UNIVERSIDADE D
COIMBRA

Inês Isabel Oliveira Formoso

Relatórios de Estágio e Monografia intitulada *"Nanotechnology applied to regenerative medicine"* referentes à unidade curricular "Estágio", sob a orientação do Doutor Henrique Santos, da Dra. Susana Cartaxo e da Professora Doutora Eliana Souto, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2021

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Setembro de 2021

Eu, Inês Isabel Oliveira Formoso, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2016253178, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada *"Nanotechnology applied to regenerative medicine"* 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, 6 de setembro de 2021.

Inês Isabel Oliveira Formoso

(Inês Isabel Oliveira Formoso)

Agradecimentos

À minha mãe, a responsável pela pessoa em que me tornei e por tudo o que conquistei até hoje.

Aos meus avós, em quem me inspiro todos os dias para ter força e nunca desistir perante qualquer desafio que se cruze no meu percurso.

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Às amizades de sempre, com quem posso sempre contar no regresso a casa.

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À FFUC, pelas oportunidades facultadas ao longo destes cinco anos.

A Coimbra, uma lição de aspiração e tradição.

Publicação

A monografia apresentada foi aprovada para publicação, possuindo a seguinte referência: Formoso, I.; Zielinska, A., Cano, A., Sanchez-Lopez, E., Silva, A.M., Souto, E.B., Nanotechnology applied to regenerative medicine, in: "*Nanotechnology and Regenerative Medicine: History, Techniques, Frontiers and Applications*", M. H. A. Santana, E. B. Souto (editors), Amsterdam, The Netherlands, Elsevier (2021) Chapter 1.

"One never notices what has been done; one can only see what remains to be done."

Marie Curie

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Secção 2 - Monografia

"Nanotechnology applied to regenerative medicine"

Primeira Secção

Resumo

Inicialmente é apresentado o relatório referente ao estágio decorrido na Farmácia do Altinho, na Pontinha, com princípio a 11 de fevereiro e término a 1 de maio de 2021, com orientação do Doutor Henrique Santos.

No mesmo é realizada uma análise SWOT *(Strenghts, Weaknesses, Opportunities and Threats)*, sendo ainda apresentados três casos práticos de situações que decorreram durante o exercer das minhas funções, nomeadamente no atendimento de utentes.

Abstract

Initially is presented the report referring to the internship that took place at Farmácia do Altinho, in Pontinha, in the period between 11st of February to the 1st of May 2021, under the supervision of Pharm D., Henrique Santos.

In it, a SWOT analysis (Strengths, Weaknesses, Opportunities and Threats) is presented, and three practical cases of situations that occurred during the exercise of my functions, namely pharmaceutical indication and advice, are also exposed.

Segunda Secção

Resumo

Posteriormente é apresentado o relatório referente ao estágio realizado no Laboratório de Estudos Farmacêuticos (LEF) – Infosaúde, em Barcarena, no departamento de Controlo de Qualidade, Segurança e Ambiente (QA&SHE), com início a 3 de maio e término a 30 de julho, sob orientação da Dra. Susana Cartaxo.

No mesmo é realizada uma análise SWOT *(Strenghts, Weaknesses, Opportunities and Threats)*.

Abstract

Subsequently, the report regarding the internship carried out at the Laboratórios de Estudos Farmacêuticos (LEF) – Infosaúde, in Barcarena, in the Quality Control, Safety and Environment department (QA&SHE), in the period between May 3rd and July 30th 2021, under the supervision of Susana Cartaxo. In it, a SWOT analysis (Strengths, Weaknesses, Opportunities and Threats) is performed.

Resumo

Por fim, é apresentada a monografia conduzida sob orientação da Professora Doutora Eliana Souto, denominada *"Nanotechnology applied to regenerative medicine"*.

O conhecimento em relação às nanopartículas aplicadas à Medicina regenerativa está em constante desenvolvimento, o que se deve aos resultados promissores que têm sido obtidos nos últimos anos, principalmente relacionados com patologias que atualmente não possuem um tratamento que realmente altere a sua progressão.

Esta monografia irá centrar-se nos tipos de nanopartículas que podem ser aplicadas na Medicina Regenerativa e em algumas estratégias que devem ser consideradas aquando da sua utilização.

Serão ainda apresentadas algumas patologias que poderão beneficiar do uso de terapias regenerativas, sendo elas, a Diabetes Tipo 1, a Osteoartrite, o Tumor cerebral, doenças neurodegenerativas e ainda algumas doenças Cardiovasculares, tendo sempre por base conceitos de nanotecnologia.

Palavras-chave: nanopartículas, nanodispositivos, nanomateriais, regeneração, tratamento direcionado, terapia genética.

Abstract

Finally, a monograph is presented, conducted under the guidance of Pharm D., Eliana Souto, called "Nanotechnology applied to regenerative medicine".

The knowledge related to nanoparticles applied to regenerative medicine is under constant development, which is due to the promising results that have been obtained in recent years, mainly associated to pathologies that currently do not have a treatment that actually changes their progression.

This monograph will focus on the types of nanoparticles that can be applied in Regenerative medicine and on some strategies that should be considered when using them.

Some pathologies that may benefit from the use of regenerative therapies will also be presented, namely, Type 1 Diabetes, Osteoarthritis, Brain Cancer, Neurodegenerative Diseases, and even some Cardiovascular Diseases, always based on nanotechnology concepts.

Keywords: nanoparticles, nanodevices, nanomaterials, regeneration, targeted treatment, gene therapy.

Secção I

Estágios Curriculares

Parte I

Relatório de Estágio em Farmácia Comunitária

farmácia do altinho

"Farmácia das soluções: faster, cheaper, remarkable."

Lista de Abreviaturas

COVID-19| Doença de Coronavírus 2019

FA| Farmácia do Altinho

FC| Farmácia Comunitária

FFUC| Faculdade de Farmácia da Universidade de Coimbra

IF| Indicação Farmacêutica

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

MICF| Mestrado Integrado em Ciências Farmacêuticas

MM| Medicamento Manipulado

MNSRM| Medicamento Não Sujeito a Receita Médica

MSRM| Medicamento Sujeito a Receita Médica

PIM| Preparação Individualizada da Medicação

PRM| Problemas relacionados com medicamentos

RNM| Resultados negativos associados à medicação

SF| Seguimento Farmacoterapêutico

SNS| Sistema Nacional de Saúde

SWOT| *Strenghts, Weaknesses, Opportunities and Threats*

Introdução

O presente relatório visa a análise do Estágio Curricular em Farmácia Comunitária (FC) realizado durante o período de 11 de fevereiro a 1 de maio, que teve lugar na Farmácia do Altinho (FA), na Pontinha, sob orientação do Doutor Henrique Santos. Este estágio resulta, conforme a Diretiva 2013/55/EU do Parlamento Europeu de novembro de 2013, de uma obrigatoriedade de 6 meses de estágio em FC [1].

A FA é reconhecida por apresentar algumas áreas bastante desenvolvidas e diferenciadoras, como é o caso da preparação de Medicamentos Manipulados (MM), da presença de Consultas Farmacêuticas no âmbito de alguns transtornos menores, principalmente no que diz respeito a afeções dermatológicas, da presença de um serviço de Preparação Individualizada da Medicação (PIM) e ainda pelo facto de possuir um serviço associado de Análises Clínicas em associação com o laboratório LUMILABO.

Os serviços explorados na FA, a intervenção ativa do Doutor Henrique Santos em temas de extrema importância para a profissão e ainda a localização da farmácia numa área com utentes tão diferenciados, acabaram por ser os motivos que levaram à minha decisão, pelo facto de considerar que seria uma experiência que iria por à prova todas as minhas capacidades e possibilitar uma evolução, tanto a nível profissional como pessoal.

Este relatório será apresentado através de uma análise SWOT, concretizada quer a nível interno, refletindo de uma forma sustentada e crítica os pontos fortes e fracos reconhecidos, tal como a nível externo, onde são apresentadas as oportunidades e ameaças que advieram da minha experiência no estágio em questão.

Contextualização da Farmácia do Altinho

Localizada na Pontinha, uma das freguesias do município de Odivelas, na cidade de Lisboa, a FA tem como proprietário e Diretor Técnico o Doutor Henrique Santos.

Considerando a situação pandémica, existiram alterações nos horários prestados pela farmácia, sendo que, inicialmente este foi adaptado de forma a que a equipa estivesse organizada por turnos.

No decorrer do estágio a equipa era composta por seis elementos, dois farmacêuticos, duas técnicas de farmácia, uma técnica auxiliar de farmácia e por uma analista.

As instalações contemplam uma sala para atendimento ao público, dividida em duas secções, a primeira secção encontra-se situada à entrada, possibilitando a cedência e dispensa de produtos contidos essencialmente na categoria de Medicamento Não Sujeito a Receita Médica (MNSRM), adicionando as categorias de venda livre na farmácia. Na segunda secção, procede-se a um atendimento sentado, situado na parte mais interior da FA, que está mais direcionado para os Medicamentos Sujeitos a Receita Médica (MSRM), Medicamentos Não Sujeitos a

Receita Médica de Dispensa Exclusiva em Farmácias e para uma possível Revisão da Medicação e Seguimento Farmacoterapêutico (SF). Existe ainda um consultório onde são realizados os serviços de medição de parâmetros bioquímicos, administração de medicamentos injetáveis, realização de pensos e tratamentos de feridas e consultas farmacêuticas, com a finalidade de tratamento de transtornos menores que exijam um acompanhamento mais personalizado.
Adicionalmente, o espaço contém um anexo que se destina à recolha de amostras, tendo em conta a parceria da farmácia com o Laboratório LUMILABO e um laboratório reservado essencialmente à preparação de MM e à PIM. A FA é complementada pelas instalações sanitárias e pela sala de receção e entrada de encomendas.

Análise SWOT

Pontos Fortes

- Plano de estágio centrado no atendimento ao público

Após um primeiro mês direcionado para a preparação de MM, gestão da farmácia, acompanhamento do atendimento dos membros da equipa da FA e alguma pesquisa bibliográfica, os restantes meses focaram-se no desenvolvimento das minhas competências através do atendimento ao público. O incentivo manifestado pela equipa, para que realizasse os atendimentos autonomamente, proporcionou-me uma grande motivação e levou a que adquirisse um grande sentido de responsabilidade pelo ato farmacêutico e pelo impacto que pode surtir na saúde dos utentes.
O atendimento farmacêutico pode seguir diferentes direções tendo em conta a situação que nos é apresentada pelo utente. Considerando a cedência de um medicamento ou de substâncias medicamentosas, o farmacêutico pode atuar nesse sentido mediante prescrição médica, automedicação ou indicação farmacêutica, sendo necessária uma avaliação prévia da necessidade do medicamento, da adequação do medicamento ao doente, da adequação da Posologia e das condições do doente para administrar o medicamento, protegendo desta forma o utente de possíveis resultados negativos associados à medicação (RNM).
Fazer esta análise durante um atendimento exigiu um grande esforço e pesquisa constante durante a minha prestação, o que me permitiu consolidar e adquirir novos conhecimentos.

- Oferta de serviços e cuidados farmacêuticos
  - Seguimento Farmacoterapêutico

No decorrer do estágio foi-me pedida uma recolha de informação relativamente ao SF, sendo que no caso da FA é adotado o Método Dáder **[Anexo I]**. Neste manual, o SF é descrito como um serviço que tem como finalidade detetar Problemas Relacionados com os Medicamentos (PRM), para prevenir e resolver RNM, o que implica um compromisso

contínuo, em colaboração com o utente e com outros profissionais do sistema de saúde, com o objetivo de atingir resultados que melhorem a qualidade de vida do doente. De forma a classificar os RNM é essencial considerar os três princípios que a farmacoterapia utilizada pelos doentes deve obedecer: necessária, efetiva e segura.

Garantir o processo de uso de um medicamento não garante que se atinjam resultados positivos no doente, o que torna indispensável que toda a componente assistencial se centre nos resultados produzidos na saúde do doente, que são os que realmente demarcam o grau de benefício ou dano para o mesmo [2].

Tendo em conta a pandemia, estas consultas tiveram uma afluência reduzida e, existindo limitações do número de pessoas dentro de espaços fechados não me foi possível assistir a nenhum, no entanto, a pesquisa que me foi pedida foi muito útil para aprofundar os meus conhecimentos nesta área.

- Preparação de Medicamentos Manipulados

Um MM define-se como qualquer fórmula magistral ou preparado oficinal preparado e dispensado sob a responsabilidade de um farmacêutico [3] estando sujeito a prescrição médica, embora apenas os presentes no anexo do Despacho n.º 18694/2010, de 16 de dezembro sejam comparticipados pelo SNS. O PVP é calculado recorrendo à consulta da Portaria n.º 769/2004, de 1 de julho.

A FA destaca-se pelo elevado número de MM preparados diariamente, principalmente com finalidades veterinárias ou cosmetológicas. De notar que todas as matérias-primas em *stock* que possam ser utilizadas para a preparação dos medicamentos manipulados possuem uma ficha com os respetivos dados de segurança.

Os MM são concebidos e rotulados, no laboratório da FA, tendo em conta as indicações do Formulário Galénico Português, pelo farmacêutico sob supervisão do Diretor técnico. No caso de dúvida relativamente à preparação do medicamento, a mesma deve ser reportada ao Laboratório de Estudos Farmacêuticos ou, caso necessário, a mesma deve ser esclarecida junto ao prescritor. No decorrer da sua produção é preenchida uma ficha de preparação onde constam dados do utente, das matérias primas e material utilizado, do modo de preparação e dos ensaios de controlo de qualidade realizados **[Anexo 2]**.

No ato da dispensa, deve garantir-se que são fornecidas todas as informações necessárias, nomeadamente referentes à posologia, condições de conservação e prazo de utilização.

Durante o primeiro mês na FA tive oportunidade de acompanhar a preparação de vários MM e ainda de perceber como se efetua a gestão de *stock* de matérias-primas, o que me permitiu aplicar conhecimentos adquiridos principalmente na disciplina de Farmácia Galénica.

- Preparação individualizada da medicação

Define-se como PIM o serviço prestado pedo farmacêutico, com a finalidade de organizar as formas farmacêuticas sólidas, para uso oral, considerando a posologia prescrita, utilizando um dispositivo de múltiplas divisões, que posteriormente é selado na farmácia. Além do acondicionamento, é prestada uma explicação de forma escrita e oralmente, com o objetivo de garantir o uso responsável do medicamento promovendo assim uma maior adesão à terapêutica [4].

A PIM é aplicada a vários doentes da FA, tendo um custo de 2,5€ por semana. Este serviço acaba por trazer grandes benefícios aos aderentes, considerando que apesar de os fármacos serem cada vez mais seguros, existem ainda muitas falhas na forma como são tomados, o que se acentua nos utentes polimedicados.

No decorrer do estágio tive a possibilidade de realizar a PIM de vários utentes habituais e também de comunicar este serviço a novos utentes, que, possuindo um tratamento muito complexo, no decorrer do atendimento revelaram muitas preocupações. Através do *feedback* disponibilizado pelos usufruidores deste serviço foi-me possível constatar a relevância e utilidade que o mesmo pode representar na qualidade de vida do seu beneficiário.

Pontos Fracos

- Programa Informático – Logitools®

A FA recorre ao programa informático Logitools® como ferramenta de gestão da farmácia. Este programa oferece um controlo da gestão e de indicadores que permitem, verificar a complexidade do negócio, tendo em vista a desmaterialização de todos os processos.

Apesar dos pontos referidos, tendo anteriormente manuseado outros programas, como é o caso do SIFARMA2000® e do Winphar®, comparativamente, o Logitools® apresenta algumas lacunas, tais como, a ausência de informações técnico-científicas relativas às posologias, dosagens, precauções e possíveis efeitos secundários, o que por vezes dificulta o atendimento, tendo em conta a necessidade de consulta de outros meios de forma a garantir um aconselhamento mais rigoroso e completo.

- Implementação de estratégias de dinamização de vendas

Atualmente, a FC encontra-se sujeita a várias ameaças e a um constante aumento da competitividade no mercado, o que acresce o facto do consumidor atual ser cada vez mais informado e exigente, procurando qualidade, mas sempre focado no valor do serviço. Deste modo, o farmacêutico comunitário precisa de assumir um papel adicional de gestor, focando-se não só no utente, mas também na estabilidade da sua farmácia, tendo sempre em conta os valores éticos associados à profissão.

A FA não possui qualquer ferramenta digital que apresente os serviços prestados, nem onde possa divulgar as campanhas ou promoções de produtos, que por si só também são escassas. Sendo que as técnicas de *merchandising* não se encontram muito desenvolvidas na FA, não tive oportunidade de desenvolver as minhas competências nesta área no decorrer do meu estágio.

Oportunidades

- Formações, aquisição e consolidação de conhecimentos técnico-científicos

Existiram algumas áreas em senti mais dificuldade no aconselhamento, no entanto, através das situações apresentadas durante o atendimento, através do auxílio da equipa, e também através das formações que tive oportunidade de assistir fui consolidando novos conhecimentos.

Dentro das diversas formações posso destacar a proporcionada pela marca medi®, que acabou por revelar-se bastante útil, passando desta forma a estar familiarizada com a distinção entre meias de compressão e meias de descanso, com as diferentes malhas que podem ser oferecidas, medições necessárias para escolha de tamanho, e ainda com os acessórios que podem ser utilizados.

Provocado pela situação pandémica, houve um aumento de situações de olho seco, relacionadas com a permanência prolongada das pessoas em frente a ecrãs e, existindo uma grande oferta de produtos oftalmológicos, tive a necessidade de realizar uma pesquisa mais intensiva, de forma a construir um aconselhamento mais responsável.

Relativamente às preparações de uso veterinário descobri a existência de uma enorme panóplia de medicamentos e produtos para animais, sendo que, inicialmente não me senti apta para aconselhar devidamente o utente. Graças ao apoio dos meus colegas, e às formações disponibilizadas pelo laboratório KRKA®, fui adquirindo mais conhecimentos nesta área.

- Medicamentos Hospitalares

Tendo em conta o contexto de pandemia, foi necessária a adoção de medidas facilitadoras ao acesso aos medicamentos hospitalares que minimizassem a propagação e transmissão do vírus. Para a aquisição dos referidos medicamentos, muitos doentes, por vezes sem aparente razão técnica, acabam por ser obrigados a deslocar-se dezenas de quilómetros até aos hospitais, com farmácias a poucos metros de casa.

A disponibilização destes medicamentos na FC demonstrou mais uma vez que o farmacêutico comunitário possuindo as capacidades técnicas necessárias, e procedendo à avaliação do doente relativamente ao aparecimento de novos sinais ou sintomas sugestivos de agravamento da doença, interações medicamentosas ou efeitos indesejáveis e, reportando os mesmos aos serviços farmacêuticos hospitalares, conseguiu garantir que o estado de saúde dos utentes não era comprometido [5].

Pela oportunidade de assistência da receção e dispensa destes medicamentos, concluí que houve uma grande adesão dos utentes, sem descurar o seu acompanhamento e interação hospitalar, possibilitando uma centralização do sistema de saúde no doente, permitindo a satisfação das suas necessidades e expectativas.

