UNIVERSIDADE Ð COIMBRA

1 2 9 0

Bruno Alexandre Pessoa Rama

Relatório de Estágio orientado pelo Dr. Alberto Paulo Carvalho e Monografia intitulada "The Role of Nanotechnology in the Prolonged Release of Drugs by the Subcutaneous Route" orientada pelo Professor Doutor António Ribeiro 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.

Fevereiro de 2023

Bruno Alexandre Pessoa Rama

Relatório de Estágio orientado pelo Dr. Alberto Paulo Carvalho e Monografia intitulada "The Role of Nanotechnology in the Prolonged Release of Drugs by the Subcutaneous Route" orientada pelo Professor Doutor António Ribeiro referentes à Unidade Curricular "Estágio"ž apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Fevereiro de 2023

Declaração de Autoria

Eu, Bruno Alexandre Pessoa Rama, estudante do Mestrado Integrado em Ciências Farmacêuticas, com nº 2017248255, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "The Role of Nanotechnology in the Prolonged Release of Drugs by the Subcutaneous Route" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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

Coimbra, 01 de fevereiro de 2023.

Bruno Alexandre Pessoa Rama

(Bruno Alexandre Pessoa Rama)

Agradecimentos

Quando abraçamos um desafio, a motivação é o que nos faz iniciar o processo; contudo, é a disciplina que permite sermos consistentes e chegar ao fim. Por vezes, é isto que determina a conquista de objetivos.

Este trabalho é o culminar de cinco anos muito exigentes em que muitas decisões foram tomadas, por vezes, em momentos difíceis. A minha motivação na realização deste trabalho e ao longo de todo o meu percurso foi imensa, mas jamais chegaria aqui sem trabalho, esforço, dedicação e disciplina. Contudo, não dependeu só de mim. As pessoas que cruzam a nossa vida desempenham um papel que influencia as nossas decisões. Dedico este trabalho a todos aqueles que me acompanharam neste percurso.

Agradeço ao Professor Doutor António Ribeiro por me ter aceitado enquanto orientando e por me propor este desafio, pela disponibilidade, e por todos os votos de confiança que me foi dando ao longo deste trabalho. Um exemplo de profissionalismo e de ser humano.

Agradeço ao Dr. Paulo Viegas de Carvalho e a toda a equipa da farmácia Santa Clara (Cíntia, Diana, Filipa, Joana Lopes, Joana Rodrigues, Mónica, Pedro e Rita) por terem-me recebido tão bem, pela disponibilidade e paciência que tiveram durante o estágio, e pelas amizades que estabelecemos.

A Fanny Charbonnier, à toute l'équipe REQPHARM (Carol, Thomas, Rand, Beatriz, Suad, Noreddine, Najete et Ana) et à Jana Kas-Kas pour m'avoir aidé depuis le début à m'intégrer à la langue française, et pour tout le support qu'ils m'ont apporté pendant ma période Erasmus. Je le répéterais si c'était possible.

À Jéssica, pela presença constante que teve ao longo deste percurso. Pelo apoio e pela motivação que me deu, mesmo não compreendendo por vezes as minhas convicções. Foram 5 anos marcados por amor, respeito, compreensão, lealdade, e também por algumas frustrações – que enfim, fazem parte, e ajudam-nos a crescer.

Aos meus pais, por nunca terem deixado que nada me faltasse durante o meu percurso académico. Sei que desde o início do curso até agora que as ausências foram aumentando, mas espero que percebam e que estejam orgulhosos.

Ao meu irmão, que por vezes em casa teve de fazer o papel de "irmão mais velho. Apesar de não ter tido a minha devida atenção ao longo destes últimos 5 anos, sempre me viu como um exemplo. Mais tempo virá, miúdo!

Aos pais da minha namorada, que apesar de longe, são como uns "segundos pais". Não me esqueci de ti Joana!

À minha restante família – padrinhos, tios, avós e primos – por sempre me terem encorajado a seguir com as minhas convicções mas também pelos momentos que temos juntos. Sei que continuam a ver-me da mesma forma que me viam há 20 anos atrás, mas pronto. O tempo passa (mas a memórias ficam).

Aos novos amigos que a vida académica trouxe, pelo companheirismo, pela vida boémia, pelo desespero em épocas de exames, mas também pelos festejos das nossas conquistas. Aos velhos amigos, que ainda hoje desempenham um papel bastante ativo na minha vida e que permitem abstrair dos stresses e pressões da vida.

A la Faculté de Pharmacie de l'Université Paris Cité pour m'avoir accueilli dans le cadre du programme Erasmus.

À Faculdade de Farmácia da Universidade de Coimbra, por me ter proporcionado 5 anos fantásticos e inesquecíveis.

A Coimbra.

ÍNDICE GERAL

ÍNDICE DE FIGURAS E TABELAS

PARTE I

Relatório de Estágio em Farmácia Comunitária

Farmácia Santa Clara

SANTA CLARA
farmácia

Sob Orientação do Dr. Paulo Viegas de Carvalho

Lista de Abreviaturas

ARS – Associação Regional de Saúde

CHUC – Centro Hospitalar e Universitário de Coimbra

FSC – Farmácia Santa Clara

MICF – mestrado integrado em ciências farmacêuticas

MNSRM – medicamentos não sujeitos a receita médica

MSRM – medicamento sujeito a receita médica

RCM – resumo das características do medicamento

SWOT – *Strenghts, Weaknesses, Opportunities and Threats*

1. Introdução

A unidade curricular "Estágio" está contemplada no plano de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF), e permite ao aluno contactar com a realidade de trabalho em farmácia comunitária antes terminar os estudos. Para além disso, é uma excelente oportunidade para o aluno colocar em prática conhecimentos teóricos que adquiriu ao longo dos cinco anos de estudos, adquirir novas competências sobretudo a nível técnico, e também para desenvolver competências pessoais e sociais.

O farmacêutico comunitário é, muitas das vezes, o profissional de saúde mais próximo dos cidadãos, e a farmácia as portas que levam o doente a cuidados de saúde mais específicos. Esta proximidade acarreta responsabilidades, não só a nível do acesso seguro, eficaz, e de qualidade ao medicamento [1] mas também a nível da promoção para a saúde da comunidade em geral. Para além de estar sempre em estreita colaboração com os outros profissionais de saúde de modo que o utente tenha o melhor plano terapêutico, o farmacêutico comunitário também é um fator chave relativamente à fluidez nos cuidados de saúde em urgências e hospitais. Se por um lado o farmacêutico tem uma formação académica que lhe permite ter conhecimento científico acerca do aconselhamento de medicamentos não sujeitos a receita médica (MNSRM) e outros produtos de saúde para o tratamento de determinadas patologias e assim diminuir a sobrecarga dos serviços médicos; por outro, ele também tem de ter consciência de que, apesar de ter competências técnico-científicas capazes de resolver determinadas patologias, existem situações que fogem da sua esfera de atuação, e portanto a melhor indicação que pode dar é encaminhar para o médico.

Tive oportunidade de realizar o meu estágio em farmácia comunitária na Farmácia Santa Clara (FSC) entre 17/05/22 e 16/09/22 sob orientação do Dr. Paulo Viegas de Carvalho. A FSC localiza-se no Intermarché Santa Clara em Coimbra e tem um horário de funcionamento extenso, o que permite, em teoria, ter um leque alargado e diversificado de utentes. Inicialmente desempenhei tarefas de *backoffice* tais como receção de encomendas e armazenamento; e após visualizar alguns atendimentos, rapidamente deram-me autonomia para realizá-los numa primeira instância acompanhado e posteriormente sozinho. Neste relatório teremos uma análise do estágio e por fim a exposição de cinco casos clínicos.

2. Análise SWOT

O presente relatório de estágio foi realizado tendo em consideração o modelo de análise SWOT (*Strenghts, Weaknesses, Opportunities and Threats*). Este modelo é uma ferramenta de gestão que permite identificar e analisar o estágio realizado de forma crítica a

nível interno – considerando os pontos fortes e fracos – e a nível externo – considerando as oportunidades e ameaças.

2.1 Pontos Fortes

Ambiente de trabalho e equipa técnica

A equipa técnica da farmácia Santa Clara é constituída atualmente por 9 profissionais onde se incluem 4 farmacêuticos e 5 técnicos superiores de saúde. Desde o primeiro momento que fui bem recebido, inicialmente pelo Dr. Paulo de Carvalho e de seguida pelos restantes elementos da equipa. O ambiente de trabalho existente na FSC é saudável e predomina a clareza, a confiança e a empatia, o que leva a uma boa comunicação e a um bom entendimento entre a equipa. Considero que este aspeto foi crucial para o meu desenvolvimento enquanto estagiário, mas também fundamental para a harmonia no trabalho realizado diariamente na farmácia.

Ferramentas Tecnológicas

Todos nós temos consciência que a tecnologia assume um papel preponderante nas nossas vidas, e a farmácia não é exceção. O *robot* BD Rowa Smart® e a caixa Cashlogy by Azkoyer permite aumentar a eficácia das atividades desempenhadas na FSC e também ter um atendimento mais centrado no utente. Ora o primeiro é possivelmente o grande "*game changer*" de qualquer farmácia uma vez que veio substituir a utilização das gavetas permitindo minimizar todos os erros associados à arrumação de medicamentos e de *stocks*. Para além disso, permite ainda otimizar o espaço de armazenamento de medicamentos na farmácia e o processo de dispensa seguindo o princípio "*first expired first out*". O segundo procura reduzir o tempo investido pelo funcionário a fazer trocos durante o atendimento e na gestão de caixa ao fim do dia. Todas as informações envolvidas na gestão de dinheiro são registadas todos os processos automatizados.

Localização da farmácia

A FSC localiza-se dentro do edifício do Intermarché na Rua Central da Mesura, que por sua vez está próxima de várias extensões de saúde tais como os Centros de Saúde de Santa Clara e São Martinho do Bispo, e o Hospital dos Covões. O facto da FSC encontrar-se dentro de um estabelecimento comercial permite, em teoria, alcançar um leque alargado e diversificado de clientes, nomeadamente os clientes de "circunstância" que estão apenas de passagem no local; e o facto de estar próxima das várias unidades de saúde permite suprimir as necessidades dos utentes que estejam a sair de urgências e de consultas médicas. Para além

disso, como a FSC era a antiga farmácia Duarte, a sua carteira de utentes manteve-se, o que significa que a FSC continua a suprimir as necessidades da população local e de aldeias vizinhas.

Horário de funcionamento e flexibilidade de horário

O facto de se encontrar dentro de um estabelecimento comercial, significa que o seu horário de funcionamento é bastante alargado. A FSC está aberta ao público de segunda-feira a sábado das 09h-21h, e domingos e feriados das 10h-20h. Uma vez que tinha flexibilidade de horário, consegui gerir o estágio de forma a realizar diversos turnos. Tive a possibilidade de fazer aberturas, fechos, fins-de-semana, feriados e serviços noturnos, e relacioná-los com o tipo e fluxo de utentes e respetivas necessidades.

2.2 Pontos Fracos

O papel proativo do farmacêutico

Apesar de, em teoria, a localização da FSC permitir alcançar um público inespecífico com necessidades heterogéneas, a maioria dos utentes pretende apenas adquirir medicação crónica; e foi essa a grande maioria dos atendimentos que realizei. Nestas situações o papel proativo do farmacêutico acaba por ser limitado – e por vezes mal interpretado pelo utente. Apesar de ter tido algumas oportunidades de colocar em prática os meus conhecimentos para um aconselhamento mais adequado, isso apenas correspondeu a uma pequena parte dos atendimentos realizados.

Espaço da farmácia

Apesar das novas instalações acolhedoras e modernas, a FSC não tem grandes dimensões. Esta limitação acaba por prejudicar no aprovisionamento de medicamentos e no atendimento. Relativamente ao primeiro a falta de espaço dificulta armazenamento, gestão e organização de excedentes que não cabem no *robot* ou nos expositores. Relativamente ao segundo, como a FSC dispõe de 6 balcões de atendimento, nem sempre é possível ter um atendimento calmo e claro com o utente respeitando a sua privacidade.

Horário de funcionamento

Ora se um horário alargado permite atrair mais utentes às instalações, por outro lado dificulta a gestão de horários dos funcionários. Apesar de ter tido flexibilidade no horário, procurei sempre compatibilizá-los com as necessidades da equipa da FSC. Por vezes é notável o cansaço dos trabalhadores, e a longo prazo isso pode refletir-se na qualidade das atividades realizadas.

Preparação de manipulados

Na FSC a preparação de manipulados é reduzida, uma vez que, atualmente, estes são adquiridos a farmácias que se dedicam especificamente a este tipo de preparações – uma vez que a sua procura é cada vez mais residual e não é vantajoso em termos económicos para a farmácia ter sempre a matéria-prima disponível. Durante o meu estágio tive a oportunidade de participar na realização de 2 manipulados; no entanto, tinham a mesma formulação, e gostaria de ter tido uma experiência mais ampla nesse sentido.

2.3 Oportunidades

Atendimento e contacto com utentes

Os utentes não todos iguais. Inicialmente a relação com o utente foi muito superficial, uma vez que ainda não dominava o *software* de atendimento nem os processos de dispensa de medicamentos. À medida que fui realizando atendimentos e que o contacto com os utentes ia aumentando, já ia sendo capaz de fazer um atendimento mais personalizado considerando o tipo de utente, as suas necessidades, crenças e convicções; e posteriormente incluindo os objetivos comerciais da farmácia.

Desenvolvimento de competências

A equipa da FSC foi o fator que determinou a minha envolvência durante o período de estágio. Várias competências foram melhoradas tais como trabalho e espírito de equipa, dando principal destaque à comunicação e gestão emocional. O primeiro porque durante o atendimento, o discurso tinha de ser adaptado ao utente que tínhamos connosco – e muitas das vezes a informação e problemas complexos tinham de ser decompostos para se tornarem de mais simples compreensão para o utente; e o segundo porque existem utentes que por vezes não têm a melhor abordagem para connosco – o que significa que temos de controlar as nossas emoções, manter a postura e a serenidade durante o atendimento.

Dispensa de medicamentos hospitalares

Desde 7 de abril de 2020, devido à pandemia de SARS-CoV-2, o governo permitiu dispensar em ambulatórios medicamentos que outrora eram de dispensa exclusiva nos serviços farmacêuticos hospitalares. Este serviço permite melhorar a acessibilidade dos medicamentos aos utentes, e permitiu ter contacto com medicação que, anteriormente, era apenas de dispensa exclusiva hospitalar.

Horário noturno

Todos os dias da semana existem duas farmácias de serviço noturno. O escalonamento anual das farmácias de serviço encontra-se no *website* da Associação Regional de Saúde (ARS) Centro. Durante o meu estágio tive oportunidade realizar algumas vezes o horário noturno. A grande maioria dos casos nesse período eram de utentes que vinham do serviço de urgências do Centro Hospitalar e Universitário de Coimbra (CHUC).

2.4 Ameaças

A desvalorização do farmacêutico

Uma questão que, a meu ver, não é recente. Após o primeiro impacto com determinados utentes, é possível percecionar a desvalorização e o pouco reconhecimento do papel do farmacêutico durante o atendimento. É complicado encontrar uma relação entre estas situações e o tipo de utentes. Contudo, é frequente acontecer com utentes que dizem estar com pressa e/ou quando já fazem a mesma medicação há muitos anos, e com utentes que de alguma forma estão envolvidos em ambientes relacionados com saúde.

A desvalorização do estagiário

Se por um lado o farmacêutico já sente dificuldades em ser proativo e reconhecido durante o atendimento com determinados utentes; por outro lado temos a falta de confiança depositada pelos utentes nos estagiários. Muitas das vezes fui confrontado com utentes que duvidavam dos medicamentos dispensados face à prescrição que traziam – mesmo cedendo aqueles que tinham levado anteriormente. Para além disso, destaco ainda a recusa por parte de alguns utentes em serem atendidos por estagiários ouvindo algumas expressões, por vezes, menos agradáveis.

Os medicamentos genéricos

Algo que nem sempre é fácil de explicar. Ainda é notório o desconhecimento por parte da população em saber o que é ao certo um medicamento genérico. A maioria tem a perceção que o medicamento genérico é a "marca branca" ou "o medicamento igual, mas mais fraco". Destaco em particular uma situação que ocorreu com o medicamento Zarator® que esteve esgotado durante quase todo o meu período de estágio, e houve utentes que preferiam não levar medicamento genérico mesmo sabendo que iam ficar sem medicação.

Concorrência e locais de venda de medicamentos não sujeitos a receita médica (MNSRM)

Podemos considerar 3 farmácias concorrentes à FSC: a Farmácia Donato, a Farmácia do Fórum e a Farmácia da Guarda Inglesa. Durante o atendimento com alguns utentes, ouvimos comparações entre a FSC e as restantes – muitas delas sem fundamento e outras que percebemos de imediato que não são verdade. Deste modo, a FSC teve de adotar estratégias de forma a atrair novos clientes – sobretudo em determinados nichos – mas também para os fidelizar – através de cartões de fidelização. Considerando os locais de venda de MNSRM, a maior ameaça é o facto de praticarem preços altamente competitivos e que por vezes as farmácias não conseguem responder da melhor forma. Isto pode levar à perda de utentes no que diz respeito à aquisição desses medicamentos e outros produtos de venda livre, e por outro lado aumenta a probabilidade de ocorrer interações medicamentosas que o utente pode associar a efeitos secundários da medicação que comprou na farmácia.

