggregate. Important to this work, cholesterol assumed responsibility for both stability and EE% of MET. Increasing cholesterol resulted in an increase in the vesicle size of the ethosomes. The mixture of isopropyl alcohol with ethanol notably reduced vesicle size, caused a high negative charge, and a high EE%, as MET is a cationic drug. Also, isopropyl alcohol has the capacity to facilitate the release of MET in the long term. When Carbopol was mixed with the ethosomes, the desired viscosity and the best bio-adhesion characteristics of the formulation were achieved.

The optimal formulation exhibited a remarkable EE% of 98.40 ± 0.35%, a PS and ZP measured at 124.01 ± 14.27 nm and - 60.08 ± 1.44 mV, respectively. From a dialysis-mediated *in vitro* assessment of the drug's release into Sorensen phosphate buffer medium, the drug release % was recorded as 55.04 ± 0.98%.

In vitro skin permeation studies were made with diffusible membranes that were collected from abdominal rat skin. The results showed that the ideal permeability behavior was observed for the formulation that had the highest ethanol and isopropyl alcohol concentration, the lower lecithin concentration and moderate concentration of cholesterol. When the concentrations of lecithin and cholesterol are increased, the rigidity of the ethosomal vesicle bilayer increased; consequently, a reduction in these two led to an increase in the permeation rate of MET.

In vivo mouse studies were conducted to evaluate antitumor activity and toxicity of MET-loaded ethosomal gel. To assess the advancement of skin cancer, the dimensions of the lesions were measured. It was noticed that the MET-loaded ethosomal gel produced a significant decrease in the lesion diameters compared with the empty-ethosome gel, empty gel, and free-MET gel, over 28 days. The curative effect was significantly enhanced in the MET-loaded ethosomal gel.

The designed ethosomal gel has demonstrated a synergistic effect with MET to increase the drug's antitumor effect for murine skin cancer. These results highlight the potential to elevate this approach to be developed as a localized application therapeutic option for the fight against skin cancer.

Exploring an alternative treatment strategy that combines photodynamic therapy (PDT) and MET repurposing on a topical formulation, Donadon *et al.* (2023)[128] evaluated the capability of monoolein (MO)-based nanodispersions to enhance the co-delivery of MET and methylene blue (MB) for topical treatment of non-melanoma skin cancer.

PDT is a modern and non-invasive clinically approved form of therapy that can use a selective cytotoxic activity against malignant cells[129; 130]. It involves the interaction between a photosensitizer (PhS), the wavelength that corresponds to an absorbance band of the sensitizer, and the presence of oxygen[129; 131]. This reaction triggers the activation of the process that leads to selective destruction of cells by necrosis or apoptosis[130; 131]. The photocytotoxic reactions occur only within the pathological tissues located in the region where the PhS is distributed, enabling precise and targeted destruction.[130] Initially, PDT relied on administering the PhS systemically, but its introduction through topical administration brought a revolutionary shift in the field[131]. Moreover, the combination of PhS with nanomaterials has also been an important field of research due to the potential to enhance the efficiency of PDT and eliminating some of its adverse effects[130].

MB is a PhS with favorable photophysical and photochemical properties. Additionally, MB demonstrates suitable photobiological qualities for PDT, inducing cell death of various types of cancerous cells, encompassing skin malignancies like BCC and melanoma[128].

In addition to the mechanisms of action mentioned above, MET may also induce a mild and specific inhibition of the mitochondrial respiratory chain complex I, resulting in a reduction in oxygen consumption by tumor cells and reversing tumor hypoxia. Tumor hypoxia can be a barrier to PDT in cancer treatment because the production of ROS depends on the availability of oxygen during PhS excitation[128].

In this research, the combination of MET and MB was introduced to enhance the effectiveness of PDT as a potential new strategy for treating skin cancer. However, neither

MET (log P = -1.43) nor MB (log P = -0.9) possess suitable physicochemical characteristics for efficient delivery through the skin. Thus, to address this limitation, the creation and evaluation of nanodispersions based on MO for co-delivery and co-localization of MET and MB within the skin represent an enhanced delivery system capable of surpassing the SC barrier and facilitating simultaneous delivery of MB and MET into the viable skin layers.

The formulation development and screening process focused on achieving optimal drug-loaded nanocarrier properties that met a PS under 150 nm, a PDI under 0.2 and a ZP less than -14 mV, as well as the drug release, skin permeation and retention profiles of MET and MB components that demonstrated a more pronounced improvement over free-MET and free-MB suspensions (controls). The dispersions with low MO content and stabilized with Kolliphor® P407 (Poloxamer 407) and sodium cholate were the most promising in maximizing cutaneous delivery as well as minimizing transdermal delivery of MB and MET.