Ameaças

- Concorrência no Setor - Locais de venda de MNSRM

O Decreto-Lei n.º 134/2005 de 16 de Agosto veio legitimar a venda de MNSRM em locais de venda fiscalizados (à semelhança das farmácias) pelo INFARMED, uma vez que o Governo reconheceu que estes locais proporcionaram um maior acesso aos medicamentos [6]. No entanto, estes locais apresentam-se como uma verdadeira ameaça à atividade farmacêutica.
Na vertente económica, estes locais efetuam grandes volumes de compras comparativamente às farmácias, o que permite a venda de medicamentos ao público a preços inferiores. Por outro lado, a facilidade de compra de um medicamento nestes locais, promove uma automedicação descomedida, sem aconselhamento por pessoas suficientemente qualificadas para oferecer um atendimento seguro e responsável.

- Alterações de preços de medicamentos

Este facto está relacionado com o surgimento de genéricos no mercado, sendo que ao existirem genéricos para um dado medicamento, o preço de referência a que se recorre para o cálculo da sua comparticipação, passa a ser o do quinto genérico mais barato, diminuindo desta forma o valor absoluto suportado pelo Estado e, consequentemente, aumentando o preço que o utente tem de pagar pelo medicamento original.
No decorrer dos atendimentos deparei-me com vários tipos de utentes: os que não sabiam o que eram genéricos, os que sabiam, mas que os rejeitavam por dizerem que não faziam tanto efeito e, por fim, os que levavam genéricos apenas de um laboratório específico. Várias vezes tive que esclarecer os utentes sobre este assunto, tentando adaptar o meu discurso consoante a pessoa em si. Estas situações constituíram uma ameaça ao funcionamento do meu estágio, tendo em conta a frustração recorrente demonstrada pelos utentes, o que se acentuava pelo facto de, por vezes, associarem a minha função de estagiária com a falta de credibilidade.

- Pandemia de COVID-19

Para muitos doentes, a pandemia acabou por se refletir num medo constante em se dirigirem aos hospitais e centros de saúde habituais, passando a recorrer principalmente à farmácia mais próxima para resolução de todos os seus problemas. Durante o período de estágio foram muitos os casos em que não foi apresentada receita médica para levantamento de medicamentos que obrigam a sua apresentação, no entanto, e segundo a alínea c) do Artigo

16º dos deveres do farmacêutico de oficina que constam no Código Deontológico, o farmacêutico tem o dever de "dispensar ao doente o medicamento em cumprimento da prescrição médica ou exercer a escolha que os seus conhecimentos permitem e que melhor satisfaça as relações benefício/risco e benefício/custo" [7].

Ao mesmo tempo, existiram situações, em que pela falta de informação do utente, a decisão de cedência e de aconselhamento se tornaram dificultadas, o que poderia ser facilmente resolvido caso fosse possível o acesso a estes dados, o que teria também permitido uma maior colaboração entre médicos e farmacêuticos.

A procura pela resolução de problemas de saúde ou de situações mais graves que inerentemente não são da competência do farmacêutico e a falta de acesso à informação de saúde do utente constituíram assim ameaças ao meu desempenho durante o estágio. No entanto, algumas situações apresentadas, acabaram por constituir uma oportunidade para aplicar os meus conhecimentos em situações que podem ser resolvidas na FC.

- A importância da comunicação na Farmácia Comunitária

Quando atendidos por um estagiário, alguns utentes adquirem uma postura distinta, colocando em causa muitos dos nossos aconselhamentos, sendo este comportamento claramente uma ameaça à aprendizagem e aplicação de conhecimentos por parte dos estagiários. Adicionando o facto anterior ao direcionamento cada vez mais acentuado dos serviços farmacêuticos para o foco no doente, há uma necessidade de novas formas de interação com o mesmo e com os restantes prestadores de cuidados de saúde, sendo a aquisição de competências comportamentais e comunicacionais fundamental para a melhoria contínua dos serviços prestados. De salientar, que o uso adequado das ferramentas de comunicação, influenciam em grande parte a satisfação dos utentes, e naturalmente a cooperação destes no processo de adesão à terapêutica.

Tendo experienciado alguma dificuldade em passar algumas mensagens, e no estabelecimento de confiança com alguns utentes, considerei pertinente aprofundar os meus conhecimentos na área da comunicação através da frequência num curso *online* de Comunicação Persuasiva da Speak and Lead®, que acabou por abrir horizontes nesta área. Sendo que esta vertente não é muito explorada no decorrer do MICF, penso que deveria ser ponderada a introdução de algumas horas referentes a este tema, incluindo exercícios práticos, de forma a preparar melhor os estudantes para a sua prestação no atendimento ao público.

Casos Práticos

1. *J.S.*, utente do sexo masculino, com 18 anos, apresenta-se na FA, queixando-se de dor de barriga, e de uma sensação de ardor na zona do epigastro, principalmente após as refeições e quando se encontra deitado. Diz ainda que apresenta estes sintomas esporadicamente. Em conversa relata que tem passado os dias sentando em frente ao computador, demonstrando um estilo de vida sedentário e uma alimentação desequilibrada.

Indicação Farmacêutica – Situação típica de um quadro de Azia (Pirose), pelo que comecei por recomendar algumas medidas não farmacológicas, como comer várias vezes ao dia e em poucas quantidades, evitar comidas gordurosas/muito temperadas, café, refrigerantes com gás, chocolates, citrinos e derivados do tomate, que são alimentos que podem aumentar a produção de acidez. Outras medidas indicadas passaram por ser evitada roupa muito justa ao nível da cintura e realizar a refeição 2 a 3 horas antes de se deitar, procurando elevar a cabeça quando deitado [8]. Como medida imediata para aliviar o seu desconforto, optei pelo Rennie® Digestif, que possui carbonato de cálcio e de magnésio na sua constituição (neutralizadores da acidez), sob a forma de comprimidos mastigáveis, aconselhando o utente uma posologia de 1 ou 2 comprimidos, 1 a 3 horas após as refeições e antes de deitar (no máximo 8 comprimidos por dia).

2. *M.F.,* utente do sexo feminino, 70 anos, obesa, queixa se de alguma comichão e ardor nas pregas da barriga e na zona inframamária. Quando observada no gabinete é visível a vermelhidão e descamação periférica da pele nas zonas descritas.

Indicação Farmacêutica – A situação descrita refere-se a uma dermatite intertriginosa, uma dermatite de contacto irritativa nas pregas cutâneas. Como medidas não farmacológicas, encorajei a utente a reduzir o seu peso, a manutenção de um ambiente seco e limpo na zona afetada, complementando com a utilização de roupa de algodão pouco apertada. Aconselhei a lavagem da área lesada recorrendo a um Syndet sem sabão (Uriage® Xémose Syndet), acrescentando a utilização regular de um creme barreira com agentes reparadores (Uriage® Bariéderm Cica creme) em associação com um creme com um corticosteróide de baixa potência, Pandermil® creme, possibilitando a redução da inflamação da pele, devendo ser utilizado 2 vezes por dia, durante 7 dias. Referi ainda que após os 7 dias, caso não existissem melhoras, deveria consultar um médico, tendo em conta que a combinação de ambiente quente, húmido e pele danificada representam condições ideais para a multiplicação de microrganismos.

3. *A.S.*, utente do sexo feminino, 26 anos**,** relata que sente ardor e dor ao urinar há 3 dias. Refere que precisa de algo urgentemente porque só consegue urinar "às pinguinhas". Adicionalmente, expõe que a sua urina não apresenta uma cor diferente do habitual, nem sangue visível e que durante estes dias não teve febre. Conta que é a primeira vez que sofre desta situação, e pergunta se o namorado lhe pode ter transmitido a infeção.

Indicação Farmacêutica – Esta situação relaciona-se com um caso de infeção urinária. A cistite na mulher é provocada essencialmente pela bactéria *Escherichia coli.* Desta forma, foi aconselhado à doente a utilização da solução de lavagem Saforelle®, que possui propriedades calmantes e suavizantes e um pH ligeiramente alcalino que respeita o pH e a flora da zona vulvar. Como tratamento farmacológico recomendei o Spasmurin®, até 2 comprimidos por dia, durante 15 dias. Este SA alega que pela ação das proantocianidinas há uma redução da adesão da *Escherichia coli* às paredes do trato urinário, ficando a mesma inativa e que naturalmente é eliminada pela urina. Salientei ainda a necessidade de beber água em abundância, lavar a vagina de frente para trás e usar roupa íntima de algodão. Foi também orientada no sentido de urinar após o ato sexual, tendo em conta que a relação sexual potencia a introdução de microrganismos para o interior da vagina e a subida dos mesmos pela uretra até a bexiga. Em caso de febre ou de aparecimento de outro sintoma de caráter sistémico referi à utente que deveria ser avaliada pelo médico [9].

Conclusão

O estágio em FC configurou uma das experiências mais enriquecedoras do meu percurso académico, uma vez que me possibilitou a aplicação dos conhecimentos adquiridos ao longo dos últimos 5 anos no contexto real do mundo de trabalho de um farmacêutico nesta área.

A FA encara o atendimento ao público como um ato de grande responsabilidade, colocando sempre em primeiro lugar o bem-estar dos seus utentes e o cumprimento das suas necessidades. A qualidade e competência de toda a equipa técnica contribuíram em grande parte para o desenvolvimento das minhas capacidades como futura farmacêutica, sendo que todos demonstraram empenho e preocupação em esclarecer as minhas dúvidas e em oferecer uma experiência positiva e gratificante. Cada uma das competências que desenvolvi e adquiri a nível pessoal e profissional vão certamente contribuir para um futuro mais promissor.

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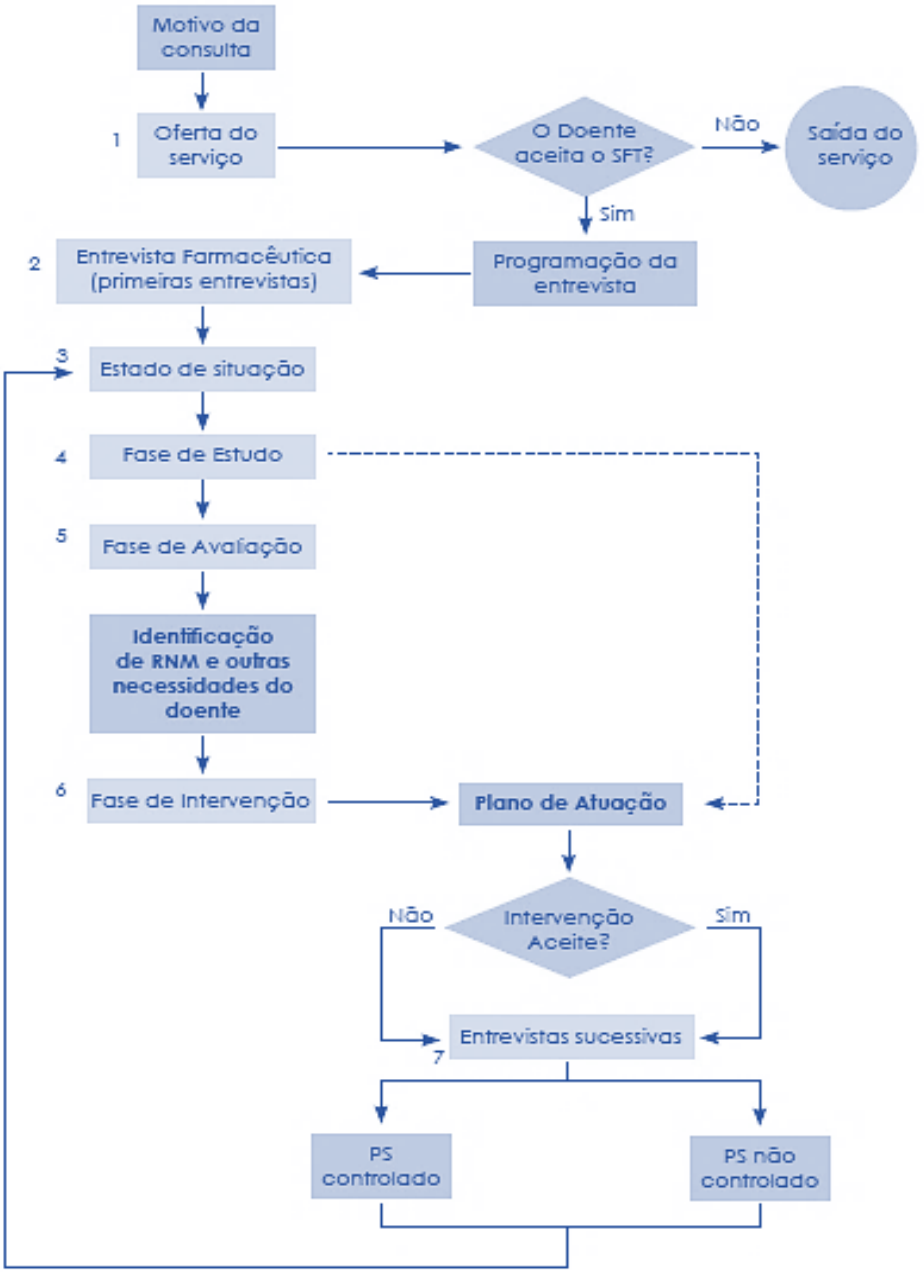
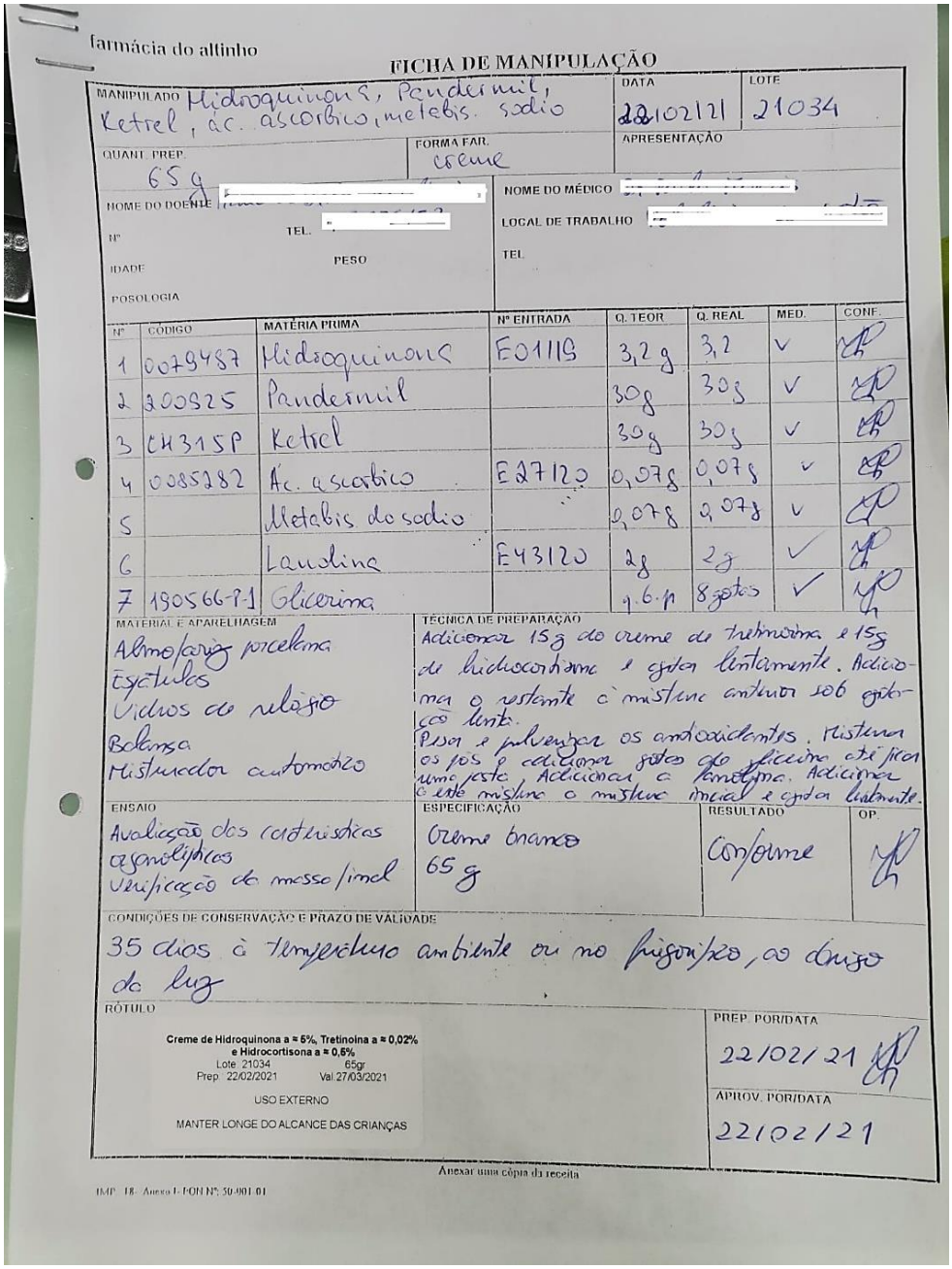
Anexos

Anexo I – *Sete etapas do Método Dáder de Seguimento Farmacoterapêutico. Adaptado de [2]*



Anexo 2 – *Ficha de Preparação do Medicamento Manipulado.*



farmácia do altinho

FICHA DE MANIPULAÇÃO

MANIPULADO: Hidroquinona?, Pandermil, Retrel, ac. ascorbico, metabis. sodio
DATA: 22/02/21 | LOTE: 21034
QUANT. PREP.: 65 g | FORMA FAR.: creme | APRESENTAÇÃO:
NOME DO DOENTE: [redacted] | NOME DO MÉDICO: [redacted]
Nº: | TEL.: [redacted] | LOCAL DE TRABALHO: [redacted]
IDADE: | PESO: | TEL.:
POSOLOGIA:

Nº	CÓDIGO	MATÉRIA PRIMA	Nº ENTRADA	Q. TEOR.	Q. REAL	MED.	CONF.
1	0079487	Hidroquinona	E01118	3,2 g	3,2	✓	
2	200325	Pandermil		30 g	30 g	✓	
3	CH315P	Retrel		30 g	30 g	✓	
4	0085282	Ac. ascorbico	E27120	0,07 g	0,07 g	✓	
5		Metabis do sodio		0,07 g	0,07 g	✓	
6		Lanolina	E43120	2 g	2 g	✓	
7	190566-71	Glicerina		q.b.p	8 gotas	✓	

MATERIAL E APARELHAGEM:
Almofariz porcelana
Espátulas
Vidros de relógio
Balança
Misturador automatico

TÉCNICA DE PREPARAÇÃO:
Adicionar 15 g do creme de tretinoina e 15 g de hidrocortisona e ajudar lentamente. Adicionar o restante à mistura anterior sob ajuda lenta.
Pesar e pulverizar os antioxidantes. Misturar os pós e adicionar gotas de glicerina até fazer uma pasta. Adicionar a lanolina. Adicionar a esta mistura a mistura inicial e ajudar lentamente.