Medicamentos esgotados

Infelizmente, os utentes associam a escassez de medicamentos à falta de empenho que a farmácia tem em satisfazer as suas necessidades. No entanto, nada disso depende dos profissionais que lá trabalham, mas sim de motivos alheios. Esta situação nem sempre é fácil de explicar à maioria dos utentes, levando por vezes a perder uma potencial venda. Estas situações foram particularmente frequentes com os medicamentos Zarator® Trental® e Ozempic®.

Local de saúde ou superfície comercial?

Na teoria as farmácias são locais de saúde e são na grande maioria das vezes o primeiro sítio onde os utentes se deslocam antes de irem ao médico ou urgências. Por isso, devemos sempre dar a melhor resposta possível aos utentes considerando sempre as suas necessidades, daí tentar-se instituir sempre um atendimento personalizado – que às vezes é *time consuming*. No entanto, a faturação de uma farmácia depende essencialmente de vendas. Ou seja, quanto mais tempo investirmos num atendimento, menor será o número de vendas num determinado período. Isto leva-nos a refletir acerca do *modus operandi* do farmacêutico comunitário. Considerando uma farmácia com uma um número considerável de utentes a aguardar pela sua vez: se por um lado eu devo ter um atendimento focado no utente; por outro tenho de pensar que existem utentes que podem cansar-se de aguardar e assim perder potenciais vendas, acabando por não lhes suprimir as necessidades.

3. Casos Clínicos

Caso Clínico I

Uma senhora dirigiu-se à farmácia queixando-se que desde sempre sentiu sensação de pernas cansadas e inchadas, mas que ultimamente devido ao calor não consegue suportar. A senhora fez questão de mostrar as pernas e verificou-se que o edema era evidente e não havia sinais de trombos nem de vermelhidão considerável. De seguida decidi questioná-la para tentar contextualizar a situação: disse ser cabeleireira desde sempre, não faz nenhuma medicação para além da contraceção oral, tem alguma dificuldade em beber água e não pratica atividade física.

Avaliando a situação, recomendei à senhora a toma diária de Daflon® 1000 mg e a aplicação de Allestax® Gel 2 vezes por dia massajando de baixo para cima. O Daflon® 1000 mg pertence ao grupo de venotónicos naturais, mais concretamente à Fração Flavonoica Purificada Micronizada. Atuam a nível da macro- e micro- circulação e exerce efeito a nível do tónus venoso, na permeabilidade capilar e no sistema linfático [2]. O facto de ser micronizado promove uma melhor absorção intestinal e consequente um melhor efeito. O Allestax® é um gel à base de extrato de Folha de Videira Vermelha e contém óleo de hortelã-pimenta e mentol e promove uma sensação refrescante [3]. Aconselhei ainda para fazer pequenas caminhadas diariamente para promover a circulação linfática, para se deitar com as pernas levantadas, e para utilizar meias de descanso durante o trabalho.

A senhora optou por levar apenas o Daflon® 1000 mg porque já tinha em casa um gel refrescante que utilizava pontualmente, e que ia seguir as recomendações não farmacológicas relativamente à caminhada e ao deitar com as pernas levantadas. Na semana seguinte, a mesma senhora voltou à farmácia a dizer que sentiu algumas melhoras e que agora queria levar o Allestax® Gel porque o gel que tinha em casa acabou e também as meias de descanso.

Caso Clínico II

Um jovem adulto com não mais de 30 anos deslocou-se à farmácia porque sentia dores musculares nas pernas e queria algo que o ajudasse uma vez que essas dores o incomodavam no seu dia-a-dia. Questionei-lhe acerca da duração das dores ao que me respondeu que tinham começado no dia anterior porque tinha ido jogar futebol com os amigos (que era habitual irem 2 vezes por semana) mas que se esforçou um bocado mais que o normal. Para além disso ainda mencionou que praticava ginásio 3 vezes por semana. Perguntei se era a primeira vez que esta situação acontecia e se tomava alguma medicação ou suplementação, ao que me respondeu "não" às questões.

A situação relatada pareceu associada ao esforço muscular. Recomendei-lhe a toma de Magnesium-K Active® uma vez que o seu estilo de vida surge associado a atividade física intensa e é normal haver necessidades aumentadas de micronutrientes que se não forem correspondidas podem comprometer o processo de recuperação muscular [4]. Contudo, salientei que seria normal continuar com dores após as primeiras tomas. Recomendei ainda a prática de uma alimentação saudável que correspondesse às suas exigências, e ter bons hábitos de sono.

Caso Clínico III

Uma jovem utente apresentou-se na farmácia num estado muito nervoso e inseguro. Diz sentir comichão nas suas zonas íntimas e alguma dor quando urina, mas não sempre. Questionei-lhe de seguida se tinha algum corrimento anormal, se havia sinais de "vermelhidão" e algum odor desagradável incomum. Respondeu-me, respetivamente, que tinha um corrimento branco "meio pastoso"; tinha alguma vermelhidão, mas não sabia se isso seria de se coçar muito porque parecia uma queimadura; e que em termos de odor estava normal. Reunindo toda esta informação – comichão, vermelhidão, corrimento branco e espesso, sensação de queimadura, e alguma dor ao urinar – temos um quadro semelhante a uma candidíase vaginal [5].

Ao transmitir esta informação à jovem, ela disse-me que de facto já teve uma candidíase "há uns tempos" e que tinha levado uns comprimidos vaginais, mas tinha consciência que houve dias que não os aplicava. Deste modo recomendei-lhe Gino-Canesten® 1 cápsula mole vaginal. Expliquei-lhe que é um tratamento de aplicação única e que dentro de 2-3 dias já haveria melhoria significativa dos sintomas [6].

De seguida, a utente questionou-me se a candidíase era transmissível para o homem ou se era apenas entre mulheres. Respondi que também é possível os homens contraírem candidíase apesar de ser menos comum e de poderem sentir alguns sintomas semelhantes. A jovem perguntou o que podia levar para o namorado uma vez que teve relações sexuais desprotegidas com ele nos últimos dias. Aconselhei então a aplicação 2-3x por semana de Gino-Canesten® creme durante duas semanas [7].

Por fim, alertei para evitarem relações sexuais e alimentos açucarados durante o período de tratamento, e em particular para a utente, aplicar a cápsula quando se for deitar o mais profundo possível e utilizar alguma proteção adicional para evitar sujar com eventuais derrames de produto.

Caso Clínico IV

Um utente chegou à farmácia com alguma pressa dizendo que pretendia uns comprimidos para dormir iguais aos que um amigo costuma levar. Após terminar de falar, colocou parte da cartonagem em cima do balcão; e correspondia ao oxazepam 15 mg. Expliquei ao senhor de forma muito sucinta que não lhe podia dispensar aquele medicamento porque era um MSRM, e o facto do amigo os utilizar não significa que sejam (a melhor solução) para adormecer e melhorar a qualidade do sono.

O senhor ficou desapontado com a resposta e disse que já tinha experimentado "muita coisa" natural para dormir e nada funcionava; e que por vezes tinha de ligar a televisão a meio da noite para ver se lhe "dava o sono". Em desespero por ter compreendido que não iria dispensar o medicamento solicitado, acrescentou ainda que ia de viagem e tem dificuldades em adormecer fora de casa.

Ouvindo estas últimas informações supos que se tratasse de um caso de insónia pontual devido ao nervosismo associado ao facto de saber que ia dormir fora de casa. Decidi então questioná-lo acerca disso, ao que me respondeu "seria bem possível porque ia sozinho e não estava a conseguir concentrar-se nas suas tarefas diárias". Decidi então sugerir-lhe a toma 3x/dia de Valdispertstress® que contém 200 mg de extrato de raiz de valeriana e 68 mg de extrato de estróbilo de lúpulo. Estas substância já foram sujeitas a estudos e demonstraram evidência na melhoria de sintomas associados à ansiedade e stress [8,9]. Indiquei-lhe também a toma de Dormidina® 25 mg, que é constituído por succinato de doxilamina, um anti-histamínico de primeira geração com elevada capacidade sedativa utilizado no tratamento a curto prazo da insónia [10,11].

Por fim referi a importância da higiene do sono e que existem determinados comportamentos que devíamos adotar e outros que devíamos evitar – de forma gradual – para melhorar a qualidade do sono: estabelecer um horário regular de deitar e acordar, evitar dispositivos eletrónicos que imitam luz azul 1 h antes de deitar, não consumir substâncias estimulantes tais como alimento açucarados e café nas 6 h antes de se deitar e criar um "ritual" que pudesse relacionar o momento antes de deitar [12].

Caso Clínico V

Uma utente veio à farmácia com uma prescrição médica para comprar a sua "medicação habitual" e queixa-se de que na última semana tem sentido muita comichão e não sabe porquê porque nunca teve alergias nem rinites. Disse que a sua medicação era toda igual exceto o Eliquis® 2,5 mg que estava a fazer à pouco mais de um mês. Questionou-me se o problema estava associado a este novo medicamento porque nota que pouco depois de o

tomar fica com comichão; no entanto, nas primeiras semanas de tratamento nunca sentiu nada disso.

A utente em questão era polimedicada e incluíam-se no seu plano terapêutico, para além do Eliquis® 2,5 mg medicamentos anti hipertensores, antidiabéticos, e moléculas com atividade no sistema nervoso central. Salientando o facto de que foi apenas após inserir o Eliquis® 2,5 mg no seu plano terapêutico que começou a sentir a comichão, procurei informação no resumo das características do medicamento (RCM) e de facto a comichão é um efeito secundário pouco frequente (afeta cerca de 1 em cada 100 pessoas) [13,14].

Expliquei a situação à utente, dizendo que a melhor solução seria falar com a sua médica de modo a adaptarem uma estratégia segura e eficaz que permitisse reduzir a comichão. Na semana seguinte, a utente surge novamente na farmácia com uma nova prescrição que tinha loratadina 10 mg [15]. A médica considerou que a comichão seria de facto um efeito secundário do medicamento. A posologia indicava tomar a loratadina 10 mg 2x/dia após cada toma de Eliquis® 2,5 mg.

4. Conclusão

O estágio na FSC foi o culminar de cinco intensos anos de formação teórica e prática proporcionada pelo MICF. Durante o período de estágio tive oportunidade de colocar os conhecimentos que fui adquirindo ao longo do curso, mas também de desenvolver outras competências – nomeadamente a nível pessoal – que permitem enriquecer a atividade farmacêutica.

Considero que a experiência que tive na FSC enquanto estagiário foi indubitavelmente enriquecedora, e permitiu-me ajustar a visão que tinha acerca do dia-a-dia da farmácia comunitária. Apesar do atendimento ser a atividade em que o farmacêutico mais se pode destacar, existe um conjunto de outras tarefas de *backoffice* que devem ser harmoniosamente organizadas e realizadas para não comprometer a fluidez do trabalho dos outros profissionais. Estes aspetos apenas são percebidos quando se chega ao terreno, uma vez que a realidade de trabalho em nada se assemelha à realidade académica.

Também foi percetível ao longo do estágio a dicotomia do papel que o farmacêutico assume relativamente à promoção da saúde: se por um lado somos o primeiro profissional de saúde que o utente procura antes de se deslocar aos centros de saúde e urgências; por outro somos encarados como "alguém que está a tentar vender". A realidade é que o farmacêutico comunitário tem de saber desempenhar os dois papeis: é verdade que devemos defender sempre os interesses do utente [1]; mas também é verdade que grande parte da faturação da farmácia depende de vendas, e se não considerarmos os objetivos económicos e comerciais

da farmácia corre-se o risco de alterar o equilíbrio e a sustentabilidade do negócio. Esta última visão não é devidamente proporcionada pelo MICF.

Em suma, quero agradecer novamente à equipa da FSC pela experiência de estágio que me proporcionaram. A integração na equipa foi sem dúvida uma mais-valia para o desenvolvimento de competências. Esta experiência permitiu-me explorar aquela que é saída profissional que mais farmacêuticos emprega, e perceber a importância que este profissional assume na comunidade. Foi um desafio que me obrigou a sair da zona de conforto e que me deu ferramentas para mais tarde entrar no mercado de trabalho.

5. Bibliografia

[1] Código Deontológico da Ordem dos Farmacêuticos. Ordem dos Farmacêuticos. https://www.ordemfarmaceuticos.pt/pt/noticias/novo-codigo-deontologico-da-ordem-dos-farmaceuticos/. (August, 2022)

[2] Ramelet AA. Clinical Benefits of Daflon 500 mg in the Most Severe Stages of Chronic Venous Insufficiency. Angiology. 2001;52:S49–S56.

[3] Committee on Herbal Medicinal Products (HMPC). European Union herbal monograph on Vitis vinifera L., folium, EMA/HMPC/464684/2016. https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-vitis-vinifera-l-folium-revision-1_en.pdf. (June, 2022)

[4] Jordan SL, Albracht-Schulte K, Robert-McComb JJ. Micronutrient deficiency in athletes and inefficiency of supplementation: Is low energy availability a culprit? PharmaNutrition. 2020;14:100229.

[5] Gino-Canesten cápsula mole vaginal | Canesten. 2022. https://www.antifungicos.bayer.pt/produtos/saude-intima-feminina/gino-canesten-capsula-mole-vaginal. (June, 2022)

[6] Resumo das Características do Medicamento: Gino-Canesten, 500 mg, Cápsula mole vaginal. https://www.bayer.com/sites/default/files/2020-11/Gino-canesten.pdf. (June, 2022)

[7] Resumo das Características do Medicamento: Gino-Canesten 10 mg/g creme vaginal https://www.bayer.com/sites/default/files/2022-01/Gino-Canesten%20creme%20vaginal_RCM.pdf. (June, 2022)

[8] Committee on Herbal Medicinal Products (HMPC). European Union herbal monograph on Valeriana officinalis L., radix, EMA/HMPC/150848/2015. https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-valeriana-officinalis-l-radix_en.pdf. (July, 2022)

[9] Committee on Herbal Medicinal Products (HMPC). Community herbal monograph on Humulus lupulus L., EMA/HMPC/682384/2013. https://www.ema.europa.eu/en/documents/herbal-monograph/final-community-herbal-monograph-humulus-lupulus-l-flos-revision-1_en.pdf. (July, 2022)

[10] Melnikov AYu, Lavrik SYu, Bikbulatova LF, et al. Effectiveness of reslip (doxylamine) in short-term insomnia: multicenter comparative randomized study. Zhurnal nevrologii i psikhiatrii im SS Korsakova. 2017;117:56.

[11] Resumo das Características do Medicamento: Dormidina 25 mg comprimidos revestidos por película. https://farmacia24.eu/content/MNSRM/5431713.pdf. (July, 2022)

[12] Healthy Sleep Habits. AASM | Sleep Education. 2020 https://sleepeducation.org/healthy-sleep/healthy-sleep-habits/. (July, 2022)

[13] Resumo das Características do Medicamento: Eliquis 25 mg comprimidos revestidos por película. https://www.ema.europa.eu/en/documents/product-information/eliquis-epar-product-information_pt.pdf. (July, 2022)

[14] Nassif D, Weatherton N, Nassif A. Medication-Induced Pruritus From Direct Oral Anticoagulants. Fed Pract. 2017;34:36–37.

[15] Folheto Informativo: Claritine 10 mg comprimidos. https://www.claritine.pt/sites/g/files/vrxlpx35581/files/2022-12/ClaritineComp_FI_MNSRM-EF_10.2022.pdf. (July, 2022)

Parte II

Monografia

"The Role of Nanotechnology in the Prolonged Release of Drugs by the Subcutaneous Route"

FACULDADE DE FARMÁCIA
UNIVERSIDADE Ð
COIMBRA

Sob orientação do Professor Doutor António José Ribeiro

List of abbreviations

AB – antibody

AI – artificial intelligence

ANN – artificial neural networks

APC – antigen-presenting cells

BGL – blood glucose level

BMP-2 – bone morphogenetic protein 2

C_{max} – maximum concentration

CINV – chemotherapy-induced nausea and vomiting

CKD – chronic kidney disease

CQA – critical quality attribute

ds-siRNA – double-stranded small interfering RNA

EMA – European Medicines Agency

ECM – extracellular matrix

EPR – enhanced permeability and retention

EX – exenatide

FBR – foreign body response

FDA – Food and Drug Administration

FPP – folate-polyethyleneimine conjugated poly(organophosphazene)

GLP-1 – glucagon-like peptide-1

HIV – human immunodeficiency virus

ICH – International Committee on Harmonization

IFN – interferon

IL – interleukin

IL-4Rα – α subunit of interleukin 4 receptor

IL-5Rα – α subunit of interleukin 5 receptor

IL-13Rα – α subunit of interleukin 13 receptor

IL-17Rα – α subunit of interleukin 17 receptor

IM – intramuscular

IV – intravenous

ML – machine learning

mRNA – messenger RNA

NS1 – nonstructural protein 1

PAEU – poly(β-amino urethane)

PCL – polycaprolactone

PCLA – poly(ε-caprolactone-co-lactide)

PDI – polydispersity index

PEG – polyethylene glycol

PHBV – poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

PLGA – poly(lactic-co-glycolic acid)

PS – particle size

PTX – paclitaxel

QbD – quality-by-design

QTPP – quality target product profile

R&D – research and development

rHuPH20 – recombinant human hyaluronidase PH20

SCT – salmon calcitonin

SC – subcutaneous

T1D – type 1 diabetes

T2D – type 2 diabetes

t_{max} – time it takes for a drug to reach the maximum concentration after administration

ABSTRACT

Subcutaneous physiology is distinct from other parenteral routes that benefit the administration of prolonged-release formulations. A prolonged-release effect is particularly convenient for treating chronic diseases because it is associated with complex and often prolonged posologies. Therefore, drug-delivery systems focused on nanotechnology are proposed as alternatives that can overcome the limitations of current therapeutic regimens and improve therapeutic efficacy.

