UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

LOUISE LACALENDOLA TUNDISI

MODULAÇÃO DO DRUG DELIVERY TÓPICO ATRAVÉS DE MATERIAIS FUNCIONAIS E NANOPARTICULADOS

CAMPINAS
2022

LOUISE LACALENDOLA TUNDISI

MODULAÇÃO DO DRUG DELIVERY TÓPICO ATRAVÉS DE MATERIAIS FUNCIONAIS E NANOPARTICULADOS

Tese apresentada à Faculdade de Ciências Farmacêuticas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências, na área de Ciências Farmacêuticas — Insumos Farmacêuticos Naturais, Biotecnológicos e Sintéticos.

Orientadora: Profª Drª Priscila Gava Mazzola

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA LOUISE LACALENDOLA TUNDISI, E ORIENTADA PELA PROFª DRª PRISCILA GAVA MAZZOLA.

CAMPINAS

2022

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Ana Paula de Morais e Oliveira - CRB 8/8985

T834m

Tundisi, Louise Lacalendola, 1992-
Modulação do *Drug Delivery* Tópico através de materiais funcionas e nanoparticulados / Louise Lacalendola Tundisi. – Campinas, SP : [s.n.], 2022.

Orientador: Priscila Gava Mazzola.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Farmacêuticas.

1. Sistemas de liberação de medicamentos. 2. Poloxamer 407. 3. Caseina. 4. Nanopartículas. 5. Hidrogel. I. Mazzola, Priscila Gava, 1979-. II. Universidade Estadual de Campinas. Faculdade de Ciências Farmacêuticas. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Topical Drug Delivery modulation through functional and nanoparticulate materials
Palavras-chave em inglês:
Drug delivery systems
Poloxamer 407
Casein
Nanoparticles
Hydrogel
Área de concentração: Ciências Farmacêuticas: insumos farmacêuticos naturais, biotecnológicos e sintéticos
Titulação: Doutora em Ciências
Banca examinadora:
Priscila Gava Mazzola [Orientador]
Frederico Pittella Silva
Marlus Chorilli
Daniele Ribeiro de Araujo
Renata Fonseca Vianna Lopez
Data de defesa: 11-02-2022
Programa de Pós-Graduação: Ciências Farmacêuticas

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-3374-6103
- Currículo Lattes do autor: http://lattes.cnpq.br/2514528588451905

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Autora Louise Lacalendola Tundisi

Orientadora Profª Drª Priscila Gava Mazzola

Tese aprovada em 11 de fevereiro de 2022

Comissão Examinadora

Profª Drª Priscila Gava Mazzola

Prof. Dr. Frederico Pittella Silva

Prof. Dr. Marlus Chorilli

Profª Drª Daniele Ribeiro de Araujo

Profª Drª Renata Fonseca Vianna Lopez

A ata de defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria de Pós-Graduação da Faculdade de Ciências de Farmacêuticas.

Campinas, 11 de fevereiro de 2022.

EPÍGAFRE

“Você é o único representante do seu sonho na face da terra”

Emicida

AGRADECIMENTOS

Os planos mais uma vez mudaram. Assim como o próprio projeto de doutorado, nada termina como se esperava no começo. Acho que no final das contas eu que tenho que aceitar a instabilidade da vida. Momentos em que ser forte não é mais um adjetivo positivo. Se não somos flexíveis, a vida nos consome. Momentos incríveis, difíceis, angustiantes, de alegria, de dúvida, de saudade, de missão cumprida. Saudade é um sentimento triste de algo feliz. O coração aperta em terminar algo que fez parte de anos da minha vida. O coração também "respira" aliviado de finalizar tudo isso. Só assim podemos seguir para novos destinos com mais altos e baixos.