ENSAIO	ESPECIFICAÇÃO	RESULTADO	OP.
Avaliação das caracteristicas organolépticas	Creme branco	Conforme	
Verificação de massa final	65 g		

CONDIÇÕES DE CONSERVAÇÃO E PRAZO DE VALIDADE:
35 dias à temperatura ambiente ou no frigorifico, ao abrigo da luz

RÓTULO:
Creme de Hidroquinona a ≈ 5%, Tretinoina a ≈ 0,02% e Hidrocortisona a ≈ 0,6%
Lote: 21034 65gr
Prep: 22/02/2021 Val.27/03/2021
USO EXTERNO
MANTER LONGE DO ALCANCE DAS CRIANÇAS

PREP. POR/DATA: 22/02/21
APROV. POR/DATA: 22/02/21

Anexar uma cópia da receita

IMP. 18 - Anexo I - PON Nº: 50-901-01

Parte 2

Relatório de Estágio em Indústria Farmacêutica

Lista de Abreviaturas

ANF| Associação Nacional das Farmácias

BPF| Boas Práticas de Fabrico

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

LEF| Laboratório de Estudos Farmacêuticos

MICF| Mestrado Integrado em Ciências Farmacêuticas

QA&SHE| Controlo de Qualidade, Segurança e Ambiente

PQR| Product Quality Review

SGQ| Sistema de Gestão de Qualidade

SWOT| *Strenghts, Weaknesses, Opportunities and Threats*

Introdução

O estágio em Indústria Farmacêutica advém do meu desejo em explorar outra realidade profissional para além da Farmácia Comunitária, podendo desta forma complementar o meu percurso académico, alargando assim as minhas perspetivas relativamente a que área pretendo seguir futuramente.

Durante o período compreendido entre 3 de maio e 30 de julho integrei o departamento de Controlo de Qualidade, Segurança e Ambiente (QA&SHE), um dos vários departamentos presentes no Laboratório de Estudos Farmacêuticos – Infosaúde, pertencente à Associação Nacional de Farmácias (ANF).

No presente relatório vou começar por contextualizar o Laboratório de Estudos Farmacêuticos (LEF) , seguindo-se uma análise SWOT, onde exponho os pontos fortes, pontos fracos, oportunidades e ameaças resultantes do estágio curricular realizado.

Contextualização do LEF

O LEF é uma unidade de negócio pertencente à ANF, atualmente com cede em Barcarena, Oeiras, tendo sido fundado em 1992. Este representa uma CRO (Instituição de Desenvolvimento de Produtos, Desenvolvimento e Validação de Métodos Analíticos) e uma CMO (Produção sob contrato e Prestação de Serviços de Análises de Controlo de Qualidade), oferecendo serviços no âmbito das Boas Práticas de Fabrico (BPF) ao setor farmacêutico, e ainda em áreas distintas como os Suplementos Alimentares, Produtos Cosméticos e de Higiene Corporal e Dispositivos Médicos.

Esta instituição tem como missão, produzir, estruturar, e disseminar conhecimento através da implementação de um Sistema de Gestão da Qualidade exigente e rigoroso, que garanta o cumprimento dos requisitos legais, técnicos e regulamentos aplicáveis, o que contribui para a satisfação dos clientes, e para a valorização científica e económica das suas atividades.

O LEF é dividido em vários departamentos: Garantia de Qualidade, Desenvolvimento Farmacêutico e Produção, Assuntos Regulamentares e Farmacovigilância, Desenvolvimento Analítico e Validação, Controlo de Qualidade e Estabilidades e Consultoria de Qualidade.

Relativamente ao sistema de documentação, os documentos são aprovados, datados e assinados por pessoas adequadas e autorizadas e são regularmente atualizados, sendo que o mesmo se baseia em três níveis **[Anexo]**.

A vantagem de possuir certificado de BPF, o *know how* dos seus colaboradores e o dinamismo da equipa faz com que todos trabalhem de acordo com os mais exigentes critérios de qualidade, apresentando-se no mercado farmacêutico com uma elevada potencialidade de crescimento futuro.

Análise SWOT

Pontos Fortes

- Importância do departamento QA&SHE na empresa

O departamento que integrei tem como missão apoiar e acompanhar as atividades relacionadas com a garantia de qualidade e ambiente do LEF. No decorrer do meu estágio as atividades que desenvolvi estiveram mais centradas na Qualidade, sendo que tive a oportunidade de elaborar Relatórios de Validação de Processo, realizei a análise critica dos Cadernos Master de Fabrico com a finalidade de elaborar o respetivo *Product Quality Review (PQR*), revi procedimentos tendo em conta Controlos de Mudança, Desvios e CAPAs (*Corrective Actions/Preventive Actions*) apresentados e, por fim, revi o Side Master File e o Manual de Gestão do LEF.

A partir desta experiência passei a encarar a qualidade como, saber olhar para a organização como um todo, ser proactivo, avaliar a *compliance* dos requisitos de qualidade, colaborar no processo de auditorias internas e externas e avaliar fornecedores e subcontratados, procurando desta forma a satisfação do cliente externo e interno. Os pontos referidos anteriormente têm como objetivo final a eficácia dos processos desenvolvidos, tendo sempre em conta os requisitos das normas adotadas pela instituição.

Tendo em conta que o departamento de QA&SHE está em constante interação com os restantes departamentos, assegurando que todas as métricas de qualidade são cumpridas, é necessário um ambiente fundamentado na colaboração e no rigor. A oportunidade de integrar este departamento permitiu-me ter outra visão relativamente aos departamentos que podem constar numa indústria farmacêutica e na importância que um bom sistema de gestão de qualidade pode refletir em todos os processos desempenhados.

- Fácil Integração na equipa

Um dos pontos fortes no decorrer dos três meses de estágio foi a forma como fui acolhida na instituição. Os colaboradores mostraram-se sempre muito prestáveis, e apesar da correria que se vivia em todos os departamentos, todos os colaboradores envolvidos nas minhas tarefas tentaram acompanhar o meu percurso. Pela possibilidade de integrar as reuniões diárias da equipa, acabei por estar em contacto com os problemas e oportunidades que podem surgir no dia-a-dia de uma empresa.

Ainda de referir que a ANF tem uma plataforma digital de bem-estar dos colaboradores que oferece, por exemplo, formações de *Mental Coaching*, de atividade física, nutrição, entre outras, o que revela a preocupação com os seus colaboradores enquanto integrantes da organização.

- Sistema de Gestão ambiental

O LEF possui um Sistema de gestão ambiental maduro e em constante monitorização. Também o seu *know how* permite influenciar positivamente os seus parceiros no seu desempenho ambiental, nomeadamente, farmácias, fornecedores, subcontratados e clientes. Ainda de referir que o LEF procura desenvolver produtos/novas metodologias tendo em conta a utilização de substâncias ambientalmente favoráveis, além de que inclui no âmbito das auditorias a fornecedores e subcontratados a avaliação das boas práticas ambientais.

Pontos Fracos

- Deficiência na informatização de processos

A inexistência de aplicação informática da gestão documental no LEF, sendo os processos tratados manualmente, conduz ao consumo de recursos humanos e materiais desnecessariamente. No entanto, está atualmente a ser estuda a implementação de um sistema informático que permita essa informatização.

No decorrer no meu estágio este fator acabou por influenciar o meu desempenho, uma vez que ao ter elaborado vários documentos sujeitos a aprovação e assinaturas consequentes, tinha constantemente que me dirigir às pessoas responsáveis para conseguir ter esses documentos aprovados, o que consumiu imenso tempo que poderia ter sido utilizado na realização de outras atividades.

- Elevada rotatividade de colaboradores

Existiu uma grande rotatividade de colaboradores do departamento QA&SHE, o que obrigou a que fosse necessária uma constante adaptação. Este fator acentuou-se pelo facto dos colaboradores que restaram terem menos possibilidade de me apoiar, visto que tiveram que assumir um maior número de funções. No entanto, é de salientar que nunca deixaram de me faltar tarefas para realizar, e que este fator acabou por me proporcionar um maior grau de autonomia e responsabilidade.

Oportunidades

- Primeiro contacto com uma empresa farmacêutica

Embora a formação académica proporcionada pelo MICF seja muito alargada, é importante que se aposte na formação mais específica, permitindo a diferenciação dos futuros farmacêuticos em áreas que preferencialmente tenham perspetiva de expansão e que requeiram mais profissionais, abrindo portas a novas "competências farmacêuticas". Assim, o estágio no LEF permitiu-me reconhecer competências que devo amplificar e/ou melhorar de forma a tornar-me profissionalmente mais competitiva.

Além de farmacêuticos, o LEF possuí colaboradores com uma formação académica muito diferenciada (gestão, química, bioquímica, engenharias, entre outras) proporcionando à empresa ideias e perspetivas alargadas, que conjugadas contribuem para o seu sucesso. A oportunidade de estar em contacto com estes profissionais tornou este estágio uma experiência única e muito enriquecedora.

- Formações disponibilizadas

Tendo sido a minha primeira experiência numa empresa farmacêutica, possibilitou-me a aquisição e consolidação de conhecimentos, quer pelas atividades realizadas por mim como pelas formações que nos foram proporcionadas, que no caso passaram pela formação no âmbito das BPF, Canábis Medicinal, Gestão ambiental, Gestão documental e Validação de processo de fabrico, entre outras.

- Autonomia na realização das tarefas

Durante o meu estágio foi-me dada a possibilidade de elaborar uma grande quantidade de documentos. Previamente à execução de qualquer tarefa, era disponibilizada uma formação interna detalhada de como deveria proceder.

Depois de cada formação a realização das minhas funções ficava totalmente da minha responsabilidade, sendo que no final era feita uma revisão detalhada, por vários colaboradores que eram influenciados pela modificação ou implementação dos respetivos documentos. Esta metodologia de aprendizagem revelou-se muito útil para aplicar os conhecimentos adquiridos teoricamente com uma experiência prática. Assim sendo criei um grande sentido de responsabilidade e exigência comigo própria, sendo necessário conciliar tudo o que aprendi num curto período de tempo, aplicar estes conhecimentos de uma forma eficaz e ainda conseguir ser crítica relativamente aos resultados obtidos nos vários processos.

- Desenvolvimento de competências informáticas (Microsoft Office®, Outlook®)

Ao longo do estágio, concluí que o Microsoft Excel®, Microsoft Word® e Outlook® são ferramentas muito utilizadas na realização da generalidade das tarefas e em vários departamentos. Também me foi solicitada a elaboração de determinados trabalhos utilizando estas ferramentas, o que inicialmente dificultou o meu desempenho, visto que apenas dominava as suas funcionalidades básicas. No entanto, através da ajuda inicial da equipa onde exerci funções, consegui rapidamente adaptar-me e executar as minhas tarefas sem grandes dificuldades.

- Canábis Medicinal (*Cannabis sativa L.*)

A vasta experiência e a representatividade do LEF como *one-stop shop* conduziu à expansão do seu portfólio de serviços prestados, tendo ultimamente apostado na Canábis Medicinal. O

LEF possui autorizações de fabrico para substâncias ativas (flor seca de *Cannabis sativa L.*) na área de controlo de qualidade físico-químico e microbiológico e ainda de fornecimento para fins específicos de derivados da planta de canábis e ainda no âmbito do desenvolvimento e fabrico de lotes experimentais à escala piloto.

Apesar das minhas funções não estarem diretamente relacionadas com esta área, através das formações disponibilizadas e pela apresentação de serviços prestados, tive contacto com uma área em crescimento exponencial, que através do estabelecimento de todos os requisitos de qualidade e seguridade pelo INFARMED, permite que estas substâncias sejam utilizadas em Portugal pelos doentes que, em situações em que as terapêuticas convencionais não produzam os efeitos esperados possam agora usufruir de outra forma de satisfazer as suas necessidades.

Ameaças

- Duração do Estágio

No início da minha experiência foi-me apresentado um plano de estágio, que apesar de ter sido cumprido, não incluía muitas das atividades que são realizadas no departamento, não podendo desta forma explorar todo o potencial do que seria integrar realmente o departamento. Tendo em conta a complexidade e a responsabilidade das funções desempenhadas pelo departamento QA&SHE, considero que o facto de este estágio ter uma duração tão reduzida se revelou um aspeto negativo para o meu desenvolvimento profissional.

- Falta de Inglês técnico durante o curso

Durante o meu estágio no LEF notei alguma dificuldade em perceber o inglês técnico utilizado, e em aplicá-lo na elaboração dos documentos solicitados. Ter a oportunidade de contactar com esta língua no decorrer no estágio permitiu familiarizar-me com muitos termos, no entanto, penso que seria útil introduzir mais conteúdos nesta língua no decorrer do MICF.

Conclusão

O farmacêutico como especialista do medicamento e um agente de saúde pública, deve ter a seu cargo, não só a cedência e o primeiro/último contacto com o utente mas, também, todos os processos que são intrínsecos ao medicamento.

Estagiar numa empresa como o LEF, salientou o esforço constante, as ameaças e oportunidades com que podemos ser deparados e, ao mesmo tempo, o quão bom é ser-se farmacêutico e querer vingar, numa atualidade que se pode tornar exigente e com oportunidades cada vez mais desafiantes. Consequentemente, considero ter sido uma experiência muito gratificante e que, certamente, me auxiliará em decisões futuras, no que diz respeito ao campo profissional pelo qual pretendo enveredar.

Anexo - Sistema de documentação no LEF.

1° Nível - Políticas Gerais	2° Nível - Processos e Procedimentos de Gestão	3° Nível - Procedimentos de Operação e Registos
• Descrevem a empresa e os sistemas de qualidade em vigor.	• Descrevem como devem ser realizadas as atividades.	• Procedimentos de funcionamento (equipamentos, métodos e produtos); • Registos das atividades realizadas.

Secção 2

Monografia

Nanotechnology applied to regenerative medicine

Abbreviations

AgNPs| Silver nanoparticles

Ang| Angiopoietin

ATP| Adenosine triphosphate

AuNPs| Gold NPs

BBB| Blood–brain barrier

BDNF| brain-derived neurotrophic factor

bFGF| Basic fibroblast growth factor

BMP-2| Bone morphogenetic protein 2

CaP| Calcium phosphate

CMs| Cardiomyocytes

CNS| Central nervous system

CNTs| Carbon nanotubes

COL1A2| Collagen Type I Alpha 2 Chain

COL2A1| Collagen Type II Alpha 1 Chain

CT| Computed tomography

DA| Dopaminergic

DNA| Deoxyribonucleic acid

ECM| Extracellular matrix

EGFR| Epidermal growth factor receptor

ESC| Embryonic stem cells

EVs| Extracellular vesicles

GBM| Glioblastoma multiforme

GLP-1| Glucagon-like peptide-1

GO| Graphene oxide

Hap| Hydroxyapatite

HEK 293 cells| Human embryonic kidney 293 cells

HSCs| Hematopoietic stem cells

IA| Intra articular

iPSCs| Induced pluripotent stem cells

iPS| Induced pluripotent stem

LbL| Layer-by-layer

LMNA| Protein laminin A/C

MI| Myocardial infarction

miRNA| microRNA

MNPs| Magnetic NPs

MRI| Magnetic resonance imaging

mRNA| Messenger RNA

MSCs| Mesenchymal stem cells

n-BGC| Bioactive glass nanoceramics

nHP| Nanohydroxyapatite

NIR| Near-infrared

NLS| Nuclear localization sequence

NPs| Nanoparticles

NSC| Neural-Stem-Cell

OTSC| Oral tissue stem cells

PCG| PolyChlorinated Biphenyls

PCU| Polycarbonate urethane

PDGF| Platelet-derived growth factor

PD| Parkinson's disease

PEG| Polyethylene glycol

PLGA| Poly Lactic-co-Glycolic acid

PLLA| Poly-L-lactic acid

PNPs| Polymeric nanoparticles

QDs| Quantum Dots

RM| Regenerative medicine

RNA| Ribonucleic acid

RUNX2| Runt-related transcription factor 2

SDF-1| Stromal cell-derived factor 1

SPIONs| Superparamagnetic iron oxide nanoparticles

siRNA| Small interfering RNA

siSOX9| siRNA against SRY-Box Transcription Factor 9

SV40| Simian vacuolating virus 40

T1D| Type-1 Diabetes

TERM| Tissue engineering and regenerative medicine

TiO_2| Titanium dioxide

VEGF| Vascular endothelial growth factor

ZnO| Zinc oxide

1. Introduction

1.1. Overview on history and evolution of nanotechnology

The flowering of nanoparticles (NPs) can be traced to the time of the Greeks and Democritus when scientists considered the question of whether a matter is continuous, and thus infinitely divisible, or composed of small, indivisible and indestructible particles, which we now call atoms. The prefix 'nano' has the meaning of 'dwarf' suggested by the Greeks as something very small that represents one thousand millionths of a meter (10^{-9}m).

We should make a distinction between nanoscience, and nanotechnology, being nanoscience the study of structures and molecules with scales varying between 1 and 100 nm and nanotechnology the ability to convert the nanoscience theory to useful applications by measuring, assembling, observing, manipulating and manufacturing at the nanometer scale. This 1 to 100 nm size range can correspond to individual molecules for polymers or other macromolecules but can also include higher-order associations into nanoparticles.

The nanotechnology concept was introduced in 1959 by Richard Feynman, laureate of The American physicist and Nobel Prize. There are two conditions that need to be verified in nanotechnology. The first is the concerned to use NPs by controlling their shape and size and the second issue has to do with the innovation that have to taking advantage of some properties because of the nanoscale.

Some characteristics like the possible formation of stable interactions with ligands, variability in size and shape, high carrier capacity, and convenience of binding of both hydrophilic and hydrophobic substances make NPs promising platforms for the target-specific and controlled delivery of micro and macromolecules in several disease treatments. Hence, the applications of nanotechnology in many biology-related areas such as diagnosis, drug delivery, molecular imaging and Regenerative Medicine (RM) are being extensively explored and are offering excellent outcomes [1].

Currently approved nanomedicine products are predominantly intended for use in cancer treatment, infectious diseases, anesthetics, cardiac/vascular disorders, inflammatory/immune disorders, endocrine/exocrine disorders and degenerative disorders. However, it should be noted that the major part of the research efforts are focused on cancer treatment [1].

To point out that nanomaterials can be employed itself or to deliver therapeutic molecules to modulate essential biological processes, like autophagy, metabolism or oxidative stress, exerting anticancer activity. Hence, nano-oncology is a very attractive application of nanoscience and allows the improvement of tumor response rates in addition to a significant reduction of the systemic toxicity associated with current chemotherapy treatments [2].

NPs have been developed to overcome the limitations of typical therapeutics and to transcend biological barriers more easily. However, the number of nanomedicines available to humans is significantly below projections for the field, partially because of a translational gap between animal and human studies. The ground for this comes from insufficient understanding of the differences in physiology and pathology between animal model species and humans, in particular how these differences affect the behavior of NPs in the body [3].

All discovery in Nanomedicine has to pass through long-lasting developmental steps, involving pre-clinical and clinical studies, in order to achieve commercialization, which makes this process more time-consuming **[Figure 1]** [1].

1.2. Designing nanoparticles for therapeutics

Nanotechnology can involve two main categories - nanodevices and nanomaterials. Nanodevices consist of miniature devices at the nanoscale, including microarrays, microscopic devices, and intelligent machines. Nanomaterials can be nanostructured or nanocrystalline, being the nanostructured subdivided into three types, polymer-based, lipid-based, and non-polymeric **[Figure 2]** [4].

Each class of NPs has numerous broad advantages and disadvantages regarding cargo, delivery and patient response.