This review presents an updated systematization of nanosystems, focusing on their applications in highly prevalent chronic diseases. Subcutaneous-delivered nanosystem-based therapies comprehensively summarize nanosystems, drugs, and diseases and their advantages, limitations, and strategies to increase their translation into clinical applications. An outline of the potential contribution of quality-by-design (QbD) and artificial intelligence (AI) to the pharmaceutical development of nanosystems is presented.

Although recent academic research and development (R&D) advances in the subcutaneous delivery of nanosystems have exhibited promising results, pharmaceutical industries and regulatory agencies need to catch up. The lack of standardized methodologies for analyzing *in vitro* data from nanosystems for subcutaneous administration and subsequent *in vivo* correlation limits their access to clinical trials. There is an urgent need for regulatory agencies to develop methods that faithfully mimic subcutaneous administration and specific guidelines for evaluating nanosystems.

KEYWORDS: chronic disease; nanoparticle; nanosystem; prolonged release; subcutaneous route.

RESUMO

A fisiologia do tecido subcutâneo é distinta de outras vias parentéricas que beneficiam da administração de formulações de libertação prolongada. Um efeito de libertação prolongada é particularmente conveniente para o tratamento de doenças crónicas uma vez que surgem associadas a posologias complexas e frequentemente prolongadas. Assim, sistemas de administração de medicamentos de base nanotecnológica são propostos como alternativas que podem superar as limitações dos tratamentos atuais e melhorar a eficácia terapêutica.

Este trabalho apresenta uma sistematização atualizada dos nanossistemas, focando as suas aplicações em doenças crónicas altamente prevalentes. As terapias baseadas em nanossistemas subcutâneos resumem de forma detalhada os nanossistemas, fármacos e doenças e as suas vantagens, limitações, e estratégias para aumentar a sua translação para aplicações clínicas. É também apresentada uma parte dedicada à potencial contribuição *Quality-by-Design* (QbD) e da inteligência artificial (IA) para o desenvolvimento farmacêutico de nanossistemas.

Embora os avanços recentes na investigação e desenvolvimento académico (I&D) na entrega subcutânea de nanossistemas tenham apresentado resultados promissores, as indústrias farmacêuticas e as agências reguladoras não acompanham o ritmo. A falta de metodologias padronizadas para análise de dados *in vitro* de nanossistemas para administração subcutânea e subsequente correlação *in vivo* limita o seu acesso a ensaios clínicos. Há uma necessidade urgente de as agências reguladoras desenvolverem métodos que representem fielmente a administração subcutânea e também *guidelines* específicas para avaliar os nanossistemas.

PALAVRAS-CHAVE: doenças crónicas; libertação prolongada; nanopartículas; nanossistemas; via subcutânea.

1. **Introduction**

The parenteral route of administration can be subdivided into three primary modalities: intramuscular (IM), intravenous (IV), and subcutaneous (SC), and is the most commonly used route for drugs when *per os* administration is not available. This route of administration has many advantages, including first-pass metabolism effect avoidance, high bioavailability, and reliable pharmaceutical dosage forms [1]. It can be the preferred route in emergencies or for noncooperative patients; however, not all parenteral routes provide the same onset of action, as the IV route provides direct access to the systemic circulation. In contrast, the IM and SC routes can promote slow drug release based on their anatomy and physiology [1]. Nevertheless, as SC tissue has fewer blood vessels than muscle tissue compared with the IM route, it can provide a better prolonged effect on drug release [2].

Given its characteristics, such as the convenience of administration [3], generally slower absorption profile [2], and lower immunogenicity after administration [4], interest in the SC route has been growing in recent years. Table 1 shows the increasing number of formulations approved by the Food and Drug Administration (FDA) for the SC route in the last 6 years, with 2021 being the year with the highest approval. However, these formulations have focused on high-molecular-weight drugs, such as semaglutide and dasiglucagon, and biotechnology-based drugs, such as monoclonal antibodies.

Nanotechnology has emerged as a potential solution to overcome the limitations of conventional drug-delivery systems, such as immediate drug release, poor therapeutic target efficacy, and unfavorable posology often associated with frequent drug administration [5]. In 1995, the FDA approved Doxil®, the first nanomedicine used for treating AIDS-related Kaposi's sarcoma. Since then, many others have been approved by the FDA and European Medicines Agency (EMA) (Table 2). However, the rhythm of the approval of nanosystem-based medicines by regulatory authorities has not followed great research and development (R&D) advances, and many do not even reach clinical trials. Therefore, there is a need to identify and characterize major barriers that may compromise the success of nanosystem-based medicine development. The idea of combining nanosystem-based medicines with prolonged-release properties has emerged to develop delivery systems with improved bioavailability and safety profiles [6]. Although prolonged-release nanosystems have been studied extensively, there are still gaps in the literature regarding their application in drug delivery via the SC route.

The relationship between nanotechnology and SC administration may play a fundamental role in developing therapeutic options for diseases that already benefit from

Table 1: Drugs approved by the FDA's Center for Drug Evaluation and Research (CDER) for SC use in the last six years.

Approval year	Trade name (Company)	DCI	Class	Target	Indication
2022	Rolvedon® (Spectrum Pharmaceuticals)	Eflapegrastim-xnst	Recombinant human granulocyte colony-stimulating factor	Granulocyte colony-stimulating factor receptors	Reduce the incidence of infection due to neutropenia
2022	Anvuttra® (Alnylam Pharmaceuticals)	Vutrisiran	ds-siRNA	Mutant and wild-type transthyretin mRNA	Polyneuropathy of hereditary transthyretin amyloidosis
2022	Mounjaro® (Eli Lilly and Co.)	Tirzepatide	GLP-I and GIP agonist	GLP-I	Type 2 diabetes mellitus
2021	Zegalogue® (Zealand Pharma)	Dasiglucagon hydrochloride	Glucagon agonist	Glucagon receptor	Hypoglycemic episodes in diabetes
2021	Empaveli® (Apellis Pharms)	Pegcetacoplan	Complement inhibitor	C3 protein	Paroxysmal nocturnal hemoglobinuria
2021	Skytrofa® (Ascendis Pharma)	Lonapeg somatropin-tcgf	Human growth hormone	Growth hormone receptor	Growth failure
2021	Besremi® (Pharmaessentia)	Ropeginterferon α-2B-NJFT	Type I IFN	IFN-α receptor	Polycythemia vera
2021	Voxzogo® (Biomarin Pharm)	Vosoritide	C-type natriuretic peptide	Natriuretic peptide receptor B	Achondroplasia
2021	Tezspire® (AstraZeneca AB)	Tezepelumab-Ekko	Human monoclonal AB (IgG2λ)	Thymic stromal lymphopoietin	Severe Asthma
2021	Leqvio® (Novartis)	Inclisiran	mRNA	PCSK9	Hypercholesterolemia
2021	Adbry® (Leo Pharma AS)	Tralokinumab-ldrm	Human monoclonal AB (IgG4)	IL-I3R α I and IL-I3R α2	Atopic dermatitis
2020	Sogroya® (Novo Nordisk)	Somapacitan-beco	Human growth hormone	Growth hormone receptor	Growth failure
2020	Oxlumo® (Alnylam Pharms)	Lumasiran sodium	ds-siRNA	HAOI mRNA	Hyperoxaluria type I
2020	Imcivree® (Rhythm)	Setmelanotide acetate	Pro-opiomelanocortin derived peptide	Melanocortin 4 receptor	Obesity

Year	Brand (Company)	Type	Target	Indication
2019	Evenity® (Amgen)	Humanized monoclonal AB (IgG2)	Sclerostin	High-risk osteoporosis in postmenopausal women
2019	Skyrizi® (Abbvie)	Humanized monoclonal AB (IgG1)	p19 subunit of IL-23	Tuberculosis
2019	Vyleesi® (Palatin Technologies)	Melanocortin agonist	Melanocortin receptor	Hypoactive sexual desire in premenopausal women
2019	Scenesse® (Clivunel)	Melanocortin I agonist	Melanocortin I receptor	Pain reliever in erythropoietic protoporphyria
2019	Reblozyl® (Celgene Corp)	Erythroid maturation agent	TGF-β superfamily ligands	Beta talassemia anemia
2019	Givlaari® (Alnylam Pharmis)	ds-siRNA	Aminolevulinate synthase I mRNA	Acute hepatic porphyria
2018	Crysvita® (Ultragenyx Pharma)	Human monoclonal AB (IgG1)	Fibroblast growth factor 23	X-linked hypophosphatemia
2018	Aimovig® (Amgen)	Human monoclonal AB (IgG2)	Calcitonin gene-related peptide	Migraine
2018	Palynziq® (Biomarin Pharma)	PEGylated phenylalanine ammonia-lyase	Phenylalanine	Adult phenylketonuria
2018	Takhzyro® (Dyax Corp)	Human monoclonal AB (IgG1/κ-light chain)	Plasma kallikrein	Hereditary angioedema
2018	Ajovy® (Teva Pharms USA)	Humanized monoclonal AB (IgG2Δa/kappa)	Calcitonin gene-related peptide	Migraine
2018	Emgality® (Eli Lilly and Co.)	Humanized monoclonal AB (IgG4)	Calcitonin gene-related peptide	Migraine
2018	TegSedi® (Akcea Theraps)	Antisense oligonucleotide	Transthyretin mRNA	Polyneuropathy of hereditary transthyretin amyloidosis
2017	Siliq® (Valeant Luxembourg)	Human monoclonal AB (IgG2κ)	IL-17Rα	Plaque psoriasis
2017	Dupixent® (Regeneron)	Human monoclonal AB (IgG4)	IL-4Rα	Atopic dermatitis
2017	Tymlos® (Radius Health)	Analog of human parathyroid hormone-related peptide	Parathyroid hormone - I receptor	High-risk osteoporosis in postmenopausal women

2017	Kevzara® (Sanofi Synthelabo)	Sarilumab	Human monoclonal AB (IgG1)	IL-6 receptors	Rheumatoid arthritis
2017	Tremfya® (Janssen Biotech)	Guselkumab	Human monoclonal AB (IgG1λ)	p19 subunit of IL-23	Plaque psoriasis
2017	Fasenra® (AstraZeneca AB)	Benralizumab	Humanized monoclonal AB (IgG1/κ-class)	IL-5Rα	Severe asthma with an eosinophilic phenotype
2017	Hemlibra® (Genentech)	Emicizumab-kxwh	Humanized monoclonal AB (IgG4)	Factors IXa and X	Hemophilia A
2017	Ozempic® (Novo Nordisk)	Semaglutide	GLP-1 analog	GLP-1 receptor	Type 2 diabetes mellitus

AB: antibody; ds-siRNA: double-stranded small interfering RNA; GLP: glucagon-like peptide; IFN: interferon; IL: interleukin; IL-4Rα: α subunit of interleukin 4 receptor; IL-5Rα: α subunit of interleukin 5 receptor; IL-13Rα: α subunit of interleukin 13 receptor; IL-17Rα: α subunit of interleukin 17 receptor.

Table 2: Clinically approved nanosystem-based therapies and diagnostics, grouped by nature and chronological order [6,8–11].

Brand name (Company)	Nature	Active ingredient	Route	Approved application/indication	First approval
Infed® (Actavis Pharma)	Inorganic	Iron dextran	IV IM	Iron deficiency anemia in CKD	FDA 1992
Dexferrum® (American Regent)	Inorganic	Iron dextran	IV	Iron deficiency anemia in CKD	FDA 1996
Ferrlecit® (Sanofi-Aventis)	Inorganic	Sodium ferric gluconate	IV	Iron deficiency anemia in CKD	FDA 1999
Venofer® (Luitpold Pharm)	Inorganic	Iron sucrose	IV	Iron deficiency anemia in CKD	FDA 2000
Feraheme® (AMAG Pharmaceuticals)	Inorganic	Ferumoxytol	IV	Iron deficiency anemia in CKD	FDA 2009
Injectafer® (Vifor)	Inorganic	Iron carboxymaltose colloid	IV	Iron deficient anemia	FDA 2013
Ambisome® (NeXstar Pharmaceuticals)	Lipid-based	Amphotericin B	IV	Fungal infections	EMA 1990
Abelcet® (Defiante Farmaceutica)	Lipid-based	Amphotericin B	IV	Fungal infections	FDA 1995
Doxil® (Johnson & Johnson)	Lipid-based	Doxorubicin pegylated	IV	HIV-associated Kaposi's sarcoma, metastatic ovarian cancer, and multiple myeloma	FDA 1995
Caelyx® (Janssen Pharmaceuticals)	Lipid-based	Doxorubicin pegylated	IV	HIV-associated Kaposi's sarcoma, metastatic ovarian cancer, and multiple myeloma	EMA 1996
DaunoXome® (Galen Ltd.)	Lipid-based	Daunorubicin	IV	Advanced HIV-related Kaposi's sarcoma	FDA 1996
Inflexal® (Crucell Berna Biotech)	Lipid-based	Inactivated influenza virus vaccine	IM	Prevents influenza infection	EMA 1997
Myocet® (Teva)	Lipid-based	Doxorubicin hydrochloride	IV	Metastatic breast cancer in adult women	EMA 2000
Visudyne® (QLT Phototherapeutics)	Lipid-based	Photosensitizer benzoporphyrin	IV	Choroidal neovascularization caused by wet age-related macular degeneration	FDA 2000

Product (Company)	Type	Active	Route	Indication	Approval
Definity® (Lantheus Medical Imaging)	Lipid-based	Perflutren	IV	Ultrasound contrast agent	FDA 2001
SonoVue® (Bracco Imaging)	Lipid-based	Phospholipid stabilized microbubble	IV	Ultrasound contrast agent	EMA 2001
Zevalin® (Bayer)	Lipid-based	90Y-ibritumomab tiuxetan	IV	Low-grade or follicular B-cell non-Hodgkin's lymphoma	FDA 2002
Marqibo® (Talon Therapeutics)	Lipid-based	Vincristine	IV	Philadelphia chromosome-negative acute lymphoblastic leukemia	FDA 2012
Lipodox® (Sun Pharma Global FZE)	Lipid-based	Doxorubicin hydrochloride	IV	HIV-associated Kaposi's sarcoma and metastatic ovarian cancer	FDA 2013
Onivyde® (Merrimack Pharmaceuticals)	Lipid-based	Irinotecan	IV	Metastatic adenocarcinoma of the pancreas	FDA 2015
Lipusu® (Luye Pharmaceuticals)	Lipid-based	Paclitaxel	IV	Breast cancer, non-small cell lung cancer, and ovarian cancer	China 2003
Vyxeos® (Jazz Pharmaceuticals)	Lipid-based	Cytarabine and daunorubicin	IV	Acute myeloid leukemia with or without myelodysplasia-related changes	FDA 2017
Comirnaty® (Pfizer)	Lipid-based	mRNA vaccine	IM	Prevents coronavirus disease 2019	FDA 2020
Spikevax® (ModernaTX Inc.)	Lipid-based	mRNA vaccine	IM	Prevents coronavirus disease 2019	FDA 2020
Epaxal® (Crucell Berna Biotech)	Nanocrystals	Inactivated hepatitis A virus vaccine	IM	Prevents hepatitis infection	Switzerland 1994
Ivemend® (Merk & Co.)	Nanocrystals	Fosaprepitant dimeglumine	IV	Prevention of nausea and vomiting	FDA 2008
Invega Sustenna® (Janssen Pharmaceuticals)	Nanocrystals	Paliperidone palmitate	IM	Schizophrenia	FDA 2009
Ryanodex® (Eagle pharm)	Nanocrystals	Dantrolene sodium	IV	Malignant hyperthermia	FDA 2014
Diprivan® (Fresenius Kabi)	Polymeric	Propofol	IV	Induce or maintenance of anesthesia or sedation	FDA 1989
Copaxone®FOGA (Teva Pharmaceutical)	Polymeric	Glatiramer acetate	SC	Multiple sclerosis	FDA 1996

Product (Company)	Type	Drug	Route	Indication	Approval
PegIntron® (Merk & Co.)	Polymeric	Alpha interferon molecule	SC	Hepatitis C	EMA 2000
Somavert® (Pfizer)	Polymeric	Pegvisomant	SC	Acromegaly	EMA 2002
Genexol-PM® (Lupin)	Polymeric	Paclitaxel	IV	Breast cancer	Korea 2007
Krystexxa® (Savient Pharmaceuticals)	Polymeric	Pegloticase	IV	Refractory chronic gout	FDA 2010
Adynovate® (Baxalta US)	Polymeric	Coagulation factor VIII	IV	Moderate vasomotor symptoms due to menopause	FDA 2015
Optison® (GE Healthcare)	Protein-based	Perflutren	IV	Ultrasound contrast agent	FDA 1997
Ontak® (Eisai)	Protein-based	Diphtheria toxin	IV	Leukemia and T-cell lymphoma	FDA 2005
Abraxane® (Celgene Pharmaceutical)	Protein-based	Paclitaxel	IV	Brest cancer, lung cancer, and metastatic pancreatic adenocarcinoma	FDA 2005

CKD: chronic kidney disease; HIV: human immunodeficiency virus; IFN: interferon; IM: intramuscular; IV: intravenous; SC: subcutaneous.

prolonged-release systems. Knowing that a prolonged release of drugs potentially reduces the number of administrations and systemic toxicity and improves pharmacokinetic parameters and patient compliance [7], this relationship can be an asset in improving the quality of the treatment of chronic diseases such as cancer and diabetes.