Esses momentos não seriam os mesmos sem o apoio, presença, ajuda, amizade, "colo", orientação, amor da Professora Priscila Gava Mazzola ou simplesmente Pri. A pessoa que entrou na minha vida e fez uma revolução. Sou imensamente grata por você existir e sempre me apoiar e me deixar aprender, seja compartilhando experiências ou sendo minha minha guia, me deixando livre para seguir meu caminho.

Agradeço também aos ilustres membros da minha banca de qualificação e de defesa pela orientação e contribuição ao meu trabalho: Laura de Oliveira Nascimento, Frederico Pittella Silva, Marco Vinicius Chaud, Marlus Chorilli, Renata Fonseca Vianna Lopez, Daniele Ribeiro de Araujo.

Agradeço à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processo n° 2017/10728-2 e processo nº 2018/05421-8, pelo apoio financeiro, à Faculdade de Ciências Farmacêuticas por me abrigar nesta pós-graduação e à secretaria do programa de Pós-Graduação em Ciências Farmacêuticas, em especial Karen Oliveira de Almeida, por toda a assistência. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Agradeço aos meus colegas do grupo de pesquisa, Amanda Canato Ferracini, Érica Mendes dos Santos, Julia Coco, Lucas Malvezzi de Macedo, Luiz Phellipe Pozzuto Borelli pelas ideias, ajuda, discussões e críticas. Em especial à Janaína Artem Ataide por ser meu par científico compartilhando dificuldades, cansaço, cafés da tarde e risadas.

Agradeço a Carla Christina Vreche e Raquel Leite pela amizade, carinho, amor e cervejas. Obrigada por me fazer voltar a acreditar que a amizade pode ser simples.

Pela parceria, amizade, correções, paciência, conselhos, agradeço ao Diego de Freitas Coelho.

Cinthia Madeira de Souza e Fernanda Almeida Machado obrigada pelas risadas, cervejas e Mario kart.

To Daniel Kohane and his whole research group, I would like to thank for having me and giving me the opportunity to increase my knowledge.

Aos ratos que fizeram parte da minha pesquisa, agradeço a contribuição.

À minha família: obrigada por sempre estarem presentes, sempre com Amor.

Carlos Augusto Liguori Filho por me estimular e respeitar o meu processo de escrita. Obrigada por compartilhar o dia a dia comigo.

LISTA DE ABREVIATURAS E SIGLAS

ALG - Alginate

ATR-FTIR – Attenuated total reflectance with Fourier transform infrared

BNC - Bacterial nanocellulose

BSA - bovine serum albumin

CF – Cellulose fiber

CHI - Chitosan

CM – Casein Micelle

DDS – Drug Delivery System

DLS – Dynamic Light Scattering

FDA – Food and Drug Administration

FTIR – Fourier transform infrared

HA – Hyaluronic Acid

HPC – Hydroxypropyl cellulose

HPLC – High pressure liquid chromatography

HPMC - Hydroxypropyl methylcellulose

HAS - Human Serum Albumin

LCST - Lower critical solution temperature

LPHN - Lipid-polymer hybrid nanoparticles

NIH – National nstitutes of Health

NLC – Nanostructured lipid carrier

NM - Nanomaterials

NP – Nanoparticle

ODF – Oral disperse film

P407 – Poloxamer 407

P18 – Poloxamer 18%

P18C5 – Poloxamer 18% Casein 5%

P18C10 - Poloxamer 18% Casein 10%

P25 - Poloxamer 25%

P25C5 - Poloxamer 25% Casein 5%

P25C10 - Poloxamer 25% Casein 10%

PBS - Phosphate-buffered saline

PCL – Polycaprolactone

PDI - polydispersity index

PEG - Poly(ethylene glycol)

PGA – Polyglycolide

PLA - Polylactic acid

PLGA – Polylactic-co-glycolic acid

PNIPAAm - Poly(N-isopropylacrylamide)