Polymeric NPs characteristics can be easily controlled and their surface can also be easily modified. Furthermore, these types of NPs payload flexibility for hydrophilic and hydrophobic cargo. However, there is the possibility of aggregation and toxicity [4].

Inorganic NPs have unique electrical, magnetic, and optical properties and can vary in size, structure, and geometry. They are well suited for theranostic applications, however, have some toxicity and solubility limitations.

Lipid-based NPs can be simply formulated with a range of physicochemical properties, have high bioavailability, and payload flexibility. However, these NPs have a low encapsulation efficiency [4].

When drugs are incorporated into NPs, their properties like solubility, biodistribution, and target tissue accumulation will no longer be constrained to the same extent by drug chemical composition, allowing greater flexibility in the conception of drug molecules themselves.

Once NPs go into the bloodstream, they are susceptible to aggregation and protein opsonization, which means that proteins can bind to their surface as a tag for the immune system to recognize. These opsonized NPs could be wiped out from the bloodstream by phagocytosis or filtration in the liver, kidney, and spleen. Hence, quick and non-specific elimination by the immune system results in reduced retention time and therefore in poor

bioavailability. By covering its surface with polyethylene glycol (PEG), carbohydrates, acetyl groups, or protein moieties like peptide and albumin, retention time can be modified [4].

2. Regenerative medicine and Nanotechnology

Tissue and organ loss through disease and damage led to the increase of therapies that can regenerate tissues and decrease the requirement of transplantations. Regenerative medicine, an multidisciplinary field that applies tissue engineering, cell therapy, and life science principles, has the potential to heal or replace tissues and organs damaged by age, disease, or damage, as well as to normalize congenital defects [5].

This field holds the potential of transforming human medicine, by actually treating diseases previously poorly managed with conventional drugs and medical procedures.

In order to regenerate tissues, we can proceed to a combination of living cells, ideally stem cells, which offer biological functionality, and several types of materials, which act as scaffolds to help cell proliferation.

Recently, the use of NPs to RM has gained a lot of interest, in part due to the realization control tests of cellular behavior, and therefore optimal tissue regeneration can be achieved by the provision of a proper nano–bio interface [6].

Nanomaterials used in biomedical applications involve NPs for the delivery of particles (drugs, growth factors, DNA), nanofibers for the construction of tissue scaffolds, and surface adjustments of implantable materials or nanodevices, as biosensors. The junction of these components within Tissue engineering and regenerative medicine (TERM) is an example of the impact that nanotechnology can have when applied to regenerative medicine [7].

A scaffold is described as a tri-dimensional porous and interconnected matrix, composed of, for example, polymeric, ceramic, or composite materials that include a controlled degradation rate, adequate mechanical properties, and supports cell functions like proliferation and differentiation from the tissue to be regenerated.

The body's innate healing response may also be exploited to promote regeneration, although adult humans retain limited regenerative capacity.

Progress in the nanotechnology field has increased advances in RM with the ability to mimic the composition and structure of tissues and organs at the nanoscale. Besides, many functional nanomaterials can be used to deliver drugs, proteins, and genes to the damaged tissues **[Figure 3]** [8].

Nanofibrous materials that reproduce the native Extracellular matrix (ECM) and promote the adhesion of cells are being developed as tissue-engineered scaffolds for the bone, cartilage, vasculature, heart, nervous system, and other tissues.

Another area that is included in the RM is cell therapy that consists of the process of introducing new cells in a tissue or organ to treat a disease or injury. Cell therapy utilizes transplanted cells, in particular stem and progenitor cells, to regenerate damaged or diseased tissue. Transplanted cells may regenerate tissues through (trans) differentiation or provide regenerative indications that facilitate regeneration through trophic factors and cell–cell connections [6].

The vascularization and innervation of regenerated tissues are factors that must be taken into account. Most cells in the body are situated within 100 μm from the nearest capillary, which allows an effective nutrient exchange and oxygen diffusion from the bloodstream. To vascularize regenerated tissues, the body's angiogenic response might be exploited via the presentation of angiogenic growth factors [6].

The main angiogenic growth factors are the vascular endothelial growth factor (VEGF), angiopoietin (Ang), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF). However, these growth factors may not be efficient without proper delivery due to their short half-life in vivo, potential toxicity, and systemic effects of bolus delivery [6]. VEGF loaded PCL NPs incorporated Poly(L-lysine)/hyaluronic acid polyelectrolyte multilayer film system has been reported as an approach for controlled delivery of VEGF for angiogenesis [9].

An alternative method to stimulate graft vascularization at the target site is to vascularize the target site before implantation.

Endothelial cells and their progenitors can self-organize into vascular structures once transplanted on an appropriate scaffold. Furthermore, proper innervation is required for appropriate function and full integration of some tissues, mostly in tissues where motor control, as in skeletal tissue [6].

An example of transplanted cells, inducing a regenerative effect is the mesenchymal stem cells (MSCs), which are being widely studied both preclinically and clinically for several diseases.

Usually, a systemic paracrine modality is sufficient to produce a therapeutic response in some situations, however, in some cases, cell–cell contact may be required. For example, MSCs can inhibit T-cell proliferation and reduce inflammation, and this effect is believed to at least partially depend on direct contact of the transplanted MSCs with host immune cells [6].

Even though the goal of RM has long been to prevent rejection of the new tissue by the host immune system, it's clear that the immune system also is important in regulating regeneration, contributing to the healing process.

Also, modifying the properties of implanted scaffolds can decrease the inflammation that accompanies their implantation. Decreasing scaffold hydrophobicity and the availability of adhesion ligands can reduce inflammatory responses, and scaffolds with aligned fibrous

structures suffer less fibrous encapsulation upon implantation. Hence, the regulation of the local immune response allows active promotion of regeneration [6].

Identifying and obtaining sufficient numbers of therapeutic cells for RM strategies is often a challenge. Stem, progenitor, and differentiated cells derived from adult tissues are widely being explored in RM due to both their ready availability and perceived safety [6].

There is great interest in finding greater numbers of stem cells from mature tissues and in identifying populations of these cells suitable for therapeutic use in tissues that was thought not to harbor stem cells.

Embryonic stem cells and induced pluripotent stem cells are possibly infinite sources of cells for regeneration.

Pluripotent Embryonic stem cells (ESC) are derived from blastocyst-stage embryos that can give rise to tissues from the three germ layers.

Induced pluripotent stem cells are developed from differentiated somatic cells exposed to transcription factors that promote pluripotency. This is an attractive cell source because they can be produced from a patient's cells, thus avoid the ethical issues of ESC and rejection of the transplanted cells [6].

ESC-like cells from adult somatic cells can be created by using a set of transcription factors. When these factors are presented in the mature cell, they initiate a cascade of events that can give rise to pluripotent stem cells, the induced pluripotent stem cells (iPSCs). iPSCs have very similar characteristics to ESCs, like morphology, expression of pluripotent markers, epigenetic modifications, ability to differentiate into cells of the three germ layers in vitro and in vivo. However, most strategies for iPSCs production are based on gene delivery to host's genome via retroviral or lentiviral vectors, which can cause a significant risk of insertional mutagenesis and oncogenic transformation. Hence, significant advances have been made with non-integrative reprogramming strategies [6].

Moreover, although various somatic cell types have been used to produce iPSCs, there is no consensus about the ideal type to be reprogrammed [10].

Nano-assisted RM that has great potential in the healthcare systems has two major objectives: utilize the self-healing capacity of endogenous stem cells by identification of signaling systems, and develop an effective targeting system for stem cell treatments, thus aiming to activate endogenous self-repair mechanisms instead of simply managing or decreasing the symptoms.

2.1. Types of nanoparticles used in regenerative medicine

Mimic ECM composition of tissue through the construction of a three-dimensional scaffold for cells with appropriate mechanical strength require a nanoscale methodology that makes easier the monitoring of cellular activities and supplying of bioactive agents.

NPs can provide high control through properties of scaffolds such as adjusting their mechanical strength and providing controlled release of bioactive agents [11].

More nanoscale products, such as nanofibers, have been used for managing cell comportment in the TERM area. Applying both therapeutic and imaging systems, adding biomaterials with higher spatiotemporal regulation inside scaffolds, moderating the release of multiple active agents like growth factors to direct the fate of stem cells and differentiation, adjusting mechanical strength of scaffolds for tough tissue requests, diminishing toxicity, and increasing biocompatibility are several applications of nanomaterials in TERM.

Some types of NPs are characterized for their utility on RM, like metallic nanoparticles (gold and silver), lipid based nanoparticles (exosomes), ceramic nanoparticles (glass nanoceramics, bioresorbable nanoceramics, bioinert nanoceramics, and magnetic nanoparticles), polymeric nanoparticles (chitosan), and no polymeric nanoparticles (nanodiamonds, quantum dots) [9].

- Metallic NPs

Metallic NPs can be produced and adapted utilizing several functional groups that deliver conjugation of antibodies, ligands, and drugs as delivery systems.

  - Gold NPs (AuNPs)

AuNPs can be characterized as a colloid of particles of gold at nanometer size. On the surface of gold, it is possible to conjugate several ligands like polypeptide sequences, antibodies, and proteins with phosphines, amines, and thiols, due to their strong affinity to gold [9].

These NPs can be use in the context of RM as a measure if the engineered tissue is restoring a damage tissue or organ. AuNPs can disturb the cell division by selectively transport particles into affected cell nuclei, which can be useful in oncologic diseases. Cytokinesis arrest caused by nuclear targeting of AuNPs in cancer cells prevents them to complete their division [12].

The *in situ* aggregation of near-infrared (NIR) absorbing plasmonic (absorbing and scattering light) AuNPs took place at the nuclei of the cells which makes these NPs a suitable candidate for a based photothermal therapy in cancer. By modifying the absorption band to NIR, AuNPs have da capacity to protect healthy tissue by reducing the heat that can cause collateral damages [13].

It was also been shown that AuNPs targeting the nucleus membrane can increase the expression of the protein laminin A/C (LMNA) and mechanical stiffness of nucleus and consequently decreased the cancer cell migration. To emphasized that LMNA is responsible for maintaining a cell's structural stability, chromosome organization, gene regulation, cell differentiation, DNA damage repair, and telomere protection [14].

These properties of AuNPs can be utilized for attacked the remaining cancer cells after tumor resection what consequently minimize cancerous cells remaining in the healthy tissue. Hence, applying these types of NPs before to implantation can offer a safety measurement to minimize the reappearance of the tumor through targeted delivery to cancer cells, and thus increase effective implantation for various TERM applications. Furthermore, AuNPs are widely used for drug supply, for example, conjugated to epidermal growth factor receptor (EGFR) through an antibody for targeting cancer cells [9].

Also, among organic and inorganic NPs, AuNPs have been used in complex gelatin scaffolds for enhancing bone regeneration, due to their potential to osteogenic differentiation of adipose derived stem cells. Thus, the key objective of utilizing AuNPs is not only to improve scaffold structures but also to manage cell behavior, improving cell differentiation and intracellular delivery [9].

- Silver NPs (AgNPs)

AgNPs can also be described as a colloid made of silver nanometer-sized particles and are widely used in the biomedical field mostly for their antimicrobial capacity, an important characteristic because infections are a significant risk with engineered tissues. Properties of AgNPs have also been investigated in TERM mostly for wound dressing applications [9].

- Polymeric NPs (PNPs)

For PNPs some aspects need to be taken into account, like formulation, size, shape, surface chemistry and charge, porosity, mechanical strength, solubility, and degradation rate that can be modified for adaptable purposes in TERM.

These NPs have low cytotoxicity, good biocompatibility, permeation and retention effect, capability to deliver less soluble drugs and sustained release of them, protecting bioactive agents from enzymatic degradation of the engineered tissue, what makes PNPs as one of the bests platforms to overcome the obstacles in RM. Recently these systems have been designed to be susceptible to different external stimuli such as magnetic field, temperature, enzymes, pH, reducing/oxidizing agents which improves the specificity and efficiency for TERM uses [9].

The types of PNPs can be established on different criteria like composition, structure and manufacturing process **[Figure 4]**. These NPs can be made in several morphologies like nanospheres, nanocapsules, polymersomes, dendrimers, polymeric micelles, and nanogels depending on their application [9].

For better properties, several PNPs have been created using biodegradable and biocompatible natural and synthetic polymers. Polysaccharides (dextran, heparin, alginate, chitosan, hyaluronic acid), proteins (albumin, gelatin, elastin, silk) and synthetic polymers (polyesters,

polyamides, polyanhydrides, polyurethanes, polyacrylates) have been used with specific functional fractions or with other materials to provide certain functionalities in TERM [9].

Focusing on chitosan **[Figure 5]**, a natural-based chitin derivate, that has been widely investigated as drug delivery carriers, because of their high abundance, nontoxicity, pH responsiveness, biocompatibility, and biodegradability. It can be easily managed in form of NPs in order to let a particular drug to be selectively released inside a certain type of cell, tissue or organ.

Chitosan is a polycationic polymer whose charge can change depending on the level of acetylation and pH. Moreover, this polymer interacts with negative molecules by electrostatic interactions. Therefore, chitosan NPs can be acquired by ionic gelation with polyphosphates or nucleic acids. However, as they have a positive charge, they can also pass through several biological barriers such as cell membranes [15].

Due to its similarity to the ECM of diverse tissues, this polymer has been mainly used in TERM, in the form of hydrogels and scaffolds. A hydrogel is a polymer complex that is able to grow and retain water, and which can behave as a tissue mimetic.

Also, several methods have been used to produce chitosan scaffolds. However, in order to be produced both as hydrogels and scaffolds, chitosan needs solubilization in acidic solutions, to certify the protonation of the primary amine.

Promising applications of chitosan hydrogels include stem cell encapsulation for supporting differentiation and its use in TERM [15].

However, it is necessary to underline the possible affinity that chitosan particles can have for endotoxins (e.g., lipopolysaccharide endotoxin) which can cause adverse biological effects [16]. Hence, the characterization, purification, and chemical modification of this polymer are a priority to use in RM [15].

- <u>Non polymeric nanoparticles</u>
    - Nanodiamonds (NDs)

NDs are a group of carbon nanomaterials which have unique characteristics that are very interesting for novel therapies in the fields of drug delivery, TERM, and bioimaging. Also, they have been widely studied for applications in RM with a special emphasis on bone and neural tissue engineering [17].

These NPs can be used for fortifying the mechanical properties of composite scaffolds to beat that of human tissue. Covalent or ionic bonds can be established with the polymeric chains of the scaffold, which allows the modulation of the mechanical properties of polymeric systems to simulate the structure of both soft and hard tissues of the body [17].

Besides, its application as bioactive coatings to improve the tribological properties and decrease the mechanical wear of orthopedic implants can be made using NDs.

To improve the therapeutic efficacy of tissue scaffolds, growth factors and proteins sustained release can be delivered by NDs. Moreover, the surface chemistry of NDs facilitates the covalent linkage of growth factors to the surface of the nanoparticle [17].

Several characteristics like biocompatibility, stiffness, wettability, and optical properties, adding to their adaptable surfaces are all favorable properties that enable the use of NDs as multifunctional tools for biomedical structures. It should be noticed that effective tissue scaffolds require materials that are able to degrade at a controlled rate to let the ingrowth of new tissue [17].

Electrospinning is one of the most popular strategies for creating polymeric nanofibers that mimic the native ECM.

NDs mechanical properties make possible the creation of soft tissue for wound regeneration or hard tissue for bone support, yet, their aggregation remains a substantial challenge. This can happen in high concentrations because the mechanical attributes of the polymer cannot be enhanced since the functional groups of the NDs establish interactions with each other instead of with the polymeric structure [17].

Some examples of NDs applications are the use of NDs complexes that facilitates the sustained release of BMP-2 from the scaffold in vivo, which can be evaluated by the upregulation of osteogenic markers such as RUNX2, COL1A2, and COL2A1. Moreover, the NDs coatings presented localized anti-inflammatory effects, inhibiting the linkage of inflammatory cells. Also, the surface coatings of NDs have been used for the delivery of angiogenic growth factors from β-tricalcium phosphate scaffolds to improve their bone healing capacity [18].

Another feature that can be explored is the utilization of reactive functional groups on the surface of NDs that can be used both to enrich the mechanical properties of polymeric scaffolds and to modulate the delivery of growth factors which can boost the tissue regeneration.

This great adaptability, which hardly can be achieved with any other current NPs, allows theranostic approaches that can overcome multiple clinical therapies challenges at the same time [17].

- Quantum Dots (QDs)

QDs are almost spherical semiconductor nanocrystals with diameters between 2 to10 nm. The semiconductor and size-dependent fluorescence of these particles have made them especially appealing for use in optoelectronic devices and biological detection. Moreover, they

provide a adaptable nanoscale scaffold for both imaging and therapeutic functions, with an additional possibility to link with targeting ligands [19].

The main applications of QDs in TERM include their imaging and therapeutic properties, the development of biocompatible nanoparticles to specific organ uptake, and escape of the reticular endothelial system capture and elimination. Also, nanotoxicology studies can be made adding to QDs fate in the body [19].

Since these NPs can be identified easily using fluorescence microscopy, they are most selected for the examination of cellular activities, like endocytosis and intracellular trafficking [19].

- Lipid based NPs
  - Exosomes

Exosomes are extracellular vesicles that are secreted by cells, composed of a lipid bilayer. These NPs comprehend many cargos, including miRNA, mRNA, DNA, and proteins, between 30 to 150 nm. Their cargo changes with the variations in parent cells and status, hence exosomes from different types of cells may show different biological effects [20].

Exosomes are by now a practical therapy for TERM across several organs. When based on parent cell type and with the right cargo loading, exosomes can increase therapeutic results and reduce side effects [20].

These NPs have proven regenerative features by decreasing inflammation and apoptosis while stimulate proliferation and angiogenesis. Some promising regenerative effects have been shown in several models, such as myocardial infarction, kidney injury, and neurological injury. MSCs derived exosomes are popular in TERM, as a result of their multipotency and self-regenerative properties what offers therapeutic benefits to several organs and tissues [20]. Paracrine effects can be induced for MSC-exosomes and their secreted factors on surrounding cells in the tissue microenvironment, offering healing and regenerative advantages. Recognition of the optimal combination of carrying capacity and culture elements will allow specific and effective exosome targeting and regeneration, increasing clinical transposition [20].

Exosomes can replace stem cells and are used to examine their related biological functions and can be applied in immunomodulation, osteogenesis, neuroprotection, nerve regeneration, and vascularization.

Several studies showed that exosomes from oral tissue stem cells are a powerful therapeutic tool that has recently gained more attention due to their great source and easy retrieval.

Stem cell-derived exosomes perform the same therapeutic advantages as their parent cells, which avoids the parent cell deficiencies and offers the convenience of storage and carriage, providing a safer option to stem cell-based treatment [20].

These NPs contain a diverse set of signaling particles, mRNAs, and miRNAs and can release these molecules directly in the cytoplasm of target cells to adjust their behavior via the stimulation of various signaling pathways.

Also, exosomes from different cell sources, cellular states, and with distinct charges, can cause different biologic effects, which must be taken into account in their formulation.

To point out that the pharmacokinetics of exosomes can be more similar to their parent cells, which allow their elimination from the circulation and body. Therefore, there is a need for exosomes sample prerequisites for clinical application [20].

The number of studies focused on oral tissue stem cell exosomes is limited in some types of stem cells. For clinical applications, more investigation is needed [9].