This review presents an updated summary of recent advances in parenteral-delivered nanotechnology-based approaches, focusing on the SC route. A comprehensive description of the SC route and the input of nanotechnology to improve the prolonged-release effect of drugs within the scope of chronic disease treatment are provided. Finally, the opportunities and challenges of nanotechnology-based medicines in drug delivery via the SC route are discussed with the trends and perspectives in this highly competitive field.

2. Characterization of the SC tissue and absorption pathways

The SC tissue or hypodermis, the deepest layer of skin, comprises adipocytes, fibroblasts, connective tissue, blood, lymphatic vessels, and macrophages, among other constituents. The aim is to deliver the drug into the interstitial space so it is essential to understand the physiology of the hypodermis and how its components affect drug absorption [12]. In studies involving the SC route of drug administration, concepts such as absorption, uptake, and dispersal are used interchangeably, which can sometimes lead to difficulties in comprehension. To facilitate the understanding of the manuscript, we proposed a definition of these concepts. Dispersal refers to the movement of the injected formulation within the hypodermis. Absorption is the transport of a drug from SC tissue to blood capillaries and/or lymphatic capillaries. Uptake is the ability of cells to capture material (nanosystem and/or drug) drugs into their interior. However, uptake may not be the most accurate term. Considering the blood capillaries, there may be entry by diffusion. In tight-junctions lacking lymphatic vessels, entry may occur by convection [4; 12]. For uniformity, the term uptake will be used in this manuscript to describe transport into the blood capillaries or lymphatic vessels, regardless of the underlying mechanism. Figure 1 illustrates the hypodermal physiology and drug absorption pathways following SC administration by the SC route.

White adipose tissue is most commonly found in the hypodermis; its functions include energy storage, heat insulation, and mechanical protection [13]. Fibroblasts are responsible for glycosaminoglycan production, such as hyaluronic acid and chondroitin sulfate; structural proteins, such as collagen and elastin; and adhesive proteins, such as laminin and fibronectin [14]. The network released by fibroblasts forms the extracellular matrix (ECM), the first biological barrier to drug absorption upon SC injection.
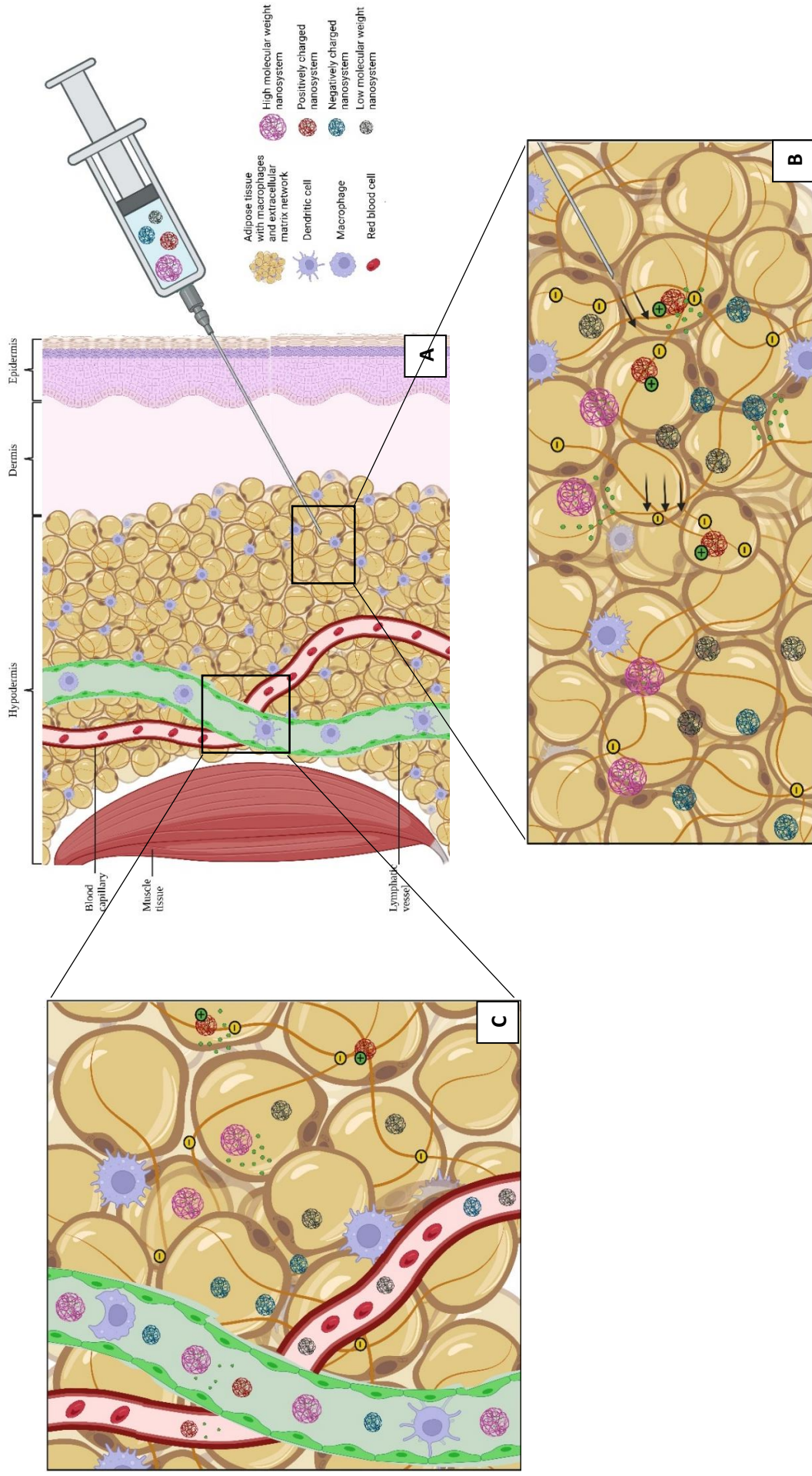
Figure 1: Fate of nanosystems and drugs after subcutaneous administrations.

The subcutaneous administration aims to inject the nanosystem into the extracellular matrix (ECM) of the hypodermis. On the syringe we have represented a hypothetical formulation consisting of nanosystems with negative (blue) and positive (red) zeta potential and with molecular weight above 16 kDa (pink) and below 1 kDa (black). The nanosystems representing the zeta potential have molecular weight between 1 and 16 kDa (a). After injection, dispersion of the formulation occurs. Nanosystems with negative zeta potential tend to move faster in the ECM compared to those with positive zeta potential, due to charge repulsion phenomena. It is also worth mentioning the possibility of drug release (green particles) by some nanosystems when moving through the ECM (b). Nanosystems with molecular weight below 1 kDa and above 16 kDa tend to be uptake by the blood and lymph capillaries, respectively. Those with molecular weight between 1 kDa and 16 kDa, can be uptake by both. The uptake by blood capillaries has direct access to the systemic circulation, where there may subsequently be contact with the liver, spleen, and kidneys. Through lymphatic uptake, the nanosystems contact with various immune cells such as macrophages, and dendritic cells, and only later reach the systemic circulation by drainage of the lymphatic duct (c).

Created with BioRender.com

Hyaluronic acid and chondroitin sulfate are the most abundant glycosaminoglycans in the ECM, and they are negatively charged under physiological conditions, which dictate ECM charge and interstitial fluid volume [4].

Therefore, negatively charged drugs are absorbed more rapidly than positively charged drugs once they do not interact with the ECM due to charge repulsion. Concerning interstitial fluid volume, ECM limits volume injection to 2 mL; therefore, higher volumes are likely to induce pain in patients [15]. Interstitial fluid is also a barrier to drug transport, as 50% of its constitution comprises protein content, including proteases that contribute to SC catabolic activity, which may lead to incomplete drug bioavailability [16].

Among several physicochemical properties of drugs influencing pathways after their dispersal, molecular weight plays a major role, as shown in Figure 1. Drugs with molecular weights less than 1 kDa are mainly uptake through blood capillary diffusion, while drugs greater than 16 kDa are primarily uptake through lymphatic vessels [17]. When drugs are between 1–16 kDa, they can be absorbed in both pathways [4]. It should be noted that by the lymphatic route, drugs undergo several lymph nodes that precede their arrival in the thoracic lymphatic duct or another duct that drains into the systemic circulation. On this path, there is contact with professional antigen-presenting cells (APC) that may negatively affect the entry of a drug into the bloodstream [12]. Despite being the molecular weight that assumes which absorption pathway to take, other factors influence bioavailability [4]. These factors may be patient-related (internal factors) or formulation-related (external factors). The relevance of patient-related factors to drug bioavailability is beyond the scope of this review, but excellent reviews have been published on this topic [4,12,18].

Formulation-related factors can be subdivided into drug properties and administrative strategies. Relative to drug properties, molecular weight and charge play significant roles in the absorption pathway. One study evaluated the absorption velocity between an anionic and a neutrally charged dextran, concluding that the anionic molecule moved at a higher rate through the interstitial fluid than the neutral one, suggesting that electrostatic repulsion reduces the interaction between the molecule and the ECM [19]. Regarding the administration strategy, parameters such as the injection site, needle length, and temperature must be addressed [20].

There has been progress in regard of knowledge of transport and uptake of nanosystems administered via the SC route. Rapamycin polymersomes measuring 100 nm drain into the lymph nodes, where their uptake takes place by APC [21]. Since the size range of nanosystems required for targeting lymphatics has been established to be between 10 and 250 nm [22], depending on the nanosystem composition, several studies have focused on

understanding the surface chemistry properties required for lymphatic targeting. Once in the ECM, the nanosystem might interact with cells, blood capillaries and lymphatic vessels. More research is needed to establish the influence of nanosystems on the flow of blood and lymphatic circulation. This is especially relevant to consider in the bifurcations and networks of the capillaries and tissues. Because not all nanosystems are spherical, it is a priority to determine and understand their flow, deposition patterns, and aggregation potential. Shape of nanosystems is an important consideration and can influence the extent of cellular uptake, circulatory system and body distribution [23].

3. Comparison between intravenous and subcutaneous routes

In the parenteral administration of drugs, three main routes can be defined: SC, IV, and IM. The rationale for choosing an administration route is hampered by factors related to the patient, desired effect after administration, and formulation [2]. The IV route allows direct drug delivery into the bloodstream, thus allowing fast drug action if needed. However, the rapid onset of drug concentration in the blood leads to higher C_{max} values and increases the risk of side effects [24]. Furthermore, the IV route requires more steps before administration and involves more human resources, which translates into higher costs for the patient and healthcare system [3]. Furthermore, the SC route can provide access to APC, among which dendritic cells can elicit tolerogenic responses upon modulation by drugs such as rapamycin [25].

The SC route is most commonly used by patients with diabetes mellitus, multiple sclerosis, primary immunodeficiency, and rheumatic arthritis, as there is no need for assistance from hospital professionals. However, cancer patients still require hospital admissions, as most anticancer drugs are delivered via the IV route. Table 2 shows the list of approved drugs. Most clinically approved nanosystem-based medicines for cancer therapy and diagnostics are delivered intravenously. In contrast, SC administration-approved drugs seek the treatment of lower-prevalence diseases, such as multiple sclerosis, hepatitis C, and acromegaly. Contrary to the IV route, the SC route under comparative conditions generally allows for a slower increase in C_{max} after injection, which means a lower risk of side effects associated with plasma drug exposure [26,27].

Despite the lower administration time and hospital professionals' assistance, the number of administrations may increase with SC injections compared to IV injections. This difference is mainly due to the formulation challenges in SC formulations. While IV

administration uses formulations with considerable volume, in the SC route, there is a volume restriction to avoid pain to the patient [28,29] while reacquiring an increase in drug concentration in the formulations. Therefore, a higher drug concentration is expected to lead to high viscosity, which can impact the formulation flow through the needle, among other constraints [30]. Among the several strategies developed to overcome this volume restriction, recombinant human hyaluronidase PH20 (rHuPH20) has been used to degrade hyaluronic acid. Currently, rHuPH20 is used as an absorption enhancer in drugs used to treat breast cancer under the marketed name Herceptin® SC, [31] and in patients with primary immunodeficiency under the marketed name Hyqvia® [32]. Enhanze® is a drug-delivery technology that allows administration volumes greater than 2 mL and promotes proper formulation flow and dispersion through the ECM with enhanced bioavailability [33].

However, a problem common to both routes of administration is the mononuclear phagocytic system, being predominant in the liver and the lymph nodes for the IV and SC routes, respectively. One study has already considered temporary inhibition of the mononuclear phagocytic system to decrease nanosystem clearance and improve efficacy and the results revealed serum levels of the nanosystem by the SC route similar to IV administration [34]. Despite the success of some SC prolonged drug-release systems, their interaction with surrounding tissues remains a major challenge if therapeutic efficacy is to be achieved [35]. Reducing host recognition and aggression (minimizing foreign body response (FBR)) cannot be neglected when developing a drug-delivery system delivered by the SC route, [36] and several approaches have been used to improve performance while minimizing FBR. A recent study discussed the possibility of a scaffold in a physiological oxidative environment with appropriate surface tension variation simultaneously releasing the nanosystem and degrading [37]. However, this topic is beyond the scope of this review, as previously described [38].

4. Application of nanotechnology-based controlled release systems by subcutaneous route

Nanotechnology-based drug-delivery systems are a relatively recent but rapidly developing science. Nanotechnology has a high drug-carrying capacity, therapeutic targeting efficacy, and pharmacokinetic and pharmacodynamic performance [39].

Among the nanosystem-based medicines approved by the FDA, lipid-based nanosystems stand out, as shown in Table 2. Their success is due to their biocompatibility, bioavailability, self-assembly, formulation simplicity, and high payload properties [40]. Liposomes are a subgroup of lipid-based nanosystems that are highly utilized because of their ability to form phospholipid uni- or multilamellar lipid bilayer structures, which incorporate both hydrophobic (in a lipidic bilayer) and hydrophilic (in their core) drugs. However, they are easily taken up by the smooth endoplasmic reticulum and usually require further surface modifications to improve their circulation time [41]. Solid lipid nanoparticles are another subgroup of lipid-based nanosystems that differ from liposomes in structure and morphology [42]. Because they are mainly used to deliver nucleic acids [43], their composition often includes ionizable lipid complexes with negatively charged genetic material, phospholipids for particle structure, cholesterol, and PEG to improve stability [44]. Upon complexation with cationic lipids, nucleic acids can improve their endosomal escape, thus increasing their residence time in the blood. However, these systems also possess low drug-loading capacity and biodistribution owing to uptake by the liver and spleen [9,43].
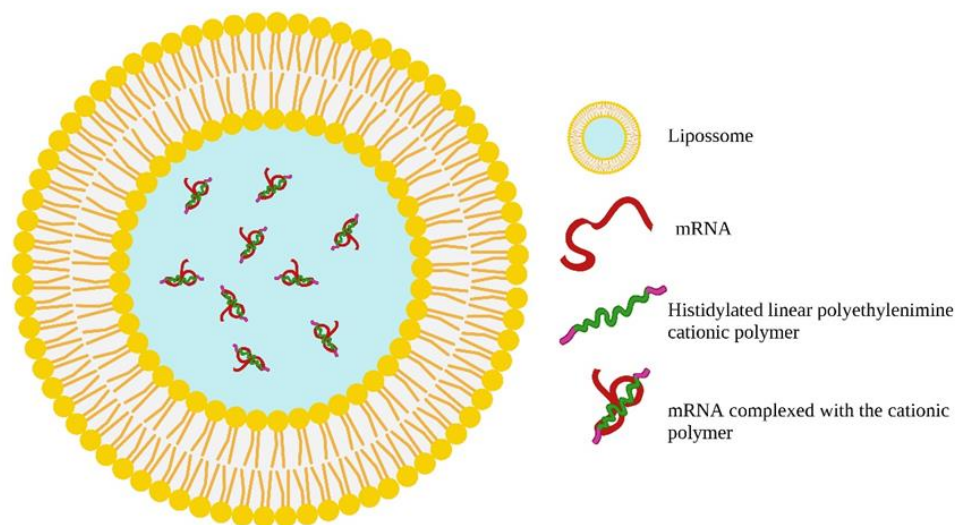
Figure 2: Example of a lipid-based nanosystem.