In vitro viability studies on A-431 cells (SCC cell line) demonstrated that the combination therapy of MET and MB in MO nanodispersions displayed significant cytotoxicity, which could be maximized by the photoactivation of MB.

The integration of nanotechnology, drug synergy, and PDT for enhanced cutaneous delivery and cytotoxic effects is an innovative approach that holds potential for enhancing treatment outcomes for non-melanoma skin cancer. The designed system evidenced positive outcomes in applying this novel strategy. The MO-based nanovesiculation of MET and MB allowed for topical administration and localized delivery of the cytotoxic agents which resulted in improved antitumor activity.

The two publications examined describe two different lipid-based nanosystems capable of enhancing skin permeation and targeted delivery of MET, therefore optimizing the antitumor effect of the drug. Moreover, the research suggests that MET has the potential to augment the desired effect of PDT when treating non-melanoma skin cancer. These two strategies proposed have successfully highlighted the value of drug delivery systems featuring nanosized configurations as well as the potential of MET repurposing to target skin cancer.

8.7. Celecoxib

Celecoxib (CEL), BCS class II drug, is a NSAID that selectively inhibits cyclooxygenase-2 (COX-2)[73; 132]. COX-2 is responsible for prostaglandin synthesis. Prostaglandins are inflammation-promoting molecules; hence, their inhibition will promote an anti-inflammatory environment[133; 134]. The analgesic, anti-inflammatory and antipyretic properties of CEL enables it to be commonly used for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute pain[133].

The use of CEL has been proven to be effective in the inhibition of the progression of skin cancer as well as enhancing the effectiveness of other chemotherapy drugs[73]. Melanoma cell growth is influenced by COX-2 activity and blocking this enzyme with CEL could offer a reasonable and clinically feasible approach to impede tumor advancement and induce tumor cell death[134].

Ahmed *et al.* (2019)[73] investigated the combination of co-loaded liposome gel with doxorubicin (DOXO) and CEL and the use of Derma roller® microneedles (MNs) as a promising therapeutic strategy for the management of melanoma. This new approach with the pretreatment of the physical penetration enhancers aims to improve the permeation of drugs through skin, thus increasing the anticancer efficacy.

DOXO stands as the most popular and widely used anthracycline antibiotic as antitumor agent, finding frequent application in the treatment of both hematological and solid tumors. However, the use of a single chemotherapeutic agent in cancer management has revealed several limitations, such as lack of sustained efficiency, drug resistance and adverse side effects. For that reason, the investigators attempted a novel strategy of co-delivery of DOXO and CEL with a synergistic effect that reduces drug dosage, thus minimizing undesirable side effects.

The utilization of MNs as a means of increasing physical penetration has been widely used in transdermal drug delivery. This approach is valued for its painless properties, no need for specialized medical proficiency (unlike intramuscular and intravenous injections) and for presenting minimal risk of infection at the application site.

The formulation developed featured a versatile and widely used gelling agent, Carbopol. The obtained co-loaded liposomes displayed a small PS (142.37 ± 0.78 nm) and a homogeneous particle size distribution (PDI = 0.27 ± 0.026). Surprisingly, the ZP was −5.04 ± 0.51 where this low value does not indicate effective electrostatic stabilization of the nanosuspension (when considering the ideal value of ±30 mV); however, the absolute value of the ZP is not as relevant since the gel formulation was intended. The EE% of DOXO was high (EE% = 98.42 ± 0.0073) as well as the EE% of CEL (98.37 ± 0.037). The process of fabrication allowed for the optimal EE% of DOXO and the EE% of CEL stems from its limited water solubility and high lipophilic nature (log P = 3.01).

In vitro studies were conducted in Franz diffusion cells equipped with a semi-permeable dialysis membrane to evaluate DOXO and CEL release from liposomes and liposomal gel in PBS. When compared with the release profile observed in liposomal drug suspensions, all liposomal gel formulations exhibited similar and sustained release behaviors, devoid of any initial burst release.

In vitro skin permeation studies were achieved using abdominal skin from mice in modified Franz diffusion cells and showed a notable increase in the penetration of DOXO and CEL through the skin layers into the receptor compartment in the groups that underwent derma roller pretreatment, as opposed to the untreated group. Moreover, the permeation and retention of DOXO improved when CEL was incorporated.