- Ceramic nanoparticles

    - Bioactive glass nanoceramics (n-BGC)

These NPs can be formed from several components such as silicone, sodium, potassium, magnesium, phosphorous, oxygen, and calcium that can be absorbed by the cells.

n-BGC can provide faster ion release due to their improved specific surface area, thus, improvement of bioactivity and adsorption of proteins can be achieved. However, the addition of lactic acid resulted in a decline in the size of n-BGs [9].

    - Bioresorbable nanoceramics

Bioresorbable nanoceramics are composed by calcium phosphate (CaP) which includes variety of materials such as hydroxyapatite (HAp), calcium aluminate, tricalcium phosphate, calcium carbonate, calcium sulfate hemihydrate, biphasic calcium phosphate, etc. HAp is a major component of natural bone, which under neutral or alkaline environments, it is the most stable form of phosphate salts.

These NPs can be applied as bone replacements, modulating characteristic features depend on the incorporated ions to the HAp particles to adjust the scaffolds for TERM applications.

Combination of HAp with some carriers like electrospun fibers, porous scaffolds, and hydrogels has been described as a nanocomposite material form to modulate the required cellular events [9].

    - Bioinert nanoceramics

Bioinert nanoceramics can be compose by titanium dioxide (TiO_2) or zinc oxide (ZnO). Mesoporous TiO_2 has been applied to magnetic-targeting for imaging and photodynamic therapy utilizing a combined approach with NIR, photodynamic therapy, chemotherapy, and gene therapy for cancer treatment.

Like AuNPs and AgNPs, these types of NPs can be used as antimicrobial agents in TERM strategy. Nanocomposite has demonstrated great antibacterial activities in the presence of $Gram^+$ and $Gram^-$ bacteria [9].

- Magnetic NPs (MNPs)

MNPs are iron oxide-based NPs, (typically Fe_3O_4 or Fe_2O_3) with lower toxicity. They have several applications like imaging cancer cells, tracking stem cells and monitoring of transplanted tissues [9].

Recently, RM has shown great interest for MNPs, because they can organize and stimulate stem cells.

Also, these NPs have been used for stem cell differentiation into the chondrogenic, adipogenic, or mesodermal cardiac lines. To achieve a therapeutic action, MNPs have to be internalized in their intracellular environment [9].

The permeability of magnetic fields into tissues and the possible influence on the MNPs at a distance can result in functions like transfer or stoppage of cells. These characteristics are being applied to move cells to specific positions, generate tissues, and control cell function.

For theranostic applications, the most used NPs are iron oxide-based, being that a naturally occurring bio-element and with their metabolic pathway in mammals. When administrated intravenously, iron oxide NPs are internalized, mostly in macrophages, and then enter the iron pool and incorporate the natural iron metabolic pathway. In the regenerative context, they can also be used for monitoring of engineered tissues [9].

However, the degradation of iron oxide NPs can result in unbound iron ions, which possibly generate reactive oxygen species, leading to oxidative stress and consequent cell injury, which for RM applications need to be avoided. Consequently, the biodegradation of these nanoparticles and the cell response to the distributed iron must be systematically evaluated.

Additionally, NPs less than 100 nm and narrow size distribution are appropriate for in vitro and in vivo biomedical applications. NPs with 10 to 50 nm size and with a certain surface coating can have prolonged circulation times and can be manipulated through an external magnetic field. Also, throw the utilization of MNPs, growth factor conjugated MNPs can be transported to the target cells to stimulate them [9].

The use of MNPs turn possible the management of individual cells up to creating cell spheroids that can be constructed magnetically in macro-scale tissues, which can result in scaffold-free replacements.

The iron oxides can transcend the blood-brain barrier; thus, they could be used conjugated with several peptide and growth factors to regenerate brain tissue.

Strategies to prevent MNPs degradation should be planned to extend theranostic ability and possibly avoid toxicity effects. For instance, modifications with a gold shell or a polymeric coating could protect these NPs from degradation and preserve their integrity and magnetic characteristics [9].

It was demonstrated that MNPs coating is primarily dissolved by lysosomal proteases, and then the iron oxides are released by quick dissolution in the acidic environment. Conversions of MNPs into stem cells have revealed that the NPs are frequently endocytosed in endosomes and then combine with lysosomes, where acid-induced degradation occurs.

Some studies have shown a dose-dependent effect of these NPs, with, at higher doses, increased DNA breaks, weakened inflammatory reaction, though no adverse effects were frequently observed [9].

To note that the shape clearly influences the physiochemical properties of MNPs, however, the geometries do not seem to have an influence on their toxicity. In vivo, nanoparticles above 100 nm will be quickly eliminated from the blood stream by the spleen and liver.

Some toxicological studies were made, and their results shown that no adverse effects are detected for less than 10 pg/cell of iron, which can be considered as the low dose threshold. For specific differentiation pathways, this low dose can increase differentiation, which has been demonstrated for adipogenesis and osteogenesis [21].

The high dose toxicity of MNPs could be established according to the interference with intracellular signaling processes, or to a modification of the cellular morphology. When at a high dose and not provoking adverse events, it was noted that MNPs improved the proliferation of stem cells [22].

Prolonged studies evaluating differentiation have shown that magnetically stem cells retain their differentiation ability as chondrocytes, adipocytes, osteocytes, myocytes, and neuron-like cells. For stem cells going through differentiation, some pathways seem more influenced than others, which occurs with toxicity, noticed under chondrogenesis and not under adipogenesis or osteogenesis for bone marrow stem cells at comparably high dose [22].

Concluding, iron oxide NPs are bio-interacting, however, they act like biocompatible particles, hence, with rigorous assessments that should be considered, these NPs can be used for biomedical applications, where their potential remains very high [22].

2.2. Delivery of Bioactive Agents

The controlled release of bioactive compounds in TERM depends on the retention of the delivery vectors in the target regenerating tissue. Creating a representative ECM which can have similar comportment to the native tissue microenvironment with comparable mechanical

strength can be achieved by selecting proper 3D scaffold construction in conjunction with the right bioactive agent carriers.

In order to improve the distribution of bioactive agents, NPs have been updated from simple delivery to multifunctional responsive systems, which can adjust the loading, targeting, and efficacy of TERM approaches [9].

Moreover, it is crucial to control microenvironment of cells through stimulus to predict and regulate the comportment of cells in tissues. Presenting proteins and drugs in the environment of cells like stimuli in the form of encapsulated NPs on your own or implanted into scaffolds such as hydrogels, fibers, and so on, can offer balanced signals for cellular activities.

The complex architecture of tissues due to their distinct cell types and multicomponent extracellular matrix turns inevitable the use of nanoscale systems for delivering bioactive factors especially growth factors in sequential and time dependent approach [9].

2.3. Imaging and Contrast Agents

Visualizing and healing techniques can be combined to achieve target tumors by utilizing NPs for diagnostic and therapeutic approaches. In TERM area it is also viable to label cells alone or in the scaffold to follow the immune, differentiated and stem cells for disease treatment, tissue healing, cell migration and differentiation for their monitorization.

Molecular imprinted polymer-based NPs which are made through surface targeting molecular models allow the creation of specific binding, 3D structures with affinity related to antibodies for cell and tissue imaging which can be very useful for regenerative strategies [9].

2.4. Nanoparticles in bioinks for 3D Printing

Approaches with 3D printing technologies can be very useful in TERM field due to their potential control on design and manufacture of 3D scaffolds. Considering bioink the main element of 3D bioprinting can be specified as a mixture of one or more biomaterials which can result in a hydrogel form with the capacity of encapsulating the required cell type.

These types of scaffolds can provide an appropriate microenvironment for encapsulation of several cells inside the hydrogels through adapting the biological, rheological and mechanical characteristics of scaffold providing delivery bioactive agents for cells and adjust scaffolds mechanical strength **[Figure 6]** [23].

Materials like synthetic polyurethanes have favorable mechanical properties, high biocompatibility, and harmonious chemical structures which allow them to be used for the 3D construction of customized scaffolds [9].

However, there are some limitations for clinical applications of 3D scaffolds, such as the lack of capacity to create 3D, extremely organized constructions for ECMs, to prepare ordered

nanoscale structures over an extensive area. Additionally, we need to consider that an ideal nanostructure for a specific cell type possibly is not the optimal for another cell type.

There are also other limitations, including cell sourcing and expansion, elevated price for most countries' healthcare systems, regulatory issues and the difficulty in scaling up some of these technologies [24].

Lately, well-ordered nanofibers, nanotubes, and nanovesicles created by chiral self-assembly peptides have been used for 3D tissue cultures to obtain primary cells and stem cells, continued release of growth factors and monoclonal antibodies, and to accelerate wound healing. To note that this self-assembling has been already used in human clinical trials for quicker wound healings [24].

2.5. Production techniques

Nanomaterials utilized in RM are normally produced by Bottom-up (e.g., polymeric and inorganic NPs, carbon nanotubes, dendrimers, QDs, layer-by-layer structures) and Top-down (e.g., nanopatterned substrates) methods **[Figure 7]**.

In addition, these methods can be divided into several subclasses depending on the operation, reaction condition and adopted techniques that can have advantages and disadvantages depending on the case **[Table 1 and 2]** [50].

Bottom-up approaches can synthesize novel materials that behave differently in bulk contrasted when in nanoscale [2].

These materials include polymeric and inorganic NPs, self-assembled materials, carbon nanotubes (CNTs), dendrimers, QDs, layer-by-layer (LbL) structures, and so on. Additionally, this method involves the establishment of nanomaterials from the bottom with molecule-by-molecule or atom-by-atom through physical and chemical methods employing controlled manipulation of self-assembly of atoms and molecules [2].

On the other hand, Top-down method is characterized for breaking down the bulk material to get nano-sized particles employing meticulous engineering and lithography.

Precision engineering is based on cubic boron nitride or diamond. This technique utilizes sensors for size management of the nanostructures and numerical control technologies.

Lithography involves the exposition of the membranes to ions, light, or electrons, and the deposition of material until the necessary material is created.

Because the cellular response to nanoscale approaches may address the establishment of proper functional tissue, it is usually needed to design biomimetic delivery matrices structurally similar to native tissue in a nanoscale range [2].

Hence, Top-down approaches involve nanoscale modifications of existing polymers and materials and are used to fabricate nanoengineered systems, such as nanopatterned substrates to provide structures that influence cell behavior and subsequent tissue formation.

To produce tough materials, chemical synthesis can be useful being that it can be used both directly in product in their bulk form, or as the building blocks of more ordered materials.

The positional assembly is the only process that allows single atoms, molecules or cluster can be arranged freely one-by-one [2].

Also, gene therapy has gained substantial interest as a strategy for specific treatment of numerous gene-associated human diseases ranging from cancer, neurodegenerative diseases to autoimmune diseases. Biodegradation is the primary factor that needs to be consider for the clinical application of nanoparticle-based gene delivery.

To note that, nanoparticle-based gene delivery are mainly based on the utilization of positive charges on the surface of cationic nanocarriers. DNA/RNA can be encapsulated in poly (lactic-co-glycolic acid) nanoparticles by double-emulsion [25].

Additionally, the PEGylation of these nanocarriers can reduce nonspecific interactions with serum proteins, preventing the recognition by immune system elements. In the case of NPs, this system will rapidly develop large aggregates leading to rapid clearance by phagocytic cells and the reticuloendothelial systems. Hence, PEGylated NPs can increase the blood circulation time and facilitate the accumulation in targeted tissues.

Protecting the charge of cationic NPs through polyanions is also a approach for stabilizing the NPs, minimizing nonspecific interactions, and prolonging circulation time in vivo.

NPs conjugated with hydrophilic polymers exhibit more steric stability with decrease aggregation and breakdown during their distribution [25].

Some peptides with cell-penetrating capability due to their translocation sequences or protein transduction domains can be introduced on the surface of nanocarriers for gene treatment, which enables a rapid cellular uptake of the genes.

However, the application of this strategy has some obstacles, starting with the complexity of each disease and the needing for precise interpretation of its pathogenesis. Besides, effective clinical gene therapy is very restricted by the safety and effectiveness of the gene delivery systems that are used [25].

3. In vitro and vivo applications of nanotechnology in tissue regeneration

3.1. Type -1 Diabetes (T1D)

A study from the International Diabetes Federation suggests that there are 425 million people suffer from diabetes, which is expected to rise to 629 million people by 2045. Approximately 10% of these people suffer from T1D [26].

T1D has an autoimmune etiology and often starts between the ages of 20 and 30 [27]. Despite the mechanisms by which the immune system damages the pancreas are not completely understood, it is recognized that the body enhances self-reactive comportment that can generate an inflammatory response in the Islets of Langerhans.

Also, the late diagnosis and inadequate control of glucose levels can cause severe complications to the patient involving nephro-, retino- and neuropathies, cardiac and vascular diseases [28]. Furthermore, there is evidence that β-cell loss in T1D can occur because of acute infectious diseases, which can be exacerbated by the genetic disposition of the patient.

The usual treatment of this pathology goes through the monitoring of daily blood glucose levels, injections of long-acting insulin, and short-acting/mealtime insulin.

Patients with recurrent life-threatening hypoglycemia usually must consider pancreas or islet transplantation as an ultimate therapeutic option. However, the necessity of immunosuppressive treatments and the low availability of human donor organs represent the limitations of the traditional approaches what reveals the needing for alternative options [26].

New treatments have been studied around biologics, including antibodies, cytokines, and cytokine receptors that can amplify or attenuate cytokine signaling. These molecules are often administered as unadulterated agents, or are integrated on carrier platforms like NPs, liposomes, and polymers [27].

Several approaches have been researched using the pancreas as the tissue supplier through repopulating decellularized pancreas, culturing islets on the decellularized pancreas, and differentiating stem cells into β-cells using β-cell line produced matrix [27].

Further, some techniques have incorporated other tissue sources and methods such as processing decellularized ECM into hydrogels and culturing islets on decellularized liver.

Biomaterials that allow the islet encapsulation, and the diffusion of nutrients/waste, oxygen, and insulin, but avoids the infiltration of immune cells and antibodies have been studied as a possible treatment of T1D for over four decades. This approach is achieved through the encapsulation of islets and the use of immune-privileged material that protects them from the adaptive immune system, while still enabling insulin to exit the capsule. Hence, both graft rejection and autoimmune attacks can be avoided [27].

One material that can be use is sodium alginate, a polysaccharide derived from algae, which can encase islets while allows nutrient and waste permutations, and enables the imbalance in osmolarity. However, are still needed further investigations (co-encapsulation, and prevascularization) for islet encapsulation to improve islet permanence [27].

Also, the use of a silicon nanopore membrane after islet encapsulation in the alginate capsule allows the protection of the islets against cytokines, improves their viability, and appropriate insulin secretion in response to glucose levels [27].

Hydrophilic nanoscale polymer systems, organized in hydrogels, are frequently employed in cell encapsulation owing to their high-water content and ability to act like ECM.

Sodium alginate is one the most widely used materials for islet encapsulation due to its biocompatible characteristics and due to the possibility of being able to be frozen under moderate conditions by complexation with divalent cations. Adding, the alginate matrix size under freezing conditions is in the nanometer range, allowing insulin and smaller molecule diffusion while excluding the transfer of larger molecules [29].

However, as alginate is derived from natural resources, polymer variations and residual endotoxin levels create concerns with its utilization [29]. In order to alleviate the concerns with variability and batch-to-batch differences in natural ECM proteins, it is viable to use expression systems to synthesize analogs.

Encaptra is a device that was developed to protect transplanted β-cells against the body's immune cells and allows the exchanges of nutrients and oxygen through the device. Also, TheraCyte/ViaCyte device was one of the first devices to show the importance of using pore sizes at the micro and nano scale [27]. As the device is going through phase 1/2 of the study, it is expected that the results will be collected in 2023.

The iNanoBIT is other project that was made to provide the missing tools for pre-clinical/clinical testing in order to enable a safe transplantation of regenerative products, currently in pre-clinical and clinical trials.

The main technical elements under development, like porcine islet cells/human beta cells, encapsulation mechanism, and safety arrays, can offer new and innovative keys to the major associated problems such as the deficiency of cell supply for human transplantation, the requirement for immunosuppression subsequent islet transplantation and complex surgical procedures. Nevertheless, more efforts are needed for a scaling-up production of transplantable cells/islets on a cost-effective approach and generate data towards regulatory acceptance [26].

Genome editing to match donor to receiver or reduce human leukocyte antigen genes has been recommended as an encouraging approach to enhance the immune compatibility of stem

cell-derived β-cells. Nanomaterials-based immune system adjustment can occur as a result of chemically modified host-interfacing surfaces, or the specific delivery of immunosuppressive drugs and biological agents [27].

In addition, encapsulated islets need an adequate nutrient supply and positive extracellular signals to survive and function in vivo. Also, insulin needs to be distributed into the bloodstream at the right amount and time for the graft to offer a therapeutic purpose [27].

ECM is somewhat responsible for the preservation of cell organization in pancreatic islets. Hence, when it was removed through the isolation procedure, cell arrangements of β- and α-cells suffer an adjustment but are then capable to return to the native arrangement following transplantation or embedding in a cell derived matrix, like Matrigel, which own sensing matrix ligands proteins including collagen I, collagen III, collagen IV, fibronectin, and laminin improving islet health [27].

Additionally, it can be useful to mention that 3D culture environments for islets generally provide islet health to a greater degree than two dimensional ones [27].

Glucagon-like peptide-1 (GLP-1), a hormone secreted by the L-cells in response to food intake, promotes insulin secretion, β-cell proliferation and prevents β-cell apoptosis. Hence, when the hydrogel structure is functionalized with GLP-1 prevents β-cells from cytokine-stimulated apoptosis. Also, insulin-like growth factor (IGF)-2 can promote the perpetuation and differentiation of islets.

The islet cells are particularly sensitive to a deficiency of blood. Considering that many of the complications occurring from hyperglycemia are related to the microvasculature, for a diabetic transplant, an undesirable environment can be present, which turns the procedure ineffective if this aspect is not considered [27].

When the objective is to vascularize the islets, it is currently not possible to require full immune defense with an encapsulation membrane. Therefore, when an immune membrane incorporates the treatment system, the aim is instead to induce vasculature growth more nearly possible to the system.

The large evaluation of islet nanoencapsulation methods demonstrates that this technology holds great potential as a platform for islet regenerative therapy [29].

Certifying cell survival, and graft function are important aspects that must be guaranteed and may be supported by materials engineering on the nanoscale size.

Encouraging data on cellular replacement therapies and limited success in the clinic stimulate the pursuit for continuous design improvements in which the nanoscale must be considered for the treatment of TID [29].

3.2. Osteoarthritis (OA)

OA is a multifactorial pathology of the entire joint which affects 140 million people worldwide having an enormous economic and social impact. Current treatments are centered on reducing pain and improving mobility, and few therapies are available to restore degraded cartilage or slow disease pathogenesis [30].

OA is correlated with genetic factors and is an age-related disease, being its incidence higher in people between 55 and 64 years. Also, their incidence is higher in males [30].

Primary treatments for patients with OA include appropriate physical activity, weight loss, braces, in combination with anti-inflammatory drugs and intraarticular corticosteroid injections. When the progression of the disease begins to affect quality of life, surgical treatments are the next step. Arthroscopy with meniscal repairs, both full and partial, increases outcomes [30].