The figure represents a lipid-based nanosystem consisting by a liposome loaded with a complex resulting from the interaction between the negative charges of the mRNA and the positive charges of the polymer. The figure was inspired by the original work "*In Vivo* bone tissue induction by freeze-dried collagen-nanohydroxyapatite matrix loaded with BMP2/NS1 mRNAs lipopolyplexes" by Pinpin Wang *et* al. in which the nanosystem was used subcutaneously.

Created with BioRender.com

Polymer-based nanosystems may have their origin in natural or synthetic polymers and can be presented in different forms: nanocapsules (with a clear separation core-shell), nanospheres (with a solid matrix), polymersomes (artificial vesicles whose membranes are made of amphiphilic block copolymers), micelles (responsive block copolymers self-assemble to form nanospheres), and dendrimers (hyperbranched polymers with a complex 3D configuration) [9]. Polymer-based nanosystems' potential is based on their biocompatibility, biodegradability, hydrophilicity, and stability upon storage. Drug release is controlled by diffusion through a polymer matrix or degradation. Even the modulation of their properties allows for better kinetics release control [45]. Furthermore, they can transport large amounts of the drug – whether encapsulated in the core or entrapped in the matrix or even conjugated with the polymer or on the surface – and their surface is easily modified, allowing for an additional targeting property. The disadvantages associated with polymer-based nanosystems are the increased risk of aggregation and the possibility of toxicity [9].
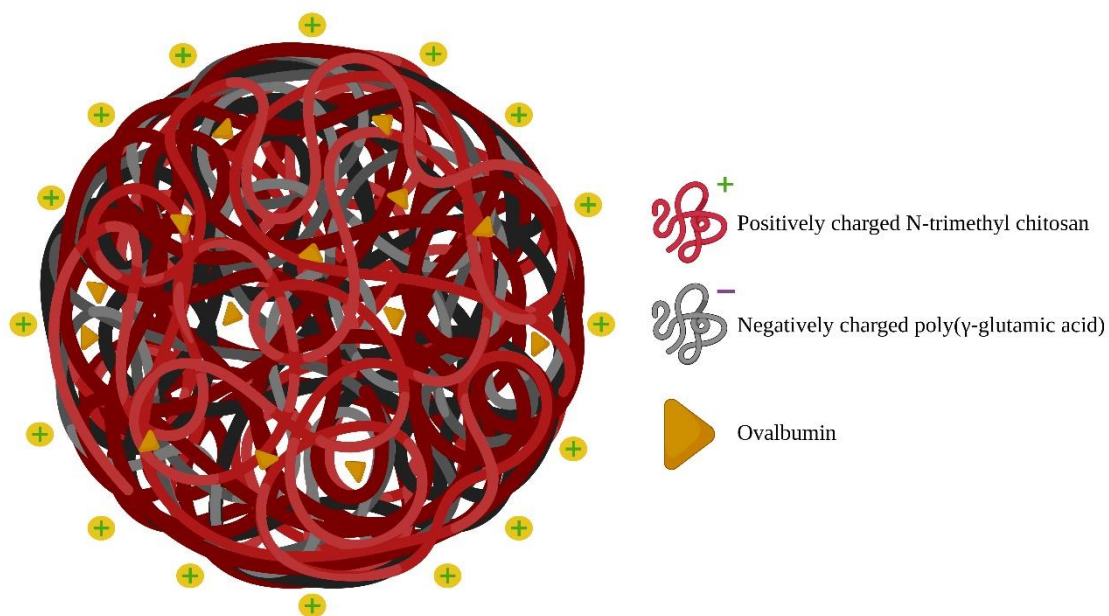
Figure 3: Example of a polymer based nanosystem.

The figure represents a polymer-based nanosystem as a result of the complexation between positively and negatively charged N-trimethyl chitosan and poly(γ-glutamic acid), respectively. The nanosystem carries ovalbumin. The figure was inspired by the original work "Single-injecting, bioinspired nanocomposite hydrogel that can recruit host immune cells in situ to elicit potent and long-lasting humoral immune responses" by Chiranjeevi Korupalli *et* al. in which the nanosystem was used subcutaneously.

Created with BioRender.com

Inorganic-based nanosystems are made of several materials, including calcium, gold, iron, and silica. They have unique electrical, magnetic, physical, and optical properties. Calcium phosphate and mesoporous silica nanosystems have been used mainly in genetic delivery [46]. Gold-based nanosystems, nanospheres, nanorods, nanostars, nanoshells, and nanocages [47], have free electrons on their surface, giving them photothermal properties, beneficial for cancer therapy [48]. Iron oxide is presented in most inorganic nanomedicines approved by the FDA. Magnetite and maghemite are magnetic iron oxides commonly used in nanosystem-based formulations due to their photothermal and superparamagnetic properties. They can also be used as contrast agents or drug-delivery vehicles [49]. However, their utilization may be compromised by their low solubility and toxicity properties, particularly in formulations with heavy metals [6].
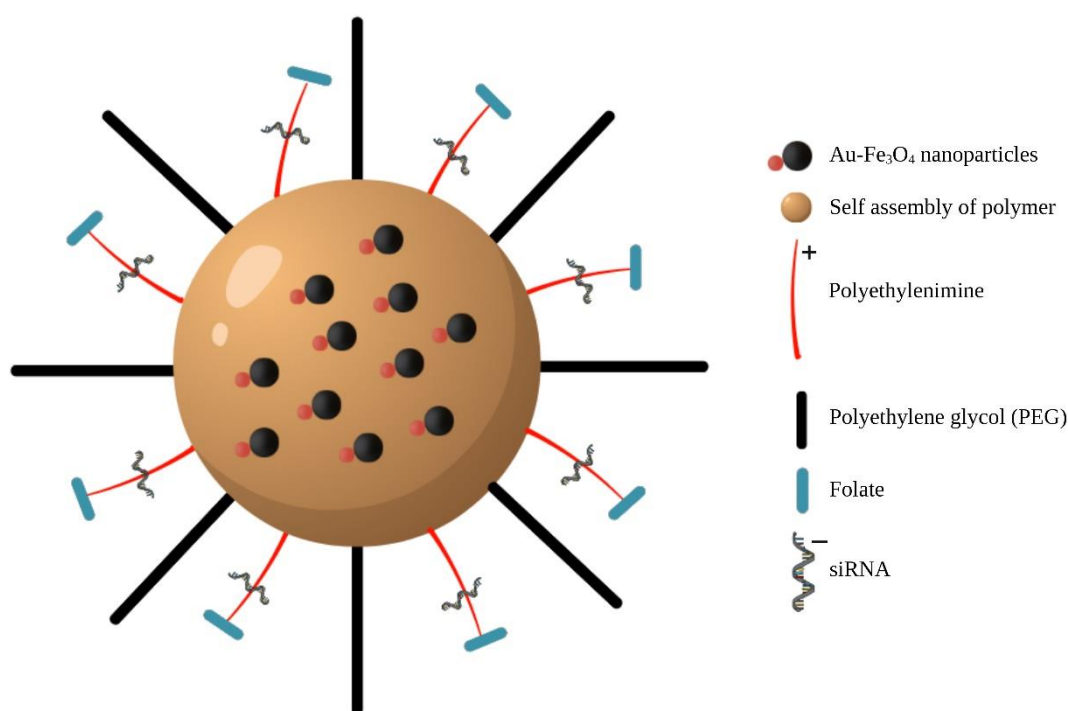
Figure 4: Example of an inorganic based nanosystem.

The figure represents an inorganic-based nanosystem loaded with Au-Fe$_3$O$_4$ and small interfering RNA (siRNA) nanoparticles through hydrophobic and electrostatic interactions, respectively. The figure was inspired by the original work "Injectable and Quadruple-Functional Hydrogel as an Alternative to Intravenous Delivery for Enhanced Tumor Targeting" by Zhi-Qiang Zhang *et al.* in which the nanosystem was used subcutaneously.

Created with BioRender.com

The classification of nanosystems could be smoother, making it difficult to classify them based on their lipid, polymeric, or inorganic nature. Therefore, the nanosystems presented in Table 3 are organized by the disease, proving that different nanosystems can be applied in various clinical contexts, regardless of their specific characteristics. The following sections present some of the most recently developed nanosystems under preclinical evaluation in high-prevalence chronic diseases.

4.1 Bone diseases

Osteoporosis is a bone disease characterized by resorption by osteoclasts, which leads to a progressive decline in bone mineral density, resulting in painful and severe fractures [75]. Besides bisphosphonates, other recommended therapies include recombinant human parathyroid hormones such as salmon calcitonin (SCT) and teriparatide. SCT is a polypeptide hormone with 32 amino acid residues capable of inhibiting bone reabsorption through interaction with osteoclasts [76]. Currently, treatment with SCT is achieved by SC injection; however, its short half-life of approximately 1 h leads to poor patient compliance [52]. The self-assembled nanosystem, based on the interactions between SCT and dipeptide aspartate-phenylalanine, continuously released 100% of SCT *in vitro*. Formulations containing other dipeptides released no more than 80% of the SCT. *In vivo* studies revealed a continuous SCT release and a decrease in calcium levels for over 1 month [52].

Teriparatide is another recombinant hormone with unique effects because osteoclast inhibition promotes osteoblast stimulation in addition to osteoclast inhibition [77]. The recommended treatment was based on daily SC injections daily for 2 years [78]. A biodegradable polymeric nanosystem containing teriparatide was loaded into a hyaluronic acid/jeffamine hydrogel to prepare a combination system for prolonged drug delivery. Despite the burst release of 12% observed after the first 12 h, a prolonged-release pattern was achieved for 50 d [51].

Table 3: Nanotechnology-based delivery systems for prolonged drug release in chronic diseases by the subcutaneous route.

Nanotechnology	Encapsulated	Prolonged release effect	REF
Lipid-based		**Bone diseases**	
Lipopolyplex loaded scaffolds	BMP-2 and NS1 mRNAs	Both mRNAs promote twice higher bone formation, with bone tissue differentiation and remodeling activity; the sustained release was observed over 120 h with a higher area under the curve values.	[46]
Polymer-based			
Alginate-PCLA hydrogel	BMP-2 protein	The nanosystem reduced the initial burst and promoted a sustained release with higher calcium deposition than nanosystems not containing alginate *in vivo*.	[50]
PHBV/PLGA nanosystem loaded hydrogel	Teriparatide	Serum calcium levels followed an ascending trend that reached a constant level after 50 days; the nanosystem reduced the initial burst, and 63% of the drug was released within 50 days.	[51]
Other			
Drug-peptide self-assembled nanosystem	SCT	SCT levels and sustained release were observed for more than one month, allowing controlled calcium levels.	[52]
		Cancer	
Polymer-based			
Polyacrylamide-drug conjugated	PTX	The nanosystem doubled the survival rate and had similar antitumoral activity to IV Taxol® at the same dose; after SC and IV administration of the nanosystem, PTX was detectable for up to 6 days.	[53]
Polypyrrole polymersomes	DOX	The nanosystem plus laser radiation nearly eliminated the tumor with an improved survival rate and low cardiotoxicity; drug release increased with the acid environment and laser radiation, releasing nearly 60% after 24 h.	[27]
PEG-PEAU hydrogel	DOX	SC administration of the nanosystem had a better inhibition of tumor growth than IV administration of free DOX; *in vitro*, a sustained release was observed over a month with slightly high values in mild acidic conditions; and *in vivo* DOX values were still detected 7 days after administration.	[54]

Material	Agent	Description	Ref.
Hyaluronic acid-drug conjugated	DOX and gemcitabine	By the SC route, the nanosystem was as effective as IV administrations in tumor growth inhibition; in vitro, sustained release for both drugs in an acidic environment was shown, but a faster release was observed for gemcitabine in neutral conditions.	[55]
Vitamin E-PEG hydrogel	Ovalbumin	The nanosystem promoted high immunization and antibody production and had the best therapeutic outcome in tumor development and survival rate; a high and sustained release of ovalbumin was observed in vitro after 21 days.	[56]
Core-shell layer-by-layer sulphonate and chitosan	IFN-α	The plasmatic values provided relatively constant IFN-α levels during 10 days with improved pharmacokinetic parameters; the total amount of IFN-α was released after 6 days.	[57]
Inorganic			
Self-assembled FPP nanosystem	Au–Fe_3O_4 and siRNA	By the SC route, the nanosystem promoted better targetability, tumor inhibition, and fewer side effects than IV administration; the sustained release and targeting of gold to tumor enabled two cycles of photothermal therapy.	[58]
Central nervous system disorders			
Polymer-based			
PHBV nanosystem loaded alginate matrix	Fingolimod	The nanosystem-based formulation reduced burst release and side effects retained at the injection site, prevented phagocytosis, and promoted an extended release over 50 days in vitro with an improved pharmacokinetic profile.	[59]
Crosslinked cellulose nanocrystal hydrogel	Donepezil	The pharmacokinetic parameters such as C_{max} and half-life were improved by comparing the donepezil solution; the initial release after 24 h was 39% and then was sustained, achieving a 79% drug release at 168 h.	[60]
Diabetes			
Polymer-based			
Polycaprolactone reservoirs	Alanine and glutamine	The graft survival of β-cells after 21 days was increased when both amino acids were sustained released by the reservoirs; both amino acids were sustained release in 2 weeks.	[61]
Chitosan nanospheres loaded hydrogel	Insulin	The nanosystem regulated insulin release and BGL according to the percentage of loaded nanospheres, showing controlled levels up to 60 h with normal weight gain.	[62]

PEG block-brush polymer	EX	The nanosystem maintained BGL controlled for 7 days and improved renal function and metabolic anomalies.	[63]
PLGA microspheres	EX nanoparticles	The nanosystem provided long-term therapeutic efficacy with a sustained drug release of up to 30 days, high bioavailability, and biocompatibility.	[64]
Protamine-conjugated poly(organophosphazene) hydrogel	Exendin-4	The nanosystem showed wide periods of reducing BGL with standard weight gain patterns and food uptake; a sustained release was achieved up to 3 weeks *in vitro*, but a better *in vivo* performance for the one with more percentage of protamine was obtained.	[65]
Immunological system: vaccination adjuvants and infections			
Polymer-based			
N-trimethyl chitosan nanoparticles loaded hydrogel	Ovalbumin	The nanosystem allowed an effective and persistent humoral immune response, with a sustained release profile over 14 days *in vitro*.	[66]
Poly (ε-caprolactone) microspheres	Selenium nanoparticles	The nanosystem revealed antibacterial activity against gram-positive bacteria with good biocompatibility; a sustained release of selenium nanoparticles was achieved *in vitro* after 11 days of incubation.	[67]
Other			
Nanocrystals	Griseofulvin	Bioavailability, C_{max}, and elimination profile were significantly improved in the SC route compared with *per os*, with a 10 h prolonged released effect that could be obtained *in vivo*.	[68]
In situ self-assembled nanosystem	Lopinavir and ritonavir	Lopinavir and ritonavir sustained release beyond 14 and 6 days, *in vitro* and *in vivo*, respectively.	[69]
Lipid-based			
Nanostructured lipid carriers	Ondansetron (CINV)	The nanosystem reduced high drug levels in the blood early and enhanced its bioavailability; a sustained release up to 96 h *in vitro*, and the drug remains in blood until 96 h.	[70]
Solid lipid nanoparticles	Bedaquiline and celecoxibe	A biphasic release profile after IM and SC administration of both nanosystems was observed with better pharmacokinetic parameters in SC administration.	[71]
Polymer-based			
PLGA nanosystem	17β-estradiol (Menopause)	Improvement in spatial working memory and spatial reference memories. Rapid release within the first 8 h followed by a slow, sustained release over 40 h *in vitro*.	[72]

Polyvinyl alcohol-coated PLGA nanosystem	Methotrexate (Rheumatoid arthritis)	The nanosystem reduced rheumatoid arthritis score and incidence and decreased Th17 cells and inflammatory factors *in vivo*. *In vitro*, methotrexate was slowly released for up to 6 h.	[73]
PEG and poly(propylene sulfide) polymersome	Rapamycin (Immunosuppression)	The SC administration repurposed the mechanism of action of rapamycin improving its immunosuppressive effect. After islet transplantation, the nanosystem maintained mice normoglycemic during 100 d.	[21]
Inorganic-based			
Mesoporous silica nanosystem	Peptide YY (Obesity)	Compared with the IV route, the nanosystem improved the sustained release and enhanced bioavailability by the SC route; *in vivo* results demonstrated a virtually complete sustained release for 4 days with absolute bioavailability near 80%.	[74]

BGL(s): blood glucose level(s); BMP-2: bone morphogenetic protein 2; CINV: chemotherapy-induced nausea and vomiting; C_{max}: maximum serum concentration; DOX: doxorubicin; EX: exenatide; FPP: folate-polyethylenimine conjugated poly(organophosphazene); IM: intramuscular; IV: intravenous; mRNA: messenger RNA; NS1: nonstructural protein 1; PAEU: poly(β-amino urethane); PCLA: poly(ε-caprolactone-co-lactide); PEG: poly(ethylene glycol); PHBV: poly (3-hydroxybutyrate-co-3-hydroxyvalerate acid); PLGA: poly(lactic-co-glycolic acid); PTX: paclitaxel; SC: subcutaneous; T1D: type 1 diabetes; T2D: type 2 diabetes.