PVA – Polyvinyl alcohol

SLN - Solid lipid nanoparticles

TEM – Transmission Electron Microscope

TNFα - Tumor Necrose Factor

UAS - Urinol Acetate Saturated

LISTA DE FIGURAS

LISTA DE TABELAS

RESUMO

A via tópica traz muitas vantagens sobre o tratamento oral por não sofrer efeito de primeira passagem, menos ou nenhuma interação medicamentosa e, normalmente, não requer monitoramento laboratorial durante o tratamento. Com uma melhoria na eficácia da administração de medicamentos tópicos, a investigação de novas formulações farmacêuticas é essencial para aumentar a adesão do paciente, facilitando o regime terapêutico. Neste contexto, o objetivo deste trabalho foi estudar um sistema de administração de medicamentos capaz de modular a liberação de fármacos tópicos usando um polímero que responde a estímulos com caseína e avaliar a adição de nanopartículas poliméricas à modulação reológica do hidrogel e ao aumento da administração de fármacos. Formulamos combinações de P407 e caseína em proporções variáveis, com a presença das duas moléculas sendo confirmada pelo FTIR. Estudamos o efeito das mudanças na proporção de caseína, na viscosidade, na bioaderência e nas propriedades mecânicas, pois isso seria um determinante importante da capacidade da formulação de se manter no lugar após a aplicação. A viscosidade a 37ºC da formulação cresceu com o aumento da proporção de caseína: 18% (p/v) de P407 com 5 e 10% de caseína foram 1,27 e 2,84 vezes mais viscosos respectivamente do que P407 somente. A adição de 10% de caseína em P407 a 25% resultou em aumento de viscosidade de 1,22 vezes. O efeito da adição de caseína sobre a bio adesão e propriedades mecânicas favoreceu a formulação de um produto tópico final mais adesivo, que visa a capacidade de espalhabilidade e contato prolongado no local. O uso da caseína retardou a liberação da droga do cloridrato de bupivicaina (anfifílico) e sulforodamina B (hidrofílico) em PBS a 37ºC. Todas as formulações foram testadas em ratos para 6 h de contato com a pele e nenhuma delas apresentou sinais de irritação ou inflamação, demonstrando seu potencial para ser testada e utilizada em humanos. As nanopartículas de PCL mostraram alta eficiência de encapsulamento (98,81%) do cloridrato de terbinafina, um bom índice de polidispersidade e não interferiu na atividade metabólica dos queratinócitos. Elas foram adicionadas ao hidrogel de caseína P407 em diferentes ordens e concentrações. A adição de nanopartículas ao hidrogel influenciou significativamente a temperatura de gelificação, as propriedades viscoelásticas e forneceu géis estáveis nos dois métodos de preparação. Além disso, a ordem de adição levou a diferentes comportamentos reológicos para a mesma concentração de nanopartículas. Tanto hidrogeis acrescidos pela adição de proteínas disponíveis quanto nanopartículas poliméricas, têm potencial como sistemas de liberação controlada de medicamentos. As propriedades desejadas para um produto tópico, reologia e liberação de ativos, podem estar na mistura inovadora de componentes já conhecidos e suas

formas. Essas melhorias podem ter um impacto na adesão do paciente, particularmente com terapias tópicas que podem durar meses e exigir muitas aplicações por dia. No entanto, parece que ainda há *plenty of room at the bottom* para permitir o desenvolvimento contínuo dos sistemas de entrega de medicamentos para as décadas vindouras.

Palavras-chave: Sistemas de administração de medicamentos, Poloxamer 407, Caseína, liberação modificada, formulações tópicas, nanopartículas, policaprolactona, hidrogel, reologia, mistura.