Positive results in cancer treatments using nanomedicines provide motivation to use these approaches to focus on OA clinical challenges [30].

Nanotubes, nanocomposites, MNPs, and other nanotechnology-based drug and gene delivery systems can be applied to molecular pathways and pathogenic mechanisms involved in the disease development.

Also, nanomedicine turns possible the design of special NPs for detection of early osteoarthritis changes in cartilage tissue, using, for example liposomes containing an antibody to type II collagen, which when combined with a color emitting near-infrared light enables detection. Moreover, NPs containing anti-inflammatory drugs and proteins allows their release in a prolonged way, ensuring sustained release and delivery.

Several bio-based materials including chitosan, bovine serum albumin, hyaluronic acid, and chondroitin sulfate can be used for the synthesis of these NPs [30].

Liposomes are highly used for drug delivery in OA due to their biodegradability, biocompatibility, elevated encapsulation capability, and ability to catch hydrophilic and lipophilic drugs [31].

Biological strategies that focus on cartilage ECM degradation, bone remodeling, inflammation, and dysfunction of skeletal muscle, and adipose tissue metabolism, are believed to be great candidates for the treatment of OA. Even though these approaches appear promising, yet there is no effective, approved treatment for OA [30].

Approaches like arthroscopic debridement, allograft application, autologous chondrocyte implantation, and matrix-based autologous chondrocyte implantation are only capable of partial restoration of mobility and improvement of the symptoms of cartilage injury. Yet, the

developed tissue, which primarily comprises a fibrocartilage, as a substitute of native hyaline cartilage, is very fragile [30].

Therefore, tissue engineering techniques applying 3D scaffolds filled with cells, and nanocompound-based drug delivery systems, represents promising approaches for the development of new treatments for cartilage injuries. Molecular elements and biochemical signals can regulate differentiated chondrocyte function and stimulate proper cartilage structure development, pore dimension, and mechanical competence for the regeneration of damaged cartilage tissue [30].

Thus, it is vital to create innovative strategies for early diagnosis and treatment of OA. These involve nanoparticle-based methods or label-free and real-time biosensors of OA biomarkers such as glycosaminoglycans, HA, cytokines, free radicals like NO, and proteinases. Also, chemical properties of AuNPs, which are being widely considered for the development of new contrast elements or biosensors. Biosensing with this type of NPs implies the interaction among a target biomarker molecule and a AuNP crosslinker or a AuNP-containing antibody [32].

Also, non-viral gene delivery using nanocarriers and scaffolds is a promising approach for OA treatment **[Table 3]**.

Alginate can be used as a scaffold for cell culture and as a nanocarrier for delivering genes to the target cells. Gene-activated alginate hydrogels are capable of gene delivery via nanohydroxyapatite (nHP) and have been developed in order to regulate differentiation capability of MSCs for cartilage or endochondral bone tissue engineering. Hence, the conjugation of MSCs and nHP and DNA, encoding TGF-ß3 and BMP-2, were encapsulated into alginate hydrogels [33].

Several studies have demonstrated that nanofiber-based polymer scaffolds improve the chondrogenic differentiation of MSCs. Additionally, hybrid peptide nanofiber-HA membrane scaffolds have been developed to conserves cartilage morphology, decreases osteophyte formation, and retains cartilage specific matrix proteins in OA models in vivo [33].

Another study has been conducted with self-assembled peptide nanofibers combined with the neuropeptide substance P as an injectable conjugate containing different concentrations of SP and applied in a rat OA model. The results revealed that these types of nanofibers can stimulate chondrogenic differentiation and decreases the progression of OA [34].

Using similar approaches, injectable hydrogel scaffolds containing chondroitin sulfate NPs and nHP were created for osteochondral regeneration and evaluated utilizing a rabbit model. This technique developed hyaline cartilage regeneration with subchondral bone creation.

Polymer-based scaffolds can fail in the delivery of proper signals to promote functional tissue regeneration. In order to overcome this limitation, it can be used other nanomaterials, like CNTs due to their exceptional electrical and mechanical features, as and versatility in the assembly of different structures [33].

CNTs have been also used to reinforce polymer-based scaffolds and when single-wall CNTs are made, they can improve the mechanical characteristics of agarose hydrogels, while providing the ideal structure to maintain cellular viability and encourage cartilaginous growth. Also, highly dispersed CNTs in polycarbonate urethane (PCU), can be used as films for chondrocyte growth manipulating at the same time the possibility to provide electrical stimulation via the conductivity of CNTs [33].

While further studies are needed to evaluate the impact of chitosan in nanofibers-based scaffolds, collagen-poly nanofiber-based scaffolds in a rabbit OA model demonstrates effective regeneration of injured joints.

One approach that can be applied to the treatment of OA, is the Kartogenin-conjugated chitosan nano-microparticles which can promote cartilage regeneration. Kartogenin is a small molecule that stimulates chondrogenesis by controlling the nuclear localization of Core Factor Binding-ß [35].

This strategy demonstrates exceptional properties in terms of prolonged-release, stimulatory effects on the expression of chondrogenic markers in vitro, and a long retention time in the knee joint after the intra-articular injection, adding inhibitory effects on cartilage degeneration in vivo.

Stimuli reactive NPs release their agent only when the proper environment requirements or triggers are present [35].

To address the challenge of loading multiple drugs with independent release profiles, it can be used a drug loaded thermo- responsive NP system composed of a chitosan modified Pluronic. The Kartogenin molecule covalently connects to anti- the amine groups of chitosan, while the molecule of diclofenac is contained within the core. Their release depends on temperature with a primary burst delivery of diclofenac after cold shock approach, supported by sustained release of Kartogenin [35].

Using this strategy in a rat model, the thermoresponsive NPs represses OA evolution and decreases COX2 expression.

Another treatment is based on the use of dendrimers that can be nanocarriers for small particles, imaging agents, proteins, peptides, and nucleic acids [36].

Recently, a study described a PEGlyated polyamidoamine dendrimer able to deliver insulin-like growth factor I directly to chondrocytes inside cartilage tissue.

In a rat model of OA, dendrimers were applied and de results demonstrated that they stayed in the joint for 30 days and significantly reduced cartilage degeneration in comparation to untreated rats at 4 weeks post-surgery [36].

Also, it was reported that a PEGlyated polyamidoamine dendrimer is capable of the cytoplasmic release of Kartogenin induced chondrogenesis of MSCs. To note that Kartogenin conjugated to PEG owns the highest expression of chondrogenic markers (Coll II, aggrecan, and SOX9).

The use of Lipid-based NPs, a quite new class of delivery structures for siRNA and DNA, have now been investigated in cartilage diseases. IA injection of lipid-based NP composed by dipalmitoyl phosphatidylcholine, cholesterol and c16 ceramide-mPEG2000, into mouse stifle joint provides a positive signal by fluorescence molecular tomography up to 72 h compared to IA administration of free beacon. In a rat OA model, treatment with lipid-based NP-siRNA complexes relate with greater safranin-O staining, and improved cellularity [36].

Another lipid-based NP approach applies the transfection reagent GenePORTE 2 to provide an endostatin plasmid to MSCs through a collagen scaffold being that overexpression of endostatin promotes cartilage repair. The results demonstrate that these specific scaffolds produce greater endostatin, compared to those seeded on control scaffolds [36].

Because the mechanical properties are vastly different between native cartilage and bone, and the cellular and biochemical characteristics are distinct as well, generating stable cartilage, and bone sections represent a central challenge to TERM.

The use of scaffolds in OA treatment are mainly connected to the incorporation of NPs within traditional scaffolds or hydrogels, the modification of traditional scaffolds to generate nanoscale features and the direct fabrication of nanofibrous scaffolds [30].

In this area, scaffolds are randomly oriented nanofibrous poly(e-caprolactone) which support MSCs chondrogenesis, when attached with the chondrogenic growth factor TGF-β1. These scaffolds have limited porosity, in order to reduce cell movement and nutrient transport. Through the incorporation of chondroitin sulfate into nanofibers, chondrogenesis can be promoted in vitro and in vivo in a rat osteochondral defect model [30].

It will be essential to clarify which scaffold-based approaches shown better functionality in vivo both in humans and in large animal investigations, which are helpful in evaluating promising methodologies before clinical trials [30].

3.3. Brain Cancer and Neurodegenerative Diseases

Recently increasing efforts in the RM area are being devoted to stem cell therapies, particularly in repair of the central nervous system (CNS). Stem-cell-mediated RM simplifies the repair of

neuronal tissues by replacing the damaged cells in the tissues of interest, or by promoting the paracrine signaling system at the damaged places in order to activate endogenous stem cells. Through carrying genes or drugs, nanomaterials can exert therapeutic effects in treating neurological diseases [37].

The blood–brain barrier (BBB) is crucial to sustain the homeostasis of brain microenvironment and to protect the neurons from toxins. These barrier limits the access of molecules with a molecular weight greater than 400 Daltons.

Also, carrier-mediated transport and receptor-mediated transport help the uptake of nutrients, but the BBB can also efflux undesirable molecules from the brain [37].

Hence, an intact BBB prevents the efficient delivery of drugs reducing their efficiency. In opposition, modifications of the BBB can exacerbate neuro-disorders by permitting the entry of neurotoxins, xenobiotics, and pathogens that can stimulate neuronal damages.

RM applied to neurodegenerative diseases has three major aims, including the replacement of diseased or lost neurons; the offer of an environmental enrichment through the supply of growth factors, by the grafted cells; and restore neural tissue or organs that were damaged owing to disease development. The impairment of the patient neurons can be compensated by replacement with exogenously derived cells that have either direct or indirect neurogenic capability [38].

Combining RM with the right nano-therapy is a process with high complexity, time, and cost. After the approach development, there is a need for reproducibility of the product itself and reproducibility in a scaling-up so the therapy can be distributed in a cost-effective way [38].

Neuroregeneration using bioactive agents and stem cells has allowed several opportunities for treating central and peripheral nervous systems associated diseases.

One field that was being explored is the use of Neural-Stem-Cell-Mediated (NSC) NPs that can provide more BBB cross and precise targeting to the brain [37].

NSCs are a population of pluripotent stem cells with that can differentiate themselves into all cell lineages in the nervous system.

The use of nanomaterials can be very useful for the manipulation of the microenvironment, fate and functions of NSCs. Also, external signals and metabolic pathways are dependent on the ECM [37].

Recently, graphene oxide was described to be useful in stimulating cell-to-cell and cell-to-matrix interactions in neurospheres when incubated with human fetal NSCs, which can accelerate the differentiation compared to untreated cells.

Also, the delivery and spatiotemporal control of the chemokine SDF-1 through NP based systems can improve the recruitment and migration of NSCs [37].

Taking into account that the inflammatory reaction and immune response in the host brain constrain the neuronal differentiation of grafted NPCs, several natural materials are being tested. As a result of these studies, it was discovered that growth-factor-reduced Matrigel is a suitable biological scaffold that can offer a microenvironment for cell survival and protection NPCs from inflammatory or immune cells in vivo [37].

Also, it is important to consider that the toxicity of nanomaterials may cause inflammation and consequently neuronal damage when they are confronted with a complex environment of resident microglial immune cells and neurons. Although nanomaterial assisted NSC therapeutics show positive results, there is still several aspects to improve **[Table 4]** [37].

Chitosan NPs have been applied to the delivery of nerve growth factors which promotes the neural differentiation of canine MSCs. Coupling nerve growth factors with SDF-1 and provide them in a controlled way could be an alternative to enhance the regeneration of central and peripheral nervous systems-related injuries [9].

Another type of NPs that has been explored, are the superparamagnetic iron oxide-Au core-shell NPs that when conjugated with nerve growth factor with low toxicity can be useful for neuron growth and differentiation. This growth factor when functionalized with NPs can provide higher neuronal growth and orientation under dynamic magnetic fields [9].

Next, some techniques used for regeneration in specific diseases in the central nervous system will be presented.

- Glioblastoma Multiforme (GBM)

In spite of the huge development in cancer therapy, glioblastoma multiforme, a lethal brain tumor, continuous with a median life expectancy of approximately fifteen months.

The typical treatment for this tumor is surgical resection followed by radiotherapy and chemotherapy, but their recurrence after surgical removal and therapeutic resistance to radiotherapies and chemotherapies results in a poor prognosis.

Existing therapies are not effective against GBM due to the presence of a BBB and endothelial membranes that strictly control paracellular and transcellular perviousness of molecules in the systemic circulation [39].

Since tumor angiogenesis is inevitable and occurs in response to the oxygen and nutrient requirement of the tumor, the structure of BBB is compromised. Hence, the increase in the number of blood vessels in microenvironment enables a bigger NP distribution in tumor tissue. In GBM, several glioma cells are capable to interact with blood vessels and get into the healthy brain cells. The gradual progression of neovascularization and growth of glioma cells breakdown the tight junctions of the BBB which can result in the formation of a permeable and heterogeneous nature of BBB, known as the blood–brain tumor barrier.

This fact permits a more effective distribution of gold NPs in the tumor brain which enables their accumulation (NPs with 10 nm preferably) in the tumor brain tissue [39].

The ability of MSCs to penetrate the BBB has been studied in order to overcome this natural obstacle of delivering drugs to brain targets, and the multi-differentiating capacity of MSCs has been proposed as a potential solution for regenerative therapy. In a clinical view, MSCs can be easy to isolate and transplant back into the patients after their proliferation.

The apprehension of possible tumorigenesis of genetically modified MSCs guided to the development of non-viral transfected MSCs centered on nanotechnology techniques for more effective cancer and regenerative treatment [39].

Importantly, MSCs, when mediated by NPs, can further enhance specific tumor sites and regenerate the damaged neurons in the central nervous system through the promotion of axon growth. Non-genetically engineered MSCs assisted by NPs have gained special attention due to their less immunotoxicity than genetically modified MSCs.

Despite some studies considered tumor promotion induced by MSCs, MSCs can stimulate apoptosis of tumor cells through the regulation of apoptotic signaling pathways [39].

Related apoptosis-inducing ligand-secreting MSCs can inhibit brain tumor by directly binding to their death receptor (DR4 and DR5) on the membrane of tumor cells. Additionally, these genetically engineered MSCs can secrete pro-inflammatory cytokines (IL-18 and IL-12) to promote cytotoxic T cell activity.

Even though the advances in NPs delivery system, using this system in animal models has been difficult due to their poor accumulation in tumor sites. In order to increase therapeutic efficacy, NPs loaded MSCs, like gold NPs loaded MSCs were developed for photothermal therapy, having also pH sensitive characteristics [39].

The regeneration of the central nervous system is inhibited by two main sources: glial scar and myelin. Glial cells are crucial for immune function when inflammatory states are present. When these cells are injured, glial scars are formed, in order to protect damaged neurons and reconstructing the blood–brain barrier. Despite this protection, the glial scar avoids axon growth by producing a mechanical barrier and inhibiting molecules. Analogous to glial scar, myelin inhibits axon regeneration by producing myelin-associated inhibitors [39].

To promote neurogenesis various growth factors, extracellular matrix degrading enzymes, and myelin neutralization have been described as capable to increase neural growth, but their application is reduced due to their low BBB penetrability.

Exploiting MSCs as cargo molecule can offer a path for prudent delivery of neurotrophic factors loaded NPs to target sites to achieve nerve regeneration. Also, the efficacy of MSCs

and neurotrophic factors as well as myelin neutralization and degrading ECM enzyme can improve axon growth [39].

- Parkinson's disease (PD)

PD is a neurodegenerative movement disorder caused by a continuous loss of dopaminergic (DA) neurons in the substantia nigra, which results in reduced dopamine levels in the dorsal striatum which consequently cause dyskinesia and dementia. Present PD treatments intended at protecting or enhancing DA neurons, however, they produce side effects, and their efficacy diminishes over time, habitually leading to the progression of motor and cognitive problems [38].

In this pathology, cell replacement approach requires purchase and implanting of the DA neurons. After DA precursor cells are grafted, they must integrate and re-create the original healthy neuronal system. However, there are challenges with inconsistency and reproducibility of this approach. Moreover, human embryonic stem cell-derived DA cells have been safe and effective in non-human primate models, however it is uncertain if the cells can be made in sufficient quantities to show comparable efficacy in the human brain [38].

Nanomaterials could innovate PD treatment by stimulating, and interacting with the target sites to trigger physiological responses while decreasing adverse reactions.

To overcome the major challenges of the treatment of this disease, NSCs with a dopaminergic phenotype can be an option to consider in order to generate neurons for transplantation of fetal brain tissues [37].

- Alzheimer's Disease (AD)

AD is a chronic neurodegenerative disease, and has an insidious start and slow progression of symptoms. The etiology of this pathology is uncertain, but amyloid-β protein and the microtubule-associated protein Tau are recognized as the targets for AD treatment. Also, AD is described by the loss of neurons and synaptic connectivity through the cortex, hippocampus, amygdala, and basal forebrain, leading to memory and cognitive impairment [37].

Nanomaterials can play an important role in the transport of stem cells, genes and drugs, directional differentiation, and real-time tracking these components [37].

A study reveals that charge reversible PCB-based therapeutic and traceable ABC/SPIONs/ siSOX9 NPs could fine-tune the differentiation of NSCs to neurons and ameliorate neurological alterations and therefore rescue the memory insufficiencies in AD mice [37].

Also, brain-derived neurotrophic factor (BDNF) proved to be an important molecule in the development of AD and plays a crucial role in the aging process, decreasing their levels over the years.

An approach conducted with models of AD where rodents received neural precursor cell grafts demonstrated a rise in hippocampal synaptic density and cognitive function related with local production of BDNF [38].

To note, that in an early stage, neuroprotection and cell-based repair may possibly be achieved through growth factor infusion or distribution of stem cells, however at advanced stages, substitution with tissue or organ prosthesis may be necessary. In more advanced stages, even the healthy grafted cells can succumb to the pathology owing to their pre-existing spread.

Recently, it has been concluded that early AD pathogenesis is accelerated by perturbed BBB function, which can be triggered by early neuroinflammation. This finding offers a possible new target for early-stage pathogenesis in AD. Hence, an early work using PNPs to target amyloid-beta plaques in the cerebral vasculature of APP transgenic mice is in progress [38].

- Huntington's disease (HD)

HD is an autosomal dominant neurodegenerative disease. Adult-onset is the most frequent form of this disorder and their early signs and symptoms includes irritability, depression, small involuntary movements, and loss of cognition capacity.

Currently, treatments that can alter the course of HD are not available. But medications can lessen some symptoms of movement and psychiatric disorders [38].

Tissue engineering has demonstrated functional recovery in several rodent and even in human HD patients. Pluripotent stem cells including ESCs, iPSCs, NSCs, and MSCs, are presently under investigation.

Nanotechnology can help resolve the limitations of stem cells use. Hence, liposomes, lipid nano capsules, poly(ethylene imine) NPs, dendrimers, and chitosan are being explored in the stem-cell-based treatment of HD [38].

Exosomes from adipose-derived stem cells have shown great promise in treatment of HD by significantly decreased mHtt aggregates in R6/2 mice-derived neuronal cells and ameliorated abnormal apoptotic protein level in an in vitro HD model [38].