4.2 Cancer

Taxols are chemotherapeutic and antimitotic agents used to treat cancer. Paclitaxel (PTX) is characterized by poor water solubility, which can translate into formulation difficulties and treatment efficacy levels [79,80]. Existing formulations are often associated with side effects and inefficient drug delivery at the appropriate site [81]. Abraxane®, overcame the hypersensitivity reactions of Taxol®, the first PTX commercial formulation [82]. The development of formulations containing chemotherapeutic agents for SC administration is particularly challenging because most cytostatic agents are lipophilic. If they remain in the SC tissue, they can lead to adverse effects, such as necrosis [83].

A nanosystem of PTX linked to the subcutaneously administered hydrophilic polymer polyacrylamide showed a higher maximum tolerated dose of PTX than IV Taxol®, with no toxicity at the administration site [53]. PTX was detected in the blood for up to 6 d after administration [53]. In terms of efficacy, the SC nanosystem was comparable to that of IV Taxol ® at the same dosage. However, as the nanosystem had higher maximum tolerated dose values, it was able to outperform Taxol® while maintaining a prolonged release [53].

Doxorubicin (DOX) belongs to the anthracycline family and is used to treat several types of cancers [84]. Despite its efficacy, its long-term use is associated with severe side effects, including cardiomyopathies [84]. Incorporating DOX into nanosystems decreases its nonspecific distribution, thus reducing toxicity and improving its pharmacokinetic profile [85]. However, DOX contains large aromatic rings that confer hydrophobic properties [86], which can lead to formulation challenges.

Polypyrrole polymer is used to develop DOX-loaded nanosystems because, in addition to incorporating DOX and promoting pH-responsive drug release [27], it also converts near-infrared light into heat energy [87]. A pH-responsive controlled drug release was observed, revealing a cumulative DOX release of 42% in 24 h [27]. *In vivo* efficacy and safety revealed an inhibition rate of almost 100% and less cardiotoxicity than free DOX [27].

Another strategy for sustaining DOX release is through a hydrophilic matrix [55,88]. The DOX-loaded hydrogel allowed prolonged release for one month *in vitro*. *In vivo* results showed that DOX was detectable seven days after SC administration. In terms of efficacy, compared with IV-free DOX, the nanosystem exhibited superior tumor growth inhibition and prolonged survival rate with low systemic toxic effects [55].

Temperature-responsive and magnetic-responsive nanosystems are also used to improve the efficiency of cancer treatment. Solid tumors' leaky vasculature and defective lymphatic drainage allow nanoparticles to migrate and accumulate in the tumor; however, it is

not sufficient to target efficiently due to tumor heterogeneity [89]. A quadruple-functional hydrogel developed to enhance tumor targeting was passively mediated by the EPR effect, actively mediated by folate, and magnetically targeted by iron oxide and gold. The prolonged release of siRNA and Au-Fe$_3$O$_4$ depended on hydrogel dissolution at body temperature, where Au-Fe$_3$O$_4$ release trended with hydrogel weight loss until 30 d and siRNA release until 18 d. Prolonged targeting of gold to tumors enabled two cycles of photothermal therapy, resulting in an enhanced silencing effect of siRNA and a considerable reduction in tumor volume [58].

4.3 Central nervous system disorders

Multiple sclerosis is an inflammatory and neurodegenerative central nervous system disease with pathological features including demyelination, gliosis, and neuroaxonal injury [90]. Fingolimod is a sphingosine 1-phosphate receptor modulator and was the first approved medication to treat this disorder [91]. However, fingolimod is associated with some severe concerns during clinical trials [92].

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) was utilized to encapsulate fingolimod and then loaded into an oleic acid-grafted-aminated alginate matrix to minimize nonspecific biodistribution [59]. The alginate matrix played a significant role in retaining the nanosystem at the injection site, preventing phagocytosis, prolonging drug diffusion, reducing side effects, and mitigating burst release [59]. Over 45% of fingolimod was released from the nanosystem loaded in the alginate matrix within 28 d, whereas a 7-day 50% release from the nanosystem alone was observed. *In vivo* efficacy was assessed by lymphocyte counting, which showed excellent results for up to 6 weeks.

4.4 Diabetes Mellitus

Diabetes mellitus affects the ability of the body to process blood glucose levels [62]. There are several forms of diabetes mellitus [93]; however, in this review, only type 1 diabetes (T1D) and type 2 diabetes (T2D) were considered. Regardless of the cause, the daily treatment of diabetes to stabilize blood glucose levels (BGL) poses a challenge for patients with this health problem. Diabetes is a long-term metabolic disorder, and its treatment lasts for the rest of the patient's life after diagnosis [65]. Thus, developing novel strategies to improve therapeutic efficiency and safety without compromising patient compliance is crucial.

T1D patients need insulin for life and inject themselves several times a day. Currently, T1D treatment focuses on providing insulin to lower the BGL to the near-normal physiological range of approximately 80–140 mg/dL [62]. A possible alternative to daily and frequent insulin administration is the encapsulation of pancreatic β-cells. A device capable of supplying

encapsulated cells, such as pancreatic β-cells, with the nutrients needed for their survival, would be a strategy to avoid immunosuppressive therapy and limit the effectiveness of transplantation. To avoid nutrient deprivation, nonporous reservoirs of amino acids were embedded in a nanoporous PEG-PLC device loaded with β-cells, in a study coordinated by Chendke. Each amino acid was encapsulated in the device, and after 18 d, a prolonged release was confirmed. The amino acid reservoirs provided greater than 80% viability of cells during both short-term (24 and 48 h) and long-term (2 weeks) *in vitro* nutrient deprivation [61]. The *in vivo* results also showed that the amino acid reservoirs of alanine and glutamine and graft survival after 21 d were better than when they were separated and when the cell culture was nutrient-depleted [61].

Another method to promote insulin delivery to the human body is based on hydrogel composites. Chitosan-insulin electrosprayed nanospheres were included in a pH-temperature-sensitive pentablock copolymer solution with *in situ* hydrogel properties [62]. After *in vivo* SC administration, the nanosystem exhibited good biocompatibility and bioresorbable properties. Two dosages of nanospheres were utilized, and both promoted prolonged-release effects. The nanosystem containing insulin at 2.50 wt% and 3.75 wt% promoted steady BGL for over 48 h and 60 h, respectively, showing a prolonged release over 2 d. Therefore, this strategy can reduce the burden of conventional T1D therapy [62].

T2D patients are characterized by a lack of insulin release, insulin resistance, or ineffective insulin production [64]. Pharmacological intervention with an oral antidiabetic agent is the second step, after diet and exercise [65].

Exenatide (EX), a synthetic exendin-4, is a GLP-1 agonist that is commonly used in T2D treatment. It was the first incretin mimetic approved by the EMA and the FDA [94]. Marketed formulations include SC twice daily (Byetta®) or once weekly (Bydureon®). Polymer-based nanosystems are likely to enhance the efficacy and bioavailability of EX while reducing the number of administrations.

PLGA microspheres containing EX-encapsulated lecithin nanoparticles were obtained by the initial fabrication of EX-loaded lecithin nanoparticles via alcohol injection, followed by encapsulation into PLGA microspheres. *In vivo* studies demonstrated a smooth plasma concentration, suggesting that this PLGA-based nanosystem can provide prolonged drug release with a reduced initial burst effect [64]. Although EX lowers the BGL, it is also used because of its role in diabetic nephropathy. Therefore, in another study, EX was loaded into a PEG block-brush polymer, and its role in drug delivery and nephropathy was assessed. In the *in vitro* release study, almost 4/5 of the EX was released after 7 d by the nanosystem. In *in vivo* experiments, the hypoglycemic action of the formulation following SC injection in diabetic

mice lasted 7 d and significantly alleviated diabetic nephropathy. This nanosystem is a potential EX nanocarrier with efficient encapsulation and prolonged-release effects [63].

Temperature-responsive poly(organophosphazene) hydrogels begin *in situ* gelification in the human body and are characterized by their high efficiency and simplicity in protein loading and administration, respectively [95]. Three polymers were synthesized: one not conjugated and two conjugated to protamine at 1% and 2%, respectively. No burst release was detected in either protamine group *in vitro*. Despite the prolonged release provided by both protamine-conjugated systems, they performed better *in vivo*, improving bioavailability and circulation time after 21 d. In addition, they also showed a significantly prolonged glucose-lowering effect, where BGL returned to the original level at 14 d after a single SC injection [65].

4.5. Immunological system: vaccination adjuvants and infections

Vaccination is a medical intervention aimed at promoting a robust and consistent humoral immune response to infectious agents. However, additional booster immunization is usually required to establish potent and long-lasting protective immunity [96,97], so it is desirable to develop a system that allows for the continuous release of antigens and thus improves immunological potency and memory [98]. Hydrogels and ovalbumin have been used to assess the effect of prolonged release on the immune response. Hyaluronic acid hydrogel loaded with an N-trimethyl chitosan nanosystem and carrying a model subunit vaccine, ovalbumin, promoted an *in vitro* prolonged release of 40% for 14 d. *In vivo* results revealed prolonged ovalbumin-specific humoral immune responses for at least 12 weeks. The nanosystem-based formulation could be a single-injection platform for strengthening and prolonging ovalbumin-specific humoral immune responses [66].

Human immunodeficiency virus (HIV) infection is a major concern worldwide. Although several published studies have recently revealed the progress and new strategies for treating HIV infection [99], there is still no effective strategy. The current treatment and prevention therapy for acquired immunodeficiency syndrome (AIDS) is oral antiretroviral therapy. Although this therapy improves patient survival and quality of life, it is expensive and has a complex etiology and several side effects [100]. One of the most potent combinations used in antiretroviral therapy is ritonavir and lopinavir, which are currently on the market under the name Kaletra® but are characterized by gastrointestinal side effects, such as diarrhea, nausea, and vomiting, which often occur.

Long-acting SC injections of ritonavir and lopinavir were developed based on their *in situ* self-assembly ability. *In vitro* studies revealed that 60% of lopinavir and 40% of ritonavir

were released after 14 d, and no burst release was observed. This nanosystem can be used to improve patient adherence and therapeutic effectiveness, although toxicological studies still need to be performed [69].

5. Regulatory challenges

Nanosystem-based medicines have complex and heterogeneous structures in which each component has a particular function that needs to be characterized. Therefore, guidelines and standardized concepts should emerge to speed up their development and subsequent approval [101]. Nanomedicines still need to have harmonized guidelines that allow standardized approval. The regulatory agencies, FDA and EMA, use a case-by-case approach based on a traditional assessment of the benefit/risk ratio [102].

The first nanosystem-based medicines that hit the market were evaluated and approved based on traditional benefit/risk principles. However, it is necessary to develop specific guidelines for nanosystems because nanotechnology-based medicines are not medicines with conventional formulations but on a smaller scale. Each nanosystem constituent performs a particular function, and each property must be explicitly rationalized for its intended purpose. A change at any stage of the manufacturing process can result in a generic nanosystem with different characteristics (mean size, size distribution, surface properties, drug loading and release profiles, aggregation status, and stability). This generic nanosystem may affect the patient's health differently from the reference formulation. For example, iron nanoparticles have been coated with carbohydrate polymers to develop parenterally administered iron nanosystems. Different coatings and changes in the manufacturing process, while maintaining the same core and coating, give rise to a formulation with other toxicological profiles [103].

The Federal Food, Drug, Cosmetic Act and Public Health Service Act regulate pharmaceuticals at the FDA. The first covers the regulation of all chemical compounds and devices, and the second covers biological derivatives. Nanosystems, as they do not fully fit into one of these two categories, are categorized as complex formulations with multiple components; therefore, they are combined products. A combination product is an association between a drug and device, device and biological, drug and biological, or even the three.

At the EMA, nanosystems are classified based on their biological or non-biological origins. For approval, a comparison with the reference formulation in terms of bioequivalence, safety, quality, and efficacy is needed [104]. However, because the interplay between nanosystem physicochemical characteristics and pharmacokinetic properties is not understood, replacing traditional animal models with more adequate ones may allow

extrapolation with greater certainty and safety. Nevertheless, the EMA has already published several reflection papers to help the industry develop its nanosystems and expedite its market entry [102]. Assessing the immunostimulatory profile of every nanosystem more precisely to determine whether the loaded drug e plays a major role may avoid interference from background immunostimulation due to the nanosystem. Unloaded blank PEG-polysulfide nanosystems elicit low immunomodulatory activity [21], whereas comparable PLGA nanosystems [105] lead to alterations of immune cell populations and coreceptor expression and changes in the inflammatory status.

An overall collaboration among several research teams working on the characterization of materials from physicochemical and biological points of view regarding the interactions of nanosystems with cells and biological surfaces is a step forward to advance the knowledge from which we will benefit through guidance and guidelines that accompany the state-of-the-art.

6. Contribution of QbD and AI

6.1 QbD approach

The potential of nanosystems to treat various pathologies has been extensively investigated in recent decades. Nevertheless, their R&D achievements at the research level do not translate into clinical applications. There are several reasons for their poor manufacturing scalability and lack of regulatory harmonization [106].

Nanosystems can be complex formulations, and it is necessary to rationalize the entire development to streamline the entire process from the R&D stage to the market. Several parameters associated with their formulation and manufacturing are involved when quality, efficacy, and safety are required to meet the requirements of nanosystem-based medicines.

A possible strategy to overcome these obstacles during the production of nanosystems is the QbD approach. QbD improves the efficiency of the development of the nanosystem and later of its manufacture, thus improving the reliability and reproducibility of manufacturing processes, and consequently leading to a greater probability of acceptance by regulatory authorities [107]. All concepts inherent to the QbD are included in the International Committee on Harmonization (ICH) Q8 guidelines [108].

Each nanosystem has unique characteristics; therefore, its characterization must be case-by-case. Nevertheless, the most common critical quality attributes (CQA) are related to general nanosystem characterization assays, including particle size (PS) [106,109], polydispersity index (PDI), morphology, surface properties, drug loading, encapsulation

efficiency, drug release, and stability [110]. Several studies have addressed the pharmaceutical development of nanosystem-based formulations using the QbD approach [110]. However, they should have targeted the SC route for the nanosystem as the target product profile. Parenteral formulations are initially made available in liquid or powder form for IV administration, ensuring a long shelf life to allow transport and storage.

Although many companies include experiments using the QbD approach, most adopt one or more QbD elements to develop their formulations. Nevertheless, as mentioned above, several publications have made QbD a successful concept in the development of nanosystem-based formulations. The full QbD approach during the development of nanosystem-based formulations in trends with other formulations must be described in regulatory dossiers. Regarding the SC route, we will only consider the PS, PDI, zeta potential, and drug-release mechanisms.

PS and PDI were the major factors to be considered. Size is one characteristic that dictates the absorption pathway [4,27]. Besides absorption, PS is still related to clearance, since when <5 nm, nanoparticles undergo renal clearance. At >150 nm, they accumulate in the lungs, liver, and spleen [108]. The PDI is the value of the PS distribution. The lower the PDI value, the more monodisperse is the nanosystem. Maintaining it at a low level is vital because it avoids heterogeneities and hurdles in the bulk properties and stability [107]. For example, a higher uptake is essential when studying *in vitro* and eventually for an *in vitro/in vivo* correlation. PDI within a certain range and under control is a significant step when the safety and efficacy of the nanosystem is a major issue [111–113].

Zeta potential is the electric charge on the surface of the nanosystem. Knowing that the cell membrane has a negative charge, nanosystem-based formulations with a positive zeta potential may allow better uptake owing to electrostatic interactions. However, the clearance of positively charged nanosystems is faster because mononuclear phagocyte system cells are negatively charged [114]. Even the ECM in SC tissue is negatively charged, which means negatively charged formulations cross the ECM more easily due to electrostatic repulsion and are more rapidly absorbed. In addition to influencing the interaction with biological tissues, the zeta potential also affects the stability of the nanosystem because it reflects the electrostatic repulsive forces between particles [115]. The surface charge can be changed by functionalization. As nanosystem functionalization can improve bioavailability and circulation time, the route of administration and intended therapeutic target should be included in QTPP [110].