ABSTRACT

Topical route brings many advantages over oral treatment, since there is no first-pass effect, less or no drug interactions and usually does not require laboratory monitoring during treatment. As an improvement in topical drug delivery effectiveness, the investigation of new pharmaceutical formulation is mandatory for increasing patient compliance, by easing the therapeutic regime. In this context, the aim of this proposal is to study a drug delivery system capable to modulate topical drug release using a stimuli-responsive polymer with casein and evaluating the the addition of polymeric nanoparticles to the hydrogel rheological modulation and drug delivery enhance. We formulated combinations of P407 and casein in varying proportions, with the presence of the two molecules being confirmed by FTIR. We studied the effect of changes in the proportion of casein, on viscosity, bio adhesiveness and mechanical properties, as this would be an important determinant of the ability of the formulation to stay in place after application. The viscosity at 37^{o}C of the formulation increased with increasing proportion of casein: 18% (w/v) P407 with 5 and 10% casein were 1.27 and 2.84 times more viscous respectively than P407 alone. At 25% P407, addition of 10% casein increased viscosity 1.22-fold. The effect of casein addition on bio adhesion and mechanical properties was favorable to a final more adhesive topical formulation which aims spreadability, prolonged contact at site. The use of casein slowed down the drug release of bupivicaine hydrochloride (amphiphilic) and sulforhodamine B (hydrophilic) in PBS at 37^{o}C. All the formulations were tested in rats for 6 h skin contact and none of them showed signs of irritation nor inflammation, demonstrating its potential to be tested and used in humans. Polycaprolactone nanoparticles showed a high encapsulation efficiency (98,81%) of terbinafine hydrochloride, a good polydispersity index and did not interfere in the metabolic activity of keratinocytes. They were added to the P407-casein hydrogel in different orders and concentrations. The nanoparticle's addition to the hydrogel significantly influenced gelation temperature, the viscoelastic properties and provided stable gels at the two preparation methods. Moreover, the nanoparticle addition order leads to different rheological behavior for the same nanoparticle concentration. Hydrogels driven by the addition of readily available proteins and polymeric nanoparticles have potential as translatable controlled drug release systems. The desired properties for a topical product, rheology and drug release, may be in the innovative mixture of already known components and their forms. These improvements may have an impact on patient compliance, particularly with topical therapies that may last for months and require many applications per

day. However, it appears that there is still "plenty of room at the bottom" to allow continued development of drug delivery systems for decades to come.

Keywords: Drug Delivery Systems, Poloxamer 407, Casein, modified release, topical formulations, nanoparticles, polycaprolactone, hydrogel, rheology, mixture.

SUMÁRIO

1. INTRODUÇÃO

O presente trabalho encontra-se dividido em capítulos redigidos sob forma de artigos, a saber:

- **CAPÍTULO I:** Artigo de revisão – Bio-polymeric delivery systems for modified-release
- **CAPÍTULO II:** Artigo de revisão - Nanotechnology as a Tool to Overcome Macromolecules Delivery Issues
- **CAPÍTULO III:** Artigo de resultado - Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein
- **CAPÍTULO IV:** Artigo de resultado - Terbinafine nanohybrid: proposing a hydrogel carrying nanoparticles for topical release

Desta forma, determinadas informações que constam em um capítulo poderão se repetir nos demais, assim como na disussão e na conclusão. Alguns dados contidos nos artigos de revisão vão além do explicitado pelo título e resumo, já que são completos e robustos, tratando de temas relevantes para esta tese.

2. JUSTIFICATIVA

Um produto dermatológico tópico visa o fornecimento ativo às várias camadas da pele, a fim de tratar as perturbações cutâneas, sendo ela o principal alvo do medicamento. Essa via tem várias vantagens no tratamento dessas perturbações, uma vez que permite o acesso direto ao local, evita o metabolismo hepático de primeira passagem, não é invasiva e reduz a toxicidade sistémica (Havlickova e Friedrich, 2008; Yang *et al.*, 2017). O desenvolvimento de um sistema apropriado de entrega tópica de medicamentos depende principalmente da barreira específica que o medicamento tem de atravessar (Yang *et al.*, 2017) (Fig.1). Os componentes presentes na formulação podem permear por caminho intercelular (entre as células), caminho transcelular (através da célula) e caminho trans apendetal (através dos folículos e glândulas sudoríparas).