Despite there are few studies on the use of nanocarriers and stem cells together for the treatment of HD, several reveals their potential, an example was the delivery of stem cells and neurotrophic factors like BDNF to drive GABAergic differentiation in vivo [38].

However, further studies are needed in this field to consolidate these data.

3.4. Cardiovascular diseases

Cardiovascular diseases represent the major cause of morbidity and mortality, accounting for 31% of all deaths. Several studies have been conducted in order to develop treatments to prevent the progression of these pathologies.

Despite the improvement made in the last decade, current therapies have poor translation into actual clinical applications. One of the major barriers of such strategies is reduced regenerative capacity of the cardiac tissue [40].

Also, once an ischemic episode occurs, the creation of fibrotic scar takes place, interfering with mechanical and electrical characteristics of the cardiac tissue. Hence, after ischemic injury the imbalance between molecular and cellular pathways, can result into adverse remodeling, culminating in heart failure [40].

Myocardial infarction (MI) is mostly caused by the obstruction of a coronary artery owing to atherosclerotic and thrombotic processes, resulting in a decrease in the blood flow to the heart muscle. The ischemic environment leads to cardiomyocyte (CMC) death, causing a defective contractile function. Consequently, progressive left ventricle [9] remodeling and scar tissue development take place.

These modifications influence the ventricular morphology, leading to a larger, thinner and more spherical heart shape.

Death that was caused by MI could be prevented by early-stage diagnosis and proper treatment [41].

Existing therapies involve surgical procedures including coronary bypass, balloon angioplasty, stents and heart transplant as a last option. These interventions are normally complemented with pharmacological treatments to improve patient outcomes. However, these approaches cannot repair the infarcted tissue.

In several investigations, angiogenesis, CMC proliferation stem cells are studied in order to understand what is the best method to enhance endogenous healing of the heart [41].

Poor results are being obtained by TERM, which justifies that organ transplantation remains the most elective treatment for end-stage heart failure.

The discovery that adult CMCs can be induced to go into the cell cycle and propagate by conjugation of miRNAs and cardioprotective medicines, including anti-inflammatory, anti-coagulants, and anti-platelets agents, stimulated pursuit for new strategies suited to achieve cardiac repair [42].

Biocompatible NPs own exceptional physicochemical characteristics that can be explored for the treatment of cardiovascular diseases and have been provided very promising outcomes, in preclinical studies, in the last years [42].

New CMCs can be generated from ESC or iPSCs and administered to the cardiac tissue as cell suspensions or upon ex vivo generation of contractile myocardial tissue. Also, the capacity of CMCs to proliferate can be accelerated by the delivery of individual genes, especially by selected miRNAs.

Recently, experimental success in large animals by both approaches demonstrates that cardiac regeneration is actually possible. However, some aspects need to be addressed and solved before the broad and successful clinical application is reached [44].

Several studies in order to understand the reason why CMC proliferation stops irreversibly after birth have been conducted, and in result, now it was known that this process is linked to sudden biochemical and mechanical events occurring immediately after birth, including, pressure overload, sudden increase in oxygen tension and oxidative stress, lack of maternal factors, changes in hormonal stimulation, and switch from glycolytic to oxidative metabolism. Some growth factors with stimulating CMC proliferation characteristics that are present during embryonic and neonatal stages have been identified. These involve interleukin-6, platelet-derived growth factor, members of the fibroblast growth factor family, follistatin-like 1, and neuregulin-1 [44].

Also, paracrine control of CMC proliferation was reported with factors secreted by T regulatory cells and cardiac monocytes.

The CMC replication was also described to respond to modifications in the ECM, that are mediated by agrin protein. This protein is a component of the neonatal ECM and can stimulate CMC division through the disassembly of the CMC membrane-associated dystrophin-glycoprotein complex [44].

Three signal transduction pathways were indicated to participate in the management of CMC proliferation during the embryonic, fetal, and neonatal life, the Wnt/ß-catenin pathway, Notch pathway, and the Hippo/YAP.

As mentioned above an interesting approach to achieve cardiac regeneration is to manipulate the CMC proliferative potential by the utilization of miRNAs [44].

The miRNAs stimulating endogenous CMC proliferation can be classified into one of three categories, pro-regenerative miRNAs, miRNAs involved in tumorigenesis, and miRNAs that can stimulate cardiac repair. The third group of miRNAs was identified through two large screenings of human miRNAs and is the most prominent and studied of these miRNAs [43].

However, there are still some issues that need to be addressed before miRNA can be used for the cardiac regeneration. One of these aspects is identifying which combination of miRNA could be the most efficient [44].

Surface adjustments of the NPs, allows specific binding to the target sites, simplifying the internalization of NPs into these cells via receptor-mediated endocytosis. Non-viral miRNA carriers use, for example, gold NPs, carbon and biocompatible silica, non-immunogenic and non-toxic materials. Additionally, PEG has been conjugated with gold and has demonstrated elevated miRNA loading capacity and low toxicity [44].

Yet, much remains to be studied, in particular, the exact mechanisms of miRNA action in cardiac differentiation and direct reprogramming and whether regulators can really be modulated in settings of chronic disease and in a tissue-specific way [44].

Also, exosomes play an important role in the development of cardiovascular diseases. Several studies have suggested that they are responsible for shuttling proteins and noncoding RNAs, specific miRNAs, between cells. Particularly, exosomes trigger signaling pathways, largely contributing to the favorable effects of stem cell treatments for cardiac hypertrophy and MI among others. c-kit+ progenitor cells, cardiosphere-derived cells, and MSCs are the most studied sources of exosomes implicated in cardiac repair [45].

These researches show that, in the cardiac field, stem cell-derived exosomes are a main vehicle for pro-reparative miRNA and protein transfer which has major importance for cardiac repair and regeneration [45].

Despite their importance in paracrine signaling for regeneration, stem cell-derived exosomes have several limitations. One major obstacle includes a supply and demand inconsistency. Cell-derived exosomes have variable, low produce that makes it challenging to meet the demand for in vivo uses.

Systems biology and computational modeling tools can be applied to understand interactions between different types of cargo (miRNA, proteins) and their respective cardiac responses such as migration, proliferation, fibrosis and angiogenesis. These computational-based approaches can be very useful when combined with exosome-engineering methods to customize-make exosome cargo with different levels of desired, potent RNA/protein to stimulate specific cardiac outcomes [45].

Another approach that has been explored is the use of MNPs and has gained increased attention as magnetic targeting of stem and development structures, scaffold-free multilayer, and spatial patterning of aggregates. In particular, magnetic fields can direct the assembly and patterning of magnetized human CMCs labeled with MNPs in collagen-based hydrogel, which can be used as a regenerative approach [46].

Poor endothelialization, can reduce the efficacy of the tissue-engineered scaffolds utilized in the regeneration of cardiovascular tissue damage. One study demonstrates that self-assembled NPs can speed up vascularization of decellularized buffalo bovine jugular vein scaffolds through sustained release of VEGF. This release had to be sustained by low molecular weight heparin and N, N, N-trimethylchitosan chloride, which experience self-assembly through non-covalent electrostatic interactions protecting the bioactivity of VEGF [9].

In another study, NPs of different compositions (PLGA and PLLA/PLGA bilayered) were used to obtain consecutive release of the Platelet-derived growth factor (PDGF) followed by co-

release of VEGF and bFGF (basic fibroblast growth factor) as angiogenesis factors for cardiac tissue regeneration. Using rat aortic ring as a model for angiogenesis it was observed a significant increase in the number of endothelial sprouts with the maximum length of angiogenesis in the sequential release group which was higher than that observed in simultaneous and only VEGF release groups [9].

Great advances have been made in the last few years, although there are still several aspects to improve and current results should be considered preliminary.

3.5. Gene therapy

Owing to advancements in genomics and biology, our understanding of the genetic basis of diseases has offered several opportunities of new targets for genetic treatments [24].

The availability of drugs or genetic material to the target cells is one of the emerging areas of biotechnology, and the transport regulated by NPs is especially interesting because of its ability to target these cells, therefore reducing the adverse effects revealed in conventional therapies with less specificity. Some NPs when carrying genetic material allow temporary or permanent reprogramming of cells.

Gene therapy implies the potential of using nucleic acids to modulate the expression of genes in cells in order to achieve therapeutic results. This designation is used irrespectively of whether it concerns gene modulation over the supply of DNA, antisense oligonucleotides, mRNA, siRNA, or miRNA [47].

Because of the negative charge and considerable larger size of the nucleic acids, the transport of these molecules, should be facilitated by gene vectors.

Due to their advantages, non-viral vectors, such as polymers, lipids, and functional vectors, have been developed and applied in gene therapy **[Figure 8]**. This type of approach was recognized as a significant tool for TERM since the initial stages of this field, and so on with the new stem cell-based treatments [47].

Using a gene-based treatment we can achieve an ideal procedure to instruct stem cell regeneration and to improve their trophic effects or their survival after implantation and also stimulate undifferentiated stem cells into a specific lineage of interest [47].

Non-viral vectors not only can provide a gene into the cells but also have the capacity of fluorescence imaging, which helps in monitoring their progress, targeted delivery, and biodegradation.

To proceed with this approach, two aspects need to be considered. The gene should be suitably delivered to the target cells, and gene carriers should protect nucleic acids from degradation by the enzymes present in the blood. Consequently, it is really a challenge to develop an effective and safe carrier for nucleic acids delivery [47].

Functional vectors can be divided into four types, including functional fluorescent and non-fluorescent vectors, targeting vectors, biodegradable vectors, and other functional vectors. Functional fluorescent and non-fluorescent vectors incorporate organic fluorescent vectors, QDs, metal complexes, and fluorescent NPs.

Knowing that the transfer of DNA into the cell nucleus is the key step for gene delivery and expression, methods that guarantee their entry should be studied [47].

The SV40 DNA targeting sequences can promote nuclear access by binding of transcription factors owning nuclear localization signals, directing nuclear entry of the non-viral plasmid-based vector.

Another approach proposed to boost the nuclear import is based on the linking of NLS-peptides or NLS-containing proteins by electrostatic, covalent, or peptide nucleic acids clamps to the DNA [47].

Also, the production of biodegradable poly (amino ester) compounds can offer higher transfection efficiency and lower cytotoxicity in HEK 293 cells, due to their biodegradable strengths and hydroxyl groups.

Below are some examples of carriers that can be used in gene therapy using nanotechnology [47].

- Exosomes

Exosomes mediate intercellular communication through the delivery of genetic information to certain cells. They are stable, biocompatible, and able to overcome natural barriers. Given these features, the use of these vesicles for therapeutic purposes is being investigated.

As they can carry DNA, RNA, proteins, and several drugs, EVs are being mainly studied in the field of cancer, but are also increasingly examined in immune-related diseases and regenerative medicine.

However, due to a lack of cell-targeting specificity, their utilization as a therapeutic agent is reduced. In order to improve this, EVs are being modified with targeting ligands, such as PEG, enable an extended circulation time and increasing EV accumulation in specific tissues, and improving cargo delivery [47].

- Nanodiamonds

To design an ND vector to deliver plasmid DNA or siRNA, these NPs should be functionalized to produce a cationic surface to which negatively charged DNA or siRNA can be linked [17]. Polyethylenimine is a cationic polymer that has high affinity to DNA and siRNA, however, when provided alone its transfection capacity is reduced because it is not easily internalized

by cells. By complexing these polymer with NDs, which can be internalized by endocytosis, extremely efficient vectors can be created [17].

Also, these complexes could be produced with fluorescent NDs, allowing the possibility to follow the delivery of the vectors in real time.

The covalent modification of NDs with lysine creates a cationic surface charge which can adsorb siRNA and prevent particle aggregation also promoting a high transfection efficiency with negligible cytotoxicity. These functionalized NDs are capable to bind to physiological proteins producing a layer of adsorbed proteins on the siRNA vectors that improved the dispersity of the vectors and gave a positive effect on cellular uptake [17].

Moreover, ND vectors produced through chemical conjugation might be preferable for the development of stable and well-dispersed nucleic acid carriers [17].

- Graphene-Based Functional Vectors

Carbon nanomaterials, like graphene and graphene oxide [10], are being exploited because of their biocompatibility as nano-carriers.

Their surface adaptations have allowed improved biostability, cellular uptake, and increase gene loading capacity.

GO nanosheets can be applied to encapsulate NPs, such as gold and nanorods.

A experience was conducted in order to transfect HeLa cells, and the results demonstrated a good DNA binding ability and condensed plasmid DNA into nanoscale particles. Despite these results, in vitro gene transfection demonstrated lower cytotoxicity and higher efficiency.

GO constructed nano-aggregates cross-linked by ATP-responsive DNA strands created for ATP-mediated regulated drug release system including elevated drug loading capacity, and ability of the target site, drug release has reveal it an encouraging aid for improved therapeutic efficacy [47].

The interest of nanostructures as alternatives for viral vectors were highlighted in a study that compared cationic lipids and adenoviral vectors for the release of DNA encoding BMP-2 to bone marrow stromal cells. Despite smaller healing times with adenoviral vectors, the researchers suggested liposomes as the gene carrier of choice because of their easier preparation, the theoretically, and the reduced safety concerns [48].

Comparably, another study was developed with polyplex nanomicelles composed of a PEG-block-catiomer incorporating DNA in order to show their promising features for bone regenerative gene therapy. This nanomicelle revealed high biocompatibility and capacity for regulated gene transfer, involving a significant increase in the bone regeneration rate in a bone deficient model [48].

In another approach that was focused on the use of devices combining a polymeric scaffold and a nanocarrier used for gene delivery, it was developed an injectable gene delivery system where calcium phosphate/DNA NPs were combined into an alginate hydrogel to allow specific distribution of the complexes to transplanted preosteoblasts and host cells at a bone deficient position. The results showed the ability of the NPs to transfect preosteoblast cells in vitro, and the ability to stimulate bone formation in vivo when a combination of NPs and cells in alginate matrices was established [48].

In the field of myocardium regeneration, the main approaches include the development of local angiogenesis to the damaged myocardium or stimulating the formation of new myocardium.

Nevertheless, gene therapy has found applications in both strategies, and were exposed above when treatments for cardiovascular diseases was addressed.

Also, the application of gene therapy to the central nervous system has the potential to benefit diseases, including Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, stroke, and spinal cord injury, which are triggered by a loss of neurons and glial cells [49].

Many neurodegenerative diseases are correlated with abnormal amounts of NSCs in the subventricular zone, which defines these cells as possible therapeutic targets.

A potential application for central nervous system gene therapy involves the release of genes that encode mitogenic factors to promote NSCs proliferation for neurogenesis.

Recently, a nonviral lipofection approach for GDNF gene overexpression in cultured human umbilical cord blood CD34+ cells was conducted. The results demonstrate that this technique might be used to promote enhanced neuroprotection in the treatment of stroke and other neurological disorders [49].

Expecting further improvements in the following years, effective application of synthetic gene nanocarriers to RM seems to be both a desirable and a reasonable objective [49].

4. Future Perspectives

New advances are shared almost on a daily basis what makes it especially hard to define where the best treatment to a clinical challenge will appear.

The most recent developments in molecular and cell biology point to a much better understanding of the molecular mechanisms under regeneration, where NPs seem to play an important role [24]. However, a better understanding of exactly how age, disease state, and the microbiome of the patient affect regeneration will be important for advancing this field in many situations.

Also, 3D human tissue culture models of disease can provide an important study tool in this area allowing tests of RM approaches in human biology, contrasted to the animal models

currently used in preclinical studies. Hence, increasing the accuracy of disease models may increase the efficacy of regenerative approaches and more easily enhance the translation to the clinic of promising treatments [6].

Some concerns will be important to solve for in order to advance with many regenerative treatments. Using, stem cells, whether isolated from mature tissue or induced, will involve strict control over their behavior to improve their safety profile and efficacy.

Additionally, the conception of microenvironments, frequently modeled on various stem cell niches with specific morphogens and physical properties, or having the capacity to genetically control target cells, can be a potential solution to promote optimal regenerative responses from therapeutic cells. The creation of a pro regeneration environment inside the patient could significantly increase outcomes of regenerative medicine strategies.

Now, focusing on the nanoscale delivery systems, they should not only be on producing advanced delivery systems but also on assessing systemic cytotoxic effect and immune responses of these systems. These approaches could provide improved comprehension of biocompatibility of nanoscale delivery systems and will direct the future studies with higher success rate in cost-effective treatments [9].

Some logistical challenges impede widespread use of cell-based regenerative therapies, and their complexity as therapeutic agents creates challenges for determining their safety and efficacy, including the potential tumorigenicity, mutagenesis, contamination, and immune rejection or immune activation that these cells can be part of.

Nanotechnology will remain to show new fundamental material characteristics and functions, hence, these discoveries will catalyze advancements in patient care as long as there is collaboration between engineers, scientists, and clinicians. Multidisciplinary teams can be very important to perform mechanistically driven in vitro, ex vivo, and in vivo experiments, and to convert their findings to patient care.

Finally, in order to accomplish the highest potential for success, clinical trial inclusion and exclusion criteria will require to be refined to decrease variability and guarantee a clean result that maximizes gains and quality of life for the patients.

5. Conclusions

The demand to apply nanotechnology in RM is being progressively recognized as a result of the better control over physical and biological features of a biomaterial than conventional technologies. Still, the production of ideal nanomaterials capable of sending signals to the diseased or damaged cells and tissues to trigger the regeneration process remains a challenge.

The careful selection of the new polymer matrix/nanomaterial combination is essential in making scaffolds with higher biomimicking ability, whose chemical composition and structure can be well-optimized.

The safety of human health using nanomaterials in RM is still a considerable concern, because this field is in its nascent stage. Hence, prior to human applications, studies on the toxic effect of these nanomaterials should be carried out in great detail.

Heterogeneity between patients can additionally restrict the success of nanomedicines, and there is currently only limited research on the interactions between nanomedicines and in stratified patient populations. In vivo maturation of engineered tissues requires a well synchronized series of events involving host immune system, circulatory system and cellular component of the implanted material, that need to be totally known.

It should be noted that it is of extreme interest to bet on regenerative therapies based on the application of nanotechnology since they can save many lives and reduce health costs on a large scale.

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Annexes

Figures

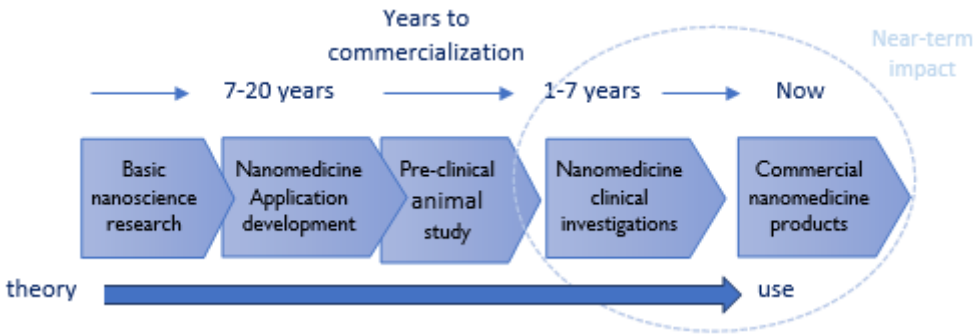

Figure 1 *- Development pipeline of nanomedicine products. Adapted from [1]*

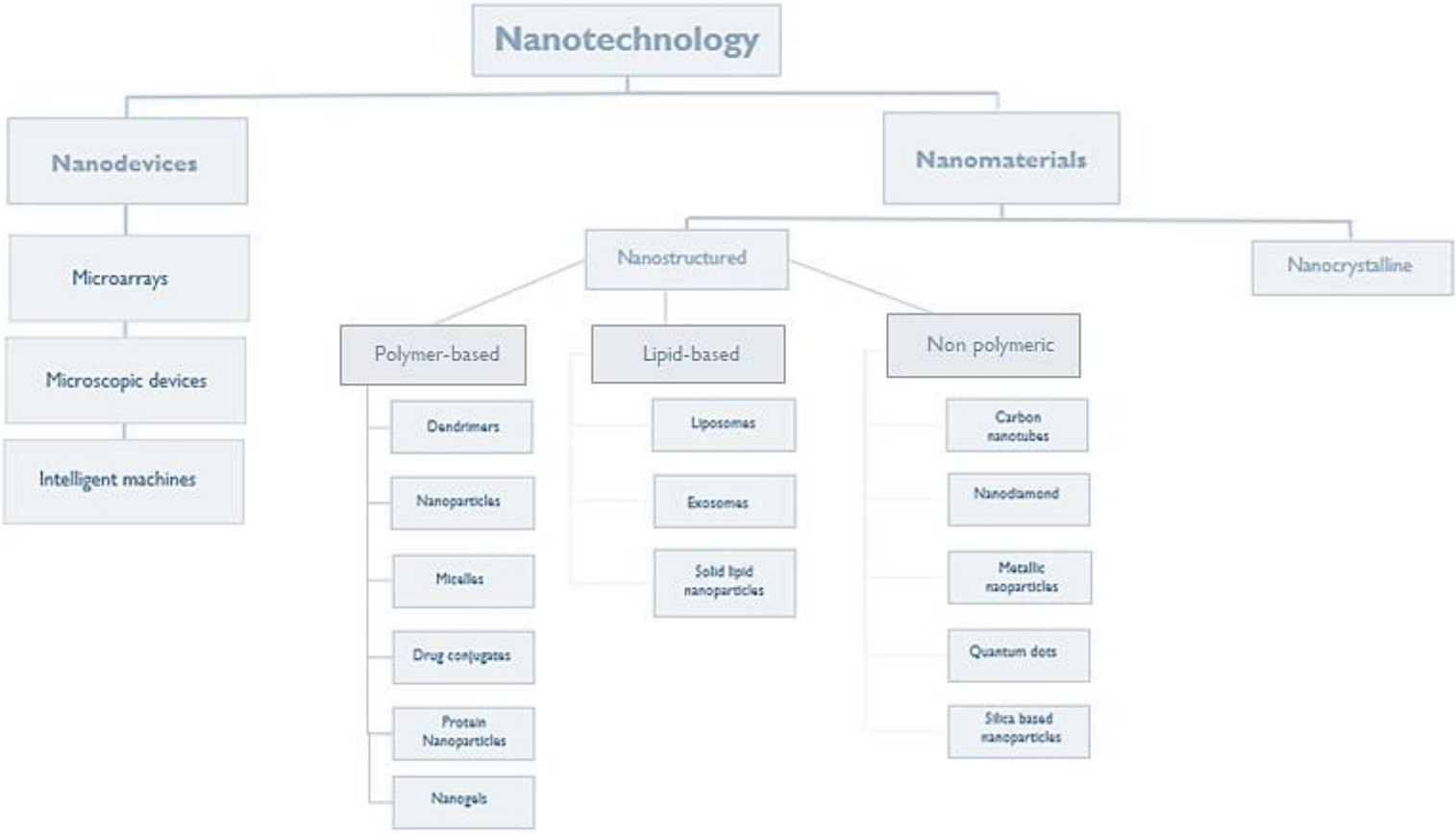

Figure 2 - *Various nanotechnology approaches, which are utilized in therapeutic applications. Adapted from [4]*

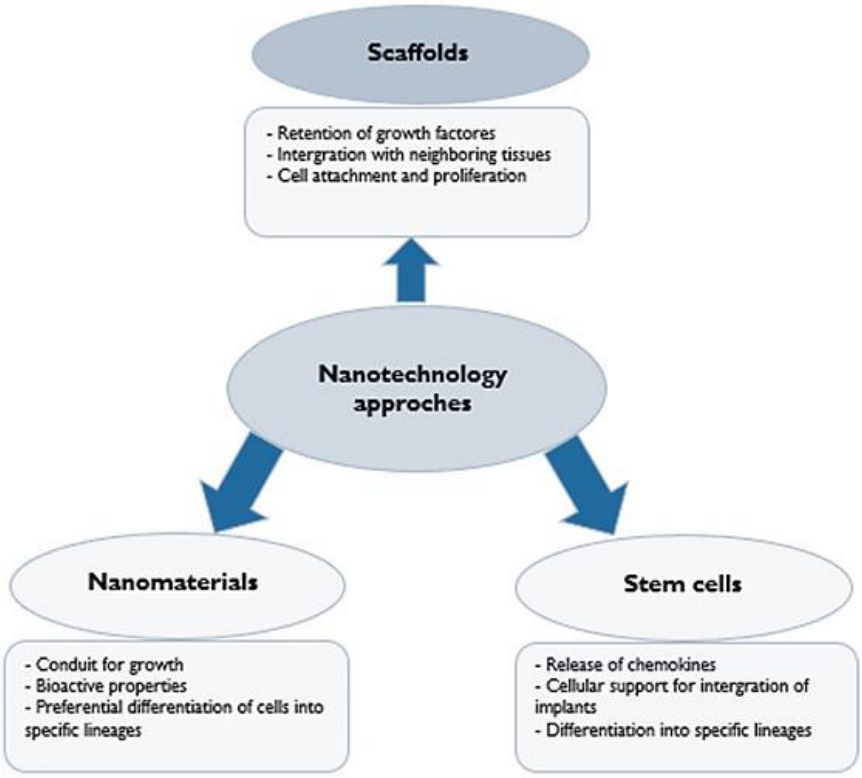

Figure 3 - *Nanotechnology approaches. Adapted from [8]*

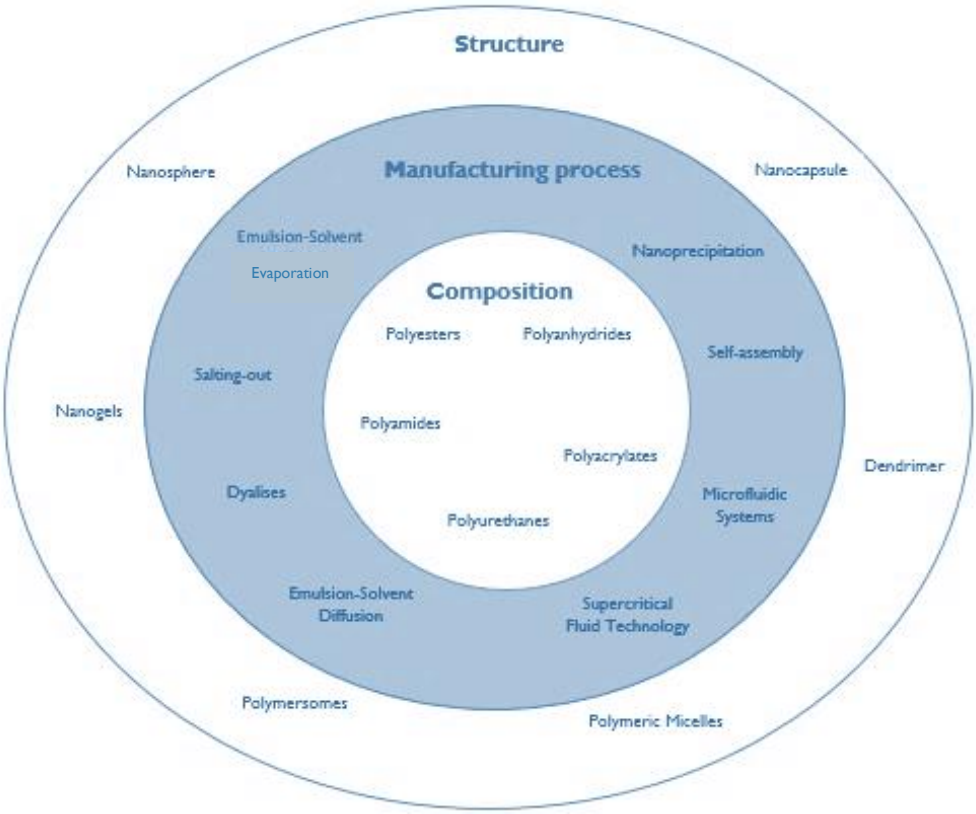

Figure 4 - *Different PNPs based on composition, manufacturing, and structure. Adapted from [9]*

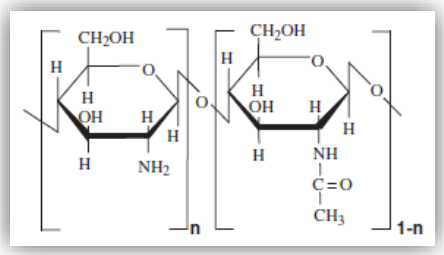

Figure 5 - *Chemical structure of chitosan. Adapted from [15]*

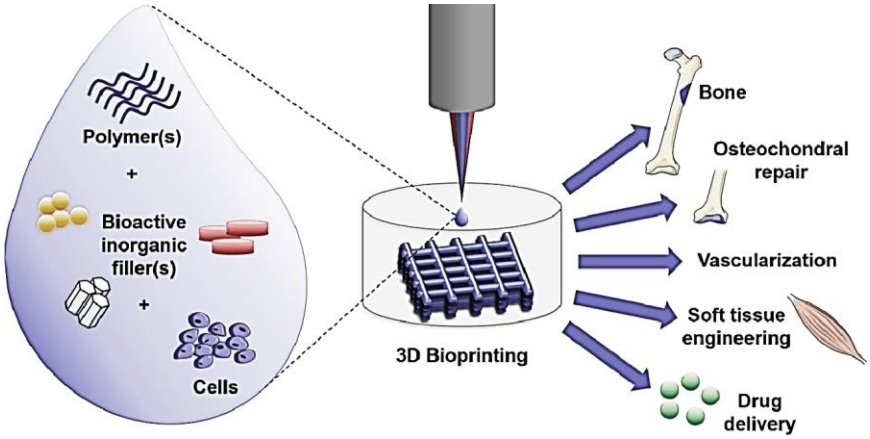

Figure 6 - *Some approaches using 3D printing technology. Adapted from [23]*

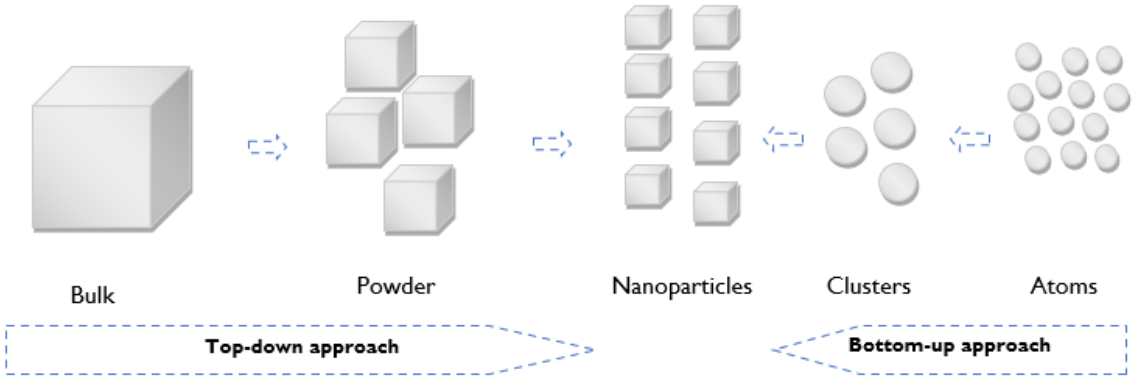

Figure 7 - *Bottom-up and Top-down approaches for the production of nanomaterials.*

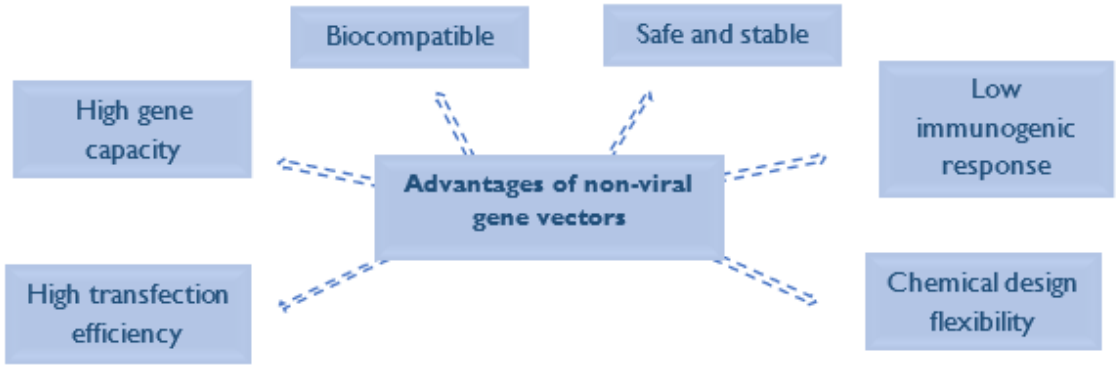

Figure 8 - *Advantages of non-viral gene vectors. Adapted from [47]*

Tables

Table 1 - *Examples of Bottom-up strategies. Adapted from [50]*

	Advantages	Disadvantages
Atomic layer deposition	Permits thickness management to the atomic level through the deposition of one atomic layer at a time, allows free nanostructured films on large areas, great reproducibility and adhesion due to the development of chemical bonds at the first atomic layer.	Slow process, expensive method owing to the use of vacuum components, tough to deposit some metals, multicomponent oxides.
Sol gel nanofabrication	Low-cost chemical production-based method, manufacture of several nanomaterials like glass, ceramic, film, fiber, and composite materials.	Not easily scalable, typically difficult to control production and the following drying steps.
Molecular self assembly	Allows self-assembly of molecular nanopatterns of thickness less than 20 nm and produces atomically nano systems.	Hard to create and fabricate nano systems unlike mechanically directed assembly.
Physical and chemical vapor phase deposition	Adaptable nanofabrication tools, controlled deposition of some materials including metal, ceramics, semiconductors, insulators, and polymers, elevated purity nanofilms, scalable process, and possibilities the deposit of porous nanofilms.	Expensive because of the use of vacuum components, high temperature method and toxic gases mainly in the case of chemical vapor deposition.
DNA scaffolding	Permits elevated precision assembling of NPs into programmable structures with much smaller dimensions.	Several aspects need to be explored, such as integration processes, line edge toughness, throughput, and cost.

Table 2 - *Examples of Top-down strategies. Adapted from [50]*

	Advantages	Disadvantages
Optical lithography	Long-standing, determined nanofabrication tool, sufficient level of resolution at high throughputs.	There is a balance between resist process sensitivity and resolution, which implicates expensive cleanroom-based complex operations.
E-beam lithography	Famous in research conditions, an extremely precise method and effective nanofabrication utensil for less than 20 nm nanostructure fabrication.	Expensive, low performance, tough for < 5 nm nanofabrication.
Soft and nanoimprint lithography	Effective nanofabrication device for fabricating ultra-small features, with less than 10 nm.	Challenging for large-scale fabrication of densely packed nanostructures, additionally dependent on other lithography techniques to create the template, and normally is not cost-effective.

	Advantages	Disadvantages
Block copolymer lithography	A high performance, low-cost, appropriate for large-scale packed nanostructures, various shapes of nanostructures, possible to fabricate including parallel assembly.	Hard to make self-assembled nanopatterns with variable periodicity, normally defect densities in block copolymer self-assembled patterns.
Scanning probe lithography	Great chemical, molecular and mechanical nanopatterning capacities, ability to manipulate big molecules and individual atoms.	Limited throughput applications and manufacturing, high-priced process.

Table 3 - *Non-viral gene delivery using nanocarriers and scaffolds for OA treatment. Adapted from [33]*

Nanocarrier	Gene	Cell line/Animal model
Iron oxide NPs	siRNA against IL-2/-15 receptor β chain	Arthritic rats
Chitosan NPs	DNA (plasmid)	Chondrocytes and synoviocytes
Calcium phosphate/liposome NPs	NF-kB targeted DNA	Arthritic rats
Hyaluronic acid/chitosan NPs	Plasmid-DNA	Chondrocytes
Chitosan NPs	IL-1Ra or IL-10 genes	Osteoarthritic rabbits
Chitosan-HA NPs	IL-1Ra	Synoviocytes
Nanohydroxyapatite (nHA)	TGF- β3 and BMP2	MSCs
Polymeric NPs	Anti-Hif-2α siRNA	Arthritic mice
HA/chitosan NPs	Cytokine response modifier A	Rat knee osteoarthritis model
Bioconjugated carbon dots with succunimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC)	Silenced TNF- α (siTnf α)	MSCs
NO-hemoglobiN@PLGA-PEG NPs	Notch1-siRNA	Macrophage

Table 4 - *Aspects to improve in NSC based therapies. Adapted from [37]*

The safety of stem cells in vivo should be guaranteed preventing the proliferation of NSCs into tumors.	- By injection of the neurons/neuroglia cells, which are differentiated in advance from NSCs in vitro, tumors can be avoid; - The injection of NSCs or their progenitor cells is preferential, but efforts should be encouraged toward research that nanomaterials loaded with specific inhibitors only allow directional differentiation of NSCs but no proliferation in vivo.
The way of transplantation of NSCs should be systematically studied.	- By intravenous injection, NSCs can travel in the bloodstream and reach the injured site spontaneously, but the quantity in the target area is always far from satisfactory for an efficient treatment because of a large capture in the lungs; - Local injection allows sufficient NSCs in the target lesion, but this compulsive behavior could destroy the surrounding normal tissues and cause an incompatible microenvironment for the poor integration of the transplanted NSCs with the peripheral neural network; - The biodistribution and the fate of injected NSCs can be visualized after being labeled with nanomaterials that can be detected by specific equipment. As such, the effect of the transplantation ways of NSCs on treatment efficacy can be investigated in real time.
Advanced labeling methods for NSCs should be explored.	- Bioluminescence is a frequently used technique in preclinical studies to image the viability of the transplanted NSCs in vivo by a transfected gene such as LacZ or green fluorescent protein. However, it is difficult for it to be transferred into clinical use. - Multifunctionalization of nanomaterials as crucial importance because they can bring added values to NSC-mediated therapy.
Biocompatibility of nanomaterials is a prerequisite.	- The toxicity, immunogenicity, and clearance of nanomaterials should be carefully investigated on all levels including cells, blood, and in vivo organs before their in vivo application.
Therapeutic mechanism study should be strengthened.	- Notch signaling pathways have critical roles in the maintenance, proliferation, and differentiation of NSCs in both developing and adult brains. This signaling pathway can be manipulated in vivo with nanostructures that are loaded with specific inhibitors or activators to adjust the ratio of neurons to neuroglia cells for optimizing treatment; - Multicolor tagging technology based on fluorescent QDs can be used to label different stem cells to image their interaction with lesions in a synchronizing fashion.