The drug-release mechanism varies depending on the nature of the nanosystem (polymeric or lipid) and its state (encapsulated and dispersed in a matrix). Drug release must

occur to have a proper therapeutic effect and avoid toxicity [53,110]. Drug loading and encapsulation efficiency are crucial parameters that affect drug-release profiles [116].

Although some publications are related to the development of nanosystems for parenteral administration through a QbD approach, studies addressing their administration specifically for the SC route still need to be made available.

6.2 AI approach

Artificial intelligence (AI) englobes a family of self-learning algorithms that only require datasets from automated synthesis practices or sources from the literature, enabling machines to mimic, develop, and demonstrate human behavior [117,118]. Machine learning (ML) is a fundamental AI component. An informatics system can analyze a large amount of data following an algorithm and statistical models, and make accurate predictions based on that information [119]. Integrating AI and ML into nanosystem-based formulation development can optimize manufacturing strategies, leading to fewer experiments and lower costs, and can even be integrated into a QbD approach to prevent undesired events and predict specific properties of nanosystems for their characterization [118]. Table 4 presents a few studies using AI to develop nanosystems.

ML has already been used to predict the ability to form self-assembling dipeptide hydrogels [120] and to predict nanocrystal PS and PDI [121]. However, one of the limitations inherent in using ML in nanotechnology is the ability in solving complex problems involving the perception of abstract information, rules, and patterns, and the lack of information [118]. It is important to note that the quantity and quality of the collected data dramatically influence the performance of the ML algorithm, and not all algorithms are suitable for all datasets, thus the distribution and volume of the collected data must be evaluated [121]. To overcome these limitations, artificial neural networks (ANNs) have been developed. ANNs are a subset of ML composed of hidden layers with connected nodes that can analyze big data and provide an outcome. ANN architecture is inspired by the human brain. They typically comprise an input layer (which corresponds to the independent factors), an output layer (which corresponds to the responses or dependent factors), and a hidden layer (which processes and extracts features from the input data and is used by the output layer to make decisions or predictions) [122]. They can also outperform response surface methodology in prediction efficiency [123]. The ANN accurately predicted the role of formulation factors that influence verapamil encapsulation in a hybrid polymer-lipid nanosystem [124]. ANN also demonstrated the capability to predict the most impactful parameters and their respective relationships, which affect the size and PDI of polymeric nanosystems based on polymer properties. The ANN was

tested three times. The first round utilized polymers known by the algorithm, the second round utilized unknown polymers unknown by the ANN but closely related to the first used polymers, and the last round utilized unfamiliar polymers. In all rounds of testing, the ANN could predict PS and PDI despite the lower efficiency of PDI [122]. Other studies concerning the successful application of ANN in the development of nanosystems have already been published [123,125–127].

Table 4: Examples where an AI strategy was applied to develop and optimize nanosystem-based formulations.

Field of application	Type of AI	Nanosystem	Aim	Results	REF
Formulation development	ANN	Silver nanoparticles	Predict particle size based on $AgNO_3$ concentration, temperature, wavelength, and montmorillonite d-spacing,	Overall R^2 and root mean square error values indicated good prediction efficiency.	[128]
Formulation development	ANN	Polymer-lipid hybrid nanoparticles	Investigate the role of formulation factors	ANN outperformed response surfaced methodology in data fit and prediction capability.	[124]
Formulation development	ANN GA	Agar nanospheres	Optimize the bupropion HCl loaded agar nanospheres characteristics	High R^2 values between predicted and real values obtained for all responses.	[123]
Formulation development	ANN	Chitosan-tripolyphosphate nanoparticles	Optimize yield of production and particle size	High R^2 values obtained for each ANN used in each response.	[129]
Formulation development	ANN	Chitosan nanoparticles	Understand the relation between process variables affecting the size, loading efficiency and cytotoxicity	R^2 values were significative for all responses, and considering train, test, and unseen rounds.	[126]
Formulation development	ANN	Polymeric nanoparticles	Predict particle size and polydispersity index	R^2, root mean square error, and % bias values indicated good predicting abilities.	[122]
Formulation development	ANN	Palmitoyl glycol chitosan nanoparticles	Formulation optimization of doxorubicin loaded nanosystem	ANN provided 5 possible formulations respecting the desired responses.	[127]
Formulation development	ML	Solid lipid nanoparticles	Predict particle size based on homogenization time, and lipid and surfactant concentrations	The lowest mean absolute error were obtained with Random Forest.	[130]
Formulation development	ML	Nanocrystals	Predict particle size and polydispersity index	Higher predictive performance for LightGBM than other algorithms.	[121]
Pharmacodynamics	ANN	Gold nanoparticles	Predict the biological fate of nanoparticles after administration	ANN predicted with great accuracy rate for both known and unknow dataset of nanoparticles.	[131]

ANN: artificial neural network; GA: genetic algorithm; ML: machine learning.

7. Conclusion and future perspectives

There have been a high number of drugs switching from the IV route to the SC route, with many advantages, such as lower healthcare costs and higher patient adherence. Given its specificity regarding its composition and eventual interaction with drugs, the SC route can be considered an administration route to be explored for developing or optimizing formulations for prolonged drug release. Nanotechnology is widely used in the development of prolonged-release systems; however, its translation into commercial applications using the SC route has been reduced. It is commonly used in patients with multiple sclerosis, hepatitis C, diabetes, and acromegaly.

Despite the potential of nanotechnology to improve the efficacy, bioavailability, targeting, and dose-response relationship of drugs, it is expensive, and not all drugs are candidates for this type of formulation. Much effort has been made to develop nanosystems with prolonged-release properties for drugs with a specific physicochemical and toxicological profile. For example, hydrophobic and extensively metabolized PTX and DOX, which have a low half-life and high toxicity, are potential candidates. Regarding SC composition, characteristics, and nanotechnology, the SC pathway can play a fundamental role in the prolonged administration of drugs, namely those negatively charged and with low molecular weight. Owing to their lower nonspecific biodistribution, nanosystems can provide a prolonged-release effect upon incorporation into hydrogels and/or microparticles.

Despite the large number of nanosystems administered subcutaneously, their potential as drug-release modifiers remain to be explored. Many of the available results were obtained using relevant characterization assays, such as *in vitro* release methodologies; however, validations regarding their correlation with the SC route still need to be improved, perhaps owing to the scarcity of models of the SC route.

Although major progress has been made toward the complete and comprehensive characterization of nanosystem-based medicines using a QbD approach, experimental approaches with the SC route in their QTPP are still lacking. In fact, given the relevance of the state-of-the-art related to drug properties and the mechanism of absorption following SC administration for drug molecules such as insulin, there is a need to fully understand the nanosystem parameters governing stability properties, such as size and aggregation during and after manufacturing. Moreover, any relationship between nanosystem-based formulation properties and their uptake following SC administration can lead to the successful formulation. Based on the assumption that the absorption pathway ultimately depends on size, the charge and shape are still properties that should be addressed. A formulation of nanosystems based

on a QbD approach should consider size, aggregation, and respective stabilities according to ICH guidelines as CQAs in the early stages of their development. This ambitious nanosystem design may become a regular practice, and major and faster breakthroughs can be achieved regarding the successful absorption pathway targeting nanoparticles via the SC route. Thus, nanotechnology can effectively contribute to the development of prolonged drug-delivery systems for SC administration.

In summary, it is expected that future studies will follow a path capable of filling the gaps that we currently have. We can consider 1) the characterization and understanding of the ECM; 2) the development of models that mimic the SC pathway; 3) the development and standardization of guidelines to evaluate and approve nanosystems; 4) studies that consider the SC pathway in the QTPP of a nanosystem and that identify and understand the CQA; and 5) the cooperation between QbD and IA to optimize the R&D process and subsequently improve the transition to clinical trials and successful market approval.

8. References

[1]   Jennifer Le. Drug Administration - Drugs - MSD Manual Consumer Version. 2016 https://www.msdmanuals.com/home/drugs/administration-and-kinetics-of-drugs/drug-administration. (April, 2022).

[2]   Zhou Q, Jin J, Zhu L, et al. The optimal choice of medication administration route regarding intravenous, intramuscular, and subcutaneous injection. Patient Prefer Adherence. 2015;9:923.

[3]   Elsamany SA, Jafal M, Hassanin F. Pharmaco-economic assessment of subcutaneous compared to intravenous trastuzumab in HER2-positive breast cancer patients: A single institution experience. J Clin Oncol. 2020;38:e19409.

[4]   Viola M, Sequeira J, Seiça R, et al. Subcutaneous delivery of monoclonal antibodies: How do we get there? J Control Rel. 2018;286:301–314.

[5]   Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: Recent developments and future prospects. J Nanobiotechnology. 2018;16:1–33.

[6]   Halwani AA. Development of Pharmaceutical Nanomedicines: From the Bench to the Market. Pharmaceutics. 2022;14:106.

[7]   Kalaydina RV, Bajwa K, Qorri B, et al. Recent advances in "smart" delivery systems for extended drug release in cancer therapy. Int J Nanomedicine. 2018;13:4727–4745.

[8]   Farjadian F, Ghasemi A, Gohari O, et al. Nanopharmaceuticals and nanomedicines currently on the market: Challenges and opportunities. Nanomedicine. 2019;14:93–126.

[9]   Mitchell MJ, Billingsley MM, Haley RM, et al. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20:101–124.

[10]   FDA Approves First COVID-19 Vaccine https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine. (June 21, 2022)

[11]   Coronavirus (COVID-19) Update: FDA Takes Key Action by Approving Second COVID-19 Vaccine https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-takes-key-action-approving-second-covid-19-vaccine. (June 21, 2022)

[12]   Richter WF, Bhansali SG, Morris ME. Mechanistic Determinants of Biotherapeutics Absorption Following SC Administration. AAPS J. 2012;14:559–570.

[13] Luo L, Liu M. Adipose tissue in control of metabolism. J Endocrinol. 2016;231:R77–R99.

[14] Kendall RT, Feghali-Bostwick CA. Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol. 2014;5:77–99.

[15] Frost GI. Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration. Expert Opin Drug Deliv. 2007;4:427–440.

[16] Varkhede N, Bommana R, Schöneich C, et al. Proteolysis and Oxidation of Therapeutic Proteins After Intradermal or Subcutaneous Administration. J Pharm Sci. 2020;109:191–205.

[17] Rasmussen CH, Røge RM, Ma Z, et al. Insulin aspart pharmacokinetics: An assessment of its variability and underlying mechanisms. Eur J Pharm Sci. 2014;62:65–75.

[18] Bittner B, Richter W, Schmidt J. Subcutaneous Administration of Biotherapeutics: An Overview of Current Challenges and Opportunities. BioDrugs. 2018;32:425–440.

[19] Reddy ST, Berk DA, Jain RK, et al. A sensitive in vivo model for quantifying interstitial convective transport of injected macromolecules and nanoparticles. J Appl Physiol. 2006;101:1162–1169.

[20] Collins DS, Kourtis LC, Thyagarajapuram NR, et al. Optimizing the Bioavailability of Subcutaneously Administered Biotherapeutics Through Mechanochemical Drivers. Pharm Res. 2017;34:2000–2011.

[21] Burke JA, Zhang X, Bobbala S, et al. Subcutaneous nanotherapy repurposes the immunosuppressive mechanism of rapamycin to enhance allogeneic islet graft viability. Nat Nanotechnol. 2022;17:319–330.

[22] McCright J, Naiknavare R, Yarmovsky J, et al. Targeting Lymphatics for Nanoparticle Drug Delivery. Front Pharmacol. 2022;13:887402.

[23] Ke X, Howard GP, Tang H, et al. Physical and chemical profiles of nanoparticles for lymphatic targeting. Adv Drug Deliv Rev. 2019;151–152:72–93.

[24] Vijayakumar A, Sharon E, Teena J, et al. A clinical study on drug-related problems associated with intravenous drug administration. J Basic Clin Pharm. 2014;5:49–53.

[25]   Haeri A, Osouli M, Bayat F, et al. Nanomedicine approaches for sirolimus delivery: a review of pharmaceutical properties and preclinical studies. Artif Cells Nanomed Biotechnol. 2018;46:1–14.

[26]   Davies N, Hovdal D, Edmunds N, et al. Functionalized lipid nanoparticles for subcutaneous administration of mRNA to achieve systemic exposures of a therapeutic protein. Mol Ther Nucleic Acids. 2021;24:369–384.

[27]   Wang L, Liu G, Hu Y, et al. Doxorubicin-loaded polypyrrole nanovesicles for suppressing tumor metastasis through combining photothermotherapy and lymphatic system-targeted chemotherapy. Nanoscale. 2022;14:3097–3111.

[28]   St Clair-Jones A, Prignano F, Goncalves J, et al. Understanding and Minimising Injection-Site Pain Following Subcutaneous Administration of Biologics: A Narrative Review. Rheumatol Ther. 2020;7:741–757.

[29]   Woodley WD, Morel DR, Sutter DE, et al. Clinical evaluation of large volume subcutaneous injection tissue effects, pain, and acceptability in healthy adults. Clin Transl Sci. 2022;15:92–104.

[30]   Jiskoot W, Hawe A, Menzen T, et al. Ongoing Challenges to Develop High Concentration Monoclonal Antibody-based Formulations for Subcutaneous Administration: Quo Vadis? J Pharm Sci. 2022;111:861–867.

[31]   Heo YA, Syed YY. Subcutaneous Trastuzumab: A Review in HER2-Positive Breast Cancer. Target Oncol. 2019;14:749–758.

[32]   Wasserman RL. Clinical Practice Experience with HyQvia in Adults Using Alternative Dosing Regimens and Pediatric Patients: A Retrospective Study. Adv Ther. 2020;37:1536–1549.

[33]   Locke KW, Maneval DC, LaBarre MJ. ENHANZE® drug delivery technology: a novel approach to subcutaneous administration using recombinant human hyaluronidase PH20. Drug Deliv. 2019;26:98–106.

[34]   Stack T, Liu Y, Frey M, et al. Enhancing subcutaneous injection and target tissue accumulation of nanoparticles: Via co-administration with macropinocytosis inhibitory nanoparticles (MiNP). Nanoscale Horiz. 2021;6:393–400.

[35]   Chen W, Yung BC, Qian Z, et al. Improving long-term subcutaneous drug delivery by regulating material-bioenvironment interaction. Adv Drug Deliv Rev. 2018;127:20–34.

[36]   Kastellorizios M, Tipnis N, Burgess DJ. Foreign Body Reaction to Subcutaneous Implants. Adv Exp Med Biol. Springer New York LLC; 2015. p. 93–108.

[37]   Karabin NB, Allen S, Kwon H-K, et al. Sustained micellar delivery via inducible transitions in nanostructure morphology. Nat Commun. 2018;9:624.

[38]   Kharbikar BN, Chendke GS, Desai TA. Modulating the foreign body response of implants for diabetes treatment. Adv Drug Deliv Rev. 2021;174:87–113.

[39]   Jogpal V, Sanduja M, Dutt R, et al. Advancement of nanomedicines in chronic inflammatory disorders. Inflamm Pharmacol. 2022;30:355–368.

[40]   Sercombe L, Veerati T, Moheimani F, et al. Advances and Challenges of Liposome Assisted Drug Delivery. Front Pharmacol. 2015;6:286.

[41]   Suk JS, Xu Q, Kim N, et al. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev. 2016;99:28–51.

[42]   Leung AKK, Tam YYC, Chen S, et al. Microfluidic Mixing: A General Method for Encapsulating Macromolecules in Lipid Nanoparticle Systems. J Phys Chem B. 2015;119:8698–8706.

[43]   Berraondo P, Martini PGV, Avila MA, et al. Messenger RNA therapy for rare genetic metabolic diseases. Gut. 2019;68:1323–1330.

[44]   Kulkarni JA, Witzigmann D, Leung J, et al. On the role of helper lipids in lipid nanoparticle formulations of siRNA. Nanoscale. 2019;11:21733–21739.

[45]   Volpatti LR, Matranga MA, Cortinas AB, et al. Glucose-Responsive Nanoparticles for Rapid and Extended Self-Regulated Insulin Delivery. ACS Nano. 2020;14:488–497.

[46]   Wang P, Perche F, Midoux P, et al. In Vivo bone tissue induction by freeze-dried collagen-nanohydroxyapatite matrix loaded with BMP2/NS1 mRNAs lipopolyplexes. J Control Rel. 2021;334:188–200.

[47]   Yang W, Liang H, Ma S, et al. Gold nanoparticle based photothermal therapy: Development and application for effective cancer treatment. SM&Ts. 2019;22:e00109.

[48]   Tanaka M, Hayashi M, Roach L, et al. Synthesis of near-infrared absorbing triangular Au nanoplates using biomineralisation peptides. Acta Biomater. 2021;131:519–531.

[49]   Arias L, Pessan J, Vieira A, et al. Iron Oxide Nanoparticles for Biomedical Applications: A Perspective on Synthesis, Drugs, Antimicrobial Activity, and Toxicity. Antibiotics. 2018;7:46.