In vitro cytotoxicity of the various formulations was tested on B16 murine melanoma cells. A synergistic effect was observed when CEL and DOXO were combined in solution; however, liposomal formulations co-loaded with DOXO and CEL exhibited greater cytotoxicity than the DOXO/CEL solution. Furthermore, the inhibitory rates of the DOXO/CEL co-loaded liposomes were superior in comparison to liposomes with a single drug.

Moreover, *in vivo* anticancer effect was evaluated on the skin area where B16 cells had been implanted in mice. Mice that were treated topically with co-loaded liposomal gel denoted significantly enhanced antitumor effect which can be attributed to the greater drug dispersion and the effective accumulation within tumor cells. The pretreatment with MNs also helped to increase the tumor inhibition rate by improving skin permeability from the micro-sized holes induced in the SC. Mice that received topical treatment with DOXO liposomal gel displayed visibly smaller tumor sizes compared to untreated mice; however, the tumor size in this group was still significantly larger than those treated with the DOXO/CEL co-loaded liposomal gel formulation.

To summarize, the co-loaded DOXO/CEL liposomal gel combined with the utilization of MNs was able to significantly elevate the tumor growth inhibitory rate in melanoma-grafted mice. These results demonstrated the benefit in opting for a combination therapy approach rather than single-drug administration as well as selecting topical application by nanocarrier-facilitated delivery for an increased localized effect, further enhanced by the influence of MN application.

9. Final Discussion

All studied approaches presented unique NP systems successfully integrated in topical formulations for encapsulation of repurposed drugs with the goal of improving the drug's antitumor activity towards melanoma and non-melanoma skin cancer. The proposed repurposed drugs had been evidenced to have potential anticancer activity which was evaluated in a topical delivery scenario. Most strategies focused on maximizing the localized delivery and enhancing the drugs' skin permeation and upper layer retention profiles for better

treatment efficacy. In all cases this goal was remarkably achieved by formulation optimization, namely in regarding the nanosystem. The results when evaluating the cytotoxicity of the drugs incorporated in the NP-based formulations compared to the free-drug, evidence that the designed approaches significantly outperform the basic formulations. In some cases, antitumor effect from the NPs themselves was registered providing great evidence to the benefit of nanotechnology in skin cancer therapy.

It was observed that the successful NP configurations that achieved best performance ensured a PS smaller than 200 nm. As for the system's homogeneity a PDI under 0.3 is recommended and should be targeted to be as close to 0 as possible. When the NP's surface (evaluated by ZP) was described as positively charged, an interactive behavior of the particles fusing with the negatively charged cellular membranes was considered in observance of improved drug permeation and retention profiles. The effect of the ZP in terms of suspension stability was more pronounced in liquid formulations (nanodispersions and nanoemulsions) where the repulsive behavior of the suspended particles is very important to prevent aggregation; a high absolute value was targeted with most designed systems achieving at least ±10 mV. In the case of semi-solid products, due to their higher viscosity, the ZP does not have such a strong relation to the formulation stability.

Most of the designed formulations showcased a favorable toxicological profile, highlighted by negligible cytotoxicity in healthy cells and high skin tolerability. The inclusion of biocompatible materials was certainly a priority for the formulation design process to ensure that the proposed systems can offer little to no harm to future patients.

10. Conclusion

Drug repurposing has had a tremendous impact in the discovery of new therapeutic options in the fight against skin cancer. It has allowed researchers to confidently unlock the anticancer potential of various marketed drugs with less effort and costs, backing on their known pharmacological profile from the original applications. Unlike most cancers, the location of cutaneous carcinoma allows for targeted treatment options that rely on simple technology, such as topical formulations directly applied on the affected area, that shy away from complex procedures, prioritize the localized therapeutic effect, offer convenience to the patient, and minimize systemic exposure and the adverse symptoms often associated with it; however, topical drug delivery is often focused on overcoming the greatest challenge: penetrating the skin barrier.

Nanotechnology has revolutionized drug delivery and conventional therapy, namely in the field of oncology. In the context of topical administration of repurposed drugs targeting skin cancer, nanoparticle-based therapeutics (lipid-based, polymeric, and inorganic nanoparticles) have bestowed remarkable improvements to skin permeation, therapeutic efficacy, toxicity, and stability. As highlighted in the research examined, various repurposed drugs formulated for topical application were enhanced by their incorporation in nanosystems. The skin permeability of these nanosystems allowed for enhanced antitumor effect on melanoma and non-melanoma research models. In some instances, the nanoparticles acted not only as drug carriers, but also as anticancer agents.

Despite no current commercialization of nano-based topical formulations, the research findings highly evidence the prominence of this approach to complement the currently available therapeutic strategies. I hope to see this treatment option available soon as the inexpensive and convenient nature really drives its applicability.

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