[50]   Kim SH, Thambi T, Giang Phan VH, et al. Modularly engineered alginate bioconjugate hydrogel as biocompatible injectable scaffold for in situ biomineralization. Carbohydr Polym. 2020;233 :e115832.

[51]   Bahari Javan N, Montazeri H, Rezaie Shirmard L, et al. Preparation, characterization and in vivo evaluation of a combination delivery system based on hyaluronic acid/jeffamine hydrogel loaded with PHBV/PLGA blend nanoparticles for prolonged delivery of Teriparatide. Eur J Pharm Sci. 2017;101:167–181.

[52]   Cao S, Liu Y, Shang H, et al. Supramolecular nanoparticles of calcitonin and dipeptide for long-term controlled release. J Control Rel. 2017;256:182–192.

[53]   Bordat A, Boissenot T, Ibrahim N, et al. A Polymer Prodrug Strategy to Switch from Intravenous to Subcutaneous Cancer Therapy for Irritant/Vesicant Drugs. J Am Chem Soc. 2022;144:18844–18860.

[54]   Thambi T, Giang Phan VH, Kim SH, et al. Smart injectable biogels based on hyaluronic acid bioconjugates finely substituted with poly(β-amino ester urethane) for cancer therapy. Biomater Sci. 2019;7:5424–5437.

[55]   Vogus DR, Evans MA, Pusuluri A, et al. A hyaluronic acid conjugate engineered to synergistically and sequentially deliver gemcitabine and doxorubicin to treat triple negative breast cancer. J Control Rel. 2017;267:191–202.

[56]   Lee ALZ, Yang C, Gao S, et al. Subcutaneous vaccination using injectable biodegradable hydrogels for long-term immune response. Nanomedicine. 2019;21:e102056.

[57]   Kristó K, Szekeres M, Makai Z, et al. Preparation and investigation of core-shell nanoparticles containing human interferon-α. Int J Pharm. 2020;573:e118825.

[58]   Zhang ZQ, Kim YM, Song SC. Injectable and Quadruple-Functional Hydrogel as an Alternative to Intravenous Delivery for Enhanced Tumor Targeting. ACS Appl Mater Interfaces. 2019;11:34634–34644.

[59]   Rezaie Shirmard L, Ghofrani M, Bahari Javan N, et al. Improving the in-vivo biological activity of fingolimod loaded PHBV nanoparticles by using hydrophobically modified alginate. Drug Dev Ind Pharm. 2020;46:318–328.

[60]   Jeong DI, Kim S, Kim M-H, et al. Donepezil hydrochloride-reinforced cellulose nanocrystal-aggregated gel structure for long-acting drug delivery. Carbohydr Polym. 2022;296:e119887.

[61]   Chendke GS, Faleo G, Juang C, et al. Supporting Survival of Transplanted Stem-Cell-Derived Insulin-Producing Cells in an Encapsulation Device Augmented with Controlled Release of Amino Acids. Adv Biosyst. 2019;3:e1900086.

[62]   Trinh TA, Duy Le TM, Ho HGV, et al. A novel injectable pH-temperature sensitive hydrogel containing chitosan-insulin electrosprayed nanosphere composite for an insulin delivery system in type i diabetes treatment. Biomater Sci. 2020;8:3830–3843.

[63]   Tong F. Preparation of exenatide-loaded linear poly(ethylene glycol)-brush poly(l-lysine) block copolymer: Potential implications on diabetic nephropathy. Int J Nanomedicine. 2017;12:4663–4678.

[64]   Dong N, Zhu C, Jiang J, et al. Development of composite PLGA microspheres containing exenatide-encapsulated lecithin nanoparticles for sustained drug release. Asian J Pharm Sci. 2020;15:347–355.

[65]   Seo BB, Park MR, Song SC. Sustained Release of Exendin 4 Using Injectable and Ionic-Nano-Complex Forming Polymer Hydrogel System for Long-Term Treatment of Type 2 Diabetes Mellitus. ACS Appl Mater Interfaces. 2019;11:15201–15211.

[66]   Korupalli C, Pan WY, Yeh CY, et al. Single-injecting, bioinspired nanocomposite hydrogel that can recruit host immune cells in situ to elicit potent and long-lasting humoral immune responses. Biomaterials. 2019;216:e119268.

[67]   Filipović N, Veselinović L, Ražić S, et al. Poly (ε-caprolactone) microspheres for prolonged release of selenium nanoparticles. Mat Sci Eng C. 2019;96:776–789.

[68]   Sigfridsson K, Xue A, Goodwin K, et al. Sustained release and improved bioavailability in mice after subcutaneous administration of griseofulvin as nano- and microcrystals. Int J Pharm. 2019;566:565–572.

[69]   Tanaudommongkon I, Tanaudommongkon A, Dong X. Development of In Situ Self-Assembly Nanoparticles to Encapsulate Lopinavir and Ritonavir for Long-Acting Subcutaneous Injection. Pharmaceutics. 2021;13:904.

[70] Duong V-A, Nguyen T-T-L, Maeng H-J, et al. Preparation of Ondansetron Hydrochloride-Loaded Nanostructured Lipid Carriers Using Solvent Injection Method for Enhancement of Pharmacokinetic Properties. Pharm Res. 2019;36:138.

[71] Elbrink K, van Hees S, Chamanza R, et al. Application of solid lipid nanoparticles as a long-term drug delivery platform for intramuscular and subcutaneous administration: In vitro and in vivo evaluation. Eur J Pharm Biopharm. 2021;163:158–170.

[72] Prakapenka A v., Quihuis AM, Carson CG, et al. Poly(lactic-co-glycolic Acid) Nanoparticle Encapsulated 17β-Estradiol Improves Spatial Memory and Increases Uterine Stimulation in Middle-Aged Ovariectomized Rats. Front Behav Neurosci. 2020;14:e597690.

[73] Park J-S, Lee D, Yang S, et al. Methotrexate-loaded nanoparticles ameliorate experimental model of autoimmune arthritis by regulating the balance of interleukin-17-producing T cells and regulatory T cells. J Transl Med. 2022;20:1–11.

[74] Kovalainen M, Mönkäre J, Kaasalainen M, et al. Development of porous silicon nanocarriers for parenteral peptide delivery. Mol Pharm. 2013;10:353–359.

[75] Sozen T, Ozisik L, Calik Basaran N. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4:46–56.

[76] Chesnut CH, Azria M, Silverman S, et al. Salmon calcitonin: a review of current and future therapeutic indications. Osteoporos Int. 2008;19:479–491.

[77] Canalis E. Management of endocrine disease: Novel anabolic treatments for osteoporosis. Eur J Endocrinol. 2018;178:33–44.

[78] Reid IR, Billington EO. Drug therapy for osteoporosis in older adults. The Lancet. 2022;399:1080–1092.

[79] Sun B, Luo C, Yu H, et al. Disulfide Bond-Driven Oxidation- and Reduction-Responsive Prodrug Nanoassemblies for Cancer Therapy. Nano Lett. 2018;18:3643–3650.

[80] Li N, Guo W, Li Y, et al. Construction and anti-tumor activities of disulfide-linked docetaxel-dihydroartemisinin nanoconjugates. Colloids Surf B Biointerfaces. 2020;191:e111018.

[81] Wang Z, Ling L, Du Y, et al. Reduction responsive liposomes based on paclitaxel-ss-lysophospholipid with high drug loading for intracellular delivery. Int J Pharm. 2019;564:244–255.

[82]   Lu J, Huang Y, Zhao W, et al. PEG-derivatized embelin as a nanomicellar carrier for delivery of paclitaxel to breast and prostate cancers. Biomaterials. 2013;34:1591–1600.

[83]   Barbee MS, Owonikoko TK, Harvey RD. Taxanes: vesicants, irritants, or just irritating? Ther Adv Med Oncol. 2014;6:16–20.

[84]   Zhao N, C Woodle M, Mixson AJ. Advances in Delivery Systems for Doxorubicin. J Nanomed Nanotechnol. 2018;9:519.

[85]   Kanwal U, Irfan Bukhari N, Ovais M, et al. Advances in nano-delivery systems for doxorubicin: an updated insight. J Drug Target. 2018;26:296–310.

[86]   Wang Y, Yang ST, Wang Y, et al. Adsorption and desorption of doxorubicin on oxidized carbon nanotubes. Colloids Surf B Biointerfaces. 2012;97:62–69.

[87]   Zha Z, Yue X, Ren Q, et al. Uniform Polypyrrole Nanoparticles with High Photothermal Conversion Efficiency for Photothermal Ablation of Cancer Cells. Adv Mater. 2013;25:777–782.

[88]   Wang T, Yang L, Xie Y, et al. An injectable hydrogel/staple fiber composite for sustained release of CA4P and doxorubicin for combined chemotherapy of xenografted breast tumor in mice. Nan Fang Yi Ke Da Xue Xue Bao. 2022;42:625–632.

[89]   Rosenblum D, Joshi N, Tao W, et al. Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun. 2018;9:1410.

[90]   Cohen JA, Tenenbaum N, Bhatt A, et al. Extended treatment with fingolimod for relapsing multiple sclerosis: the 14-year LONGTERMS study results. Ther Adv Neurol Disord. 2019;12:1756286419878322.

[91]   Singer BA. Initiating oral fingolimod treatment in patients with multiple sclerosis. Ther Adv Neurol Disord. 2013;6:269–275.

[92]   Vargas WS, Perumal JS. Fingolimod and cardiac risk: latest findings and clinical implications. Ther Adv Drug Saf. 2013;4:119–124.

[93]   Genuth SM, Palmer JP, Nathan DM. Classification and Diagnosis of Diabetes. Diabetes in America. National Institute of Diabetes and Digestive and Kidney Diseases (US); 2018. p. 1–39.

[94]   Hansen KB, Vilsbøll T, Knop FK. Incretin mimetics: a novel therapeutic option for patients with type 2 diabetes - a review. Diabetes Metab Syndr Obes. 2010;3:155–163.

[95]   Seo BB, Park MR, Chun CJ, et al. The biological efficiency and bioavailability of human growth hormone delivered using injectable, ionic, thermosensitive poly(organophosphazene)-polyethylenimine conjugate hydrogels. Biomaterials. 2011;32:8271–8280.

[96]   Plotkin SA. Correlates of protection induced by vaccination. Clin Vacc Immunol. 2010;17:1055–1065.

[97]   Sallusto F, Lanzavecchia A, Araki K, et al. From Vaccines to Memory and Back. Immunity. 2010;33:451–463.

[98]   Roth GA, Gale EC, Alcantara-Hernandez M, et al. Injectable hydrogels for sustained codelivery of subunit vaccines enhance humoral immunity. ACS Cent Sci. 2020;6:1800–1812.

[99]   Bailon L, Mothe B, Berman L, et al. Novel Approaches Towards a Functional Cure of HIV/AIDS. Drugs. 2020;80:859–868.

[100]   Adefolalu A, Nkosi Z. The Complex Nature of Adherence in the Management of HIV/AIDS as a Chronic Medical Condition. Diseases. 2013;1:18–35.

[101]   Zingg R, Fischer M. The consolidation of nanomedicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2019;11:1–6.

[102]   Foulkes R, Man E, Thind J, et al. The regulation of nanomaterials and nanomedicines for clinical application: Current and future perspectives. Biomater Sci. 2020;8:4653–4664.

[103]   Committee for Medicinal Products for Human Use (CHMP) EMA. Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications, EMA/CHMP/SWP/100094/2011. https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-non-clinical-studies-generic-nanoparticle-iron-medicinal-product-applications_en.pdf. (May 24, 2022).

[104]   Soares S, Sousa J, Pais A, et al. Nanomedicine: Principles, Properties, and Regulatory Issues. Front Chem. 2018;6:1–15.

[105]   Allen RP, Bolandparvaz A, Ma JA, et al. Latent, Immunosuppressive Nature of Poly(lactic- co -glycolic acid) Microparticles. ACS Biomater Sci Eng. 2018;4:900–918.

[106]   Epstein RS. Payer Perspectives on Intravenous versus Subcutaneous Administration of Drugs. ClinicoEconomics and Outcomes Research. 2021;13:801–807.

[107] Danaei M, Dehghankhold M, Ataei S, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics. 2018;10:57.

[108] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015;33:941–951.

[109] Bastogne T. Quality-by-design of nanopharmaceuticals – a state of the art. Nanomedicine. 2017;13:2151–2157.

[110] Rawal M, Singh A, Amiji MM. Quality-by-Design Concepts to Improve Nanotechnology-Based Drug Development. Pharm Res. 2019;36:1–20.

[111] Pallagi E, Ismail R, Paál TL, et al. Initial Risk Assessment as part of the Quality by Design in peptide drug containing formulation development. Eur J Pharm Sci. 2018;122:160–169.

[112] Thanki K, Zeng X, Justesen S, et al. Engineering of small interfering RNA-loaded lipidoid-poly(DL-lactic-co-glycolic acid) hybrid nanoparticles for highly efficient and safe gene silencing: A quality by design-based approach. Eur J Pharm Biopharm. 2017;120:22–33.

[113] Gupta A, Costa AP, Xu X, et al. Formulation and characterization of curcumin loaded polymeric micelles produced via continuous processing. Int J Pharm. 2020;583:e119340.

[114] Alexis F, Pridgen E, Molnar LK, et al. Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles. Mol Pharm. 2008;5:505–515.

[115] Zagalo DM, Silva BMA, Silva C, et al. A quality by design (QbD) approach in pharmaceutical development of lipid-based nanosystems: A systematic review. J Drug Deliv Sci Technol. 2022;70:103207.

[116] Omar Zaki SS, Ibrahim MN, Katas H. Particle Size Affects Concentration-Dependent Cytotoxicity of Chitosan Nanoparticles towards Mouse Hematopoietic Stem Cells. J Nanotechnol. 2015;2015:1–5.

[117] Egorov E, Pieters C, Korach-Rechtman H, et al. Robotics, microfluidics, nanotechnology and AI in the synthesis and evaluation of liposomes and polymeric drug delivery systems. Drug Deliv Transl Res. 2021;11:345–352.

[118] Villa Nova M, Lin TP, Shanehsazzadeh S, et al. Nanomedicine Ex Machina: Between Model-Informed Development and Artificial Intelligence. Front Digit Health. 2022;4:1–12.

[119] Sarker IH. Machine Learning: Algorithms, Real-World Applications and Research Directions. SN Comput Sci. 2021;2:1–21.

[120] Li F, Han J, Cao T, et al. Design of self-assembly dipeptide hydrogels and machine learning via their chemical features. PNAS. 2019;116:11259–11264.

[121] He Y, Ye Z, Liu X, et al. Can machine learning predict drug nanocrystals? J Control Rel. 2020;322:274–285.

[122] Youshia J, Ali ME, Lamprecht A. Artificial neural network based particle size prediction of polymeric nanoparticles. Eur J Pharm Biopharm. 2017;119:333–342.

[123] Zaki MR, Varshosaz J, Fathi M. Preparation of agar nanospheres: Comparison of response surface and artificial neural network modeling by a genetic algorithm approach. Carbohydr Polym. 2015;122:314–320.

[124] Li Y, Abbaspour MR, Grootendorst P v., et al. Optimization of controlled release nanoparticle formulation of verapamil hydrochloride using artificial neural networks with genetic algorithm and response surface methodology. Eur J Pharm Biopharm. 2015;94:170–179.

[125] Hashad RA, Ishak RAH, Fahmy S, et al. Chitosan-tripolyphosphate nanoparticles: Optimization of formulation parameters for improving process yield at a novel pH using artificial neural networks. Int J Biol Macromol. 2016;86:50–58.

[126] Baharifar H, Amani A. Size, Loading Efficiency, and Cytotoxicity of Albumin-Loaded Chitosan Nanoparticles: An Artificial Neural Networks Study. J Pharm Sci. 2017;106:411–417.

[127] Kanwal U, Bukhari NI, Rana NF, et al. Doxorubicin-loaded quaternary ammonium palmitoyl glycol chitosan polymeric nanoformulation: Uptake by cells and organs. Int J Nanomedicine. 2019;14:1–15.

[128] Shabanzadeh P, Senu N, Shameli K, et al. Artificial Intelligence in Numerical Modeling of Silver Nanoparticles Prepared in Montmorillonite Interlayer Space. J Chem. 2013;2013:1–8.

[129] Hashad RA, Ishak RAH, Fahmy S, et al. Chitosan-tripolyphosphate nanoparticles: Optimization of formulation parameters for improving process yield at a novel pH using artificial neural networks. Int J Biol Macromol. 2016;86:50–58.

[130] Öztürk AA, Gündüz AB, Ozisik O. Supervised Machine Learning Algorithms for Evaluation of Solid Lipid Nanoparticles and Particle Size. Comb Chem High Throughput Screen. 2018;21:693–699.

[131] Lazarovits J, Sindhwani S, Tavares AJ, et al. Supervised Learning and Mass Spectrometry Predicts the in Vivo Fate of Nanomaterials. ACS Nano. 2019;13:8023–8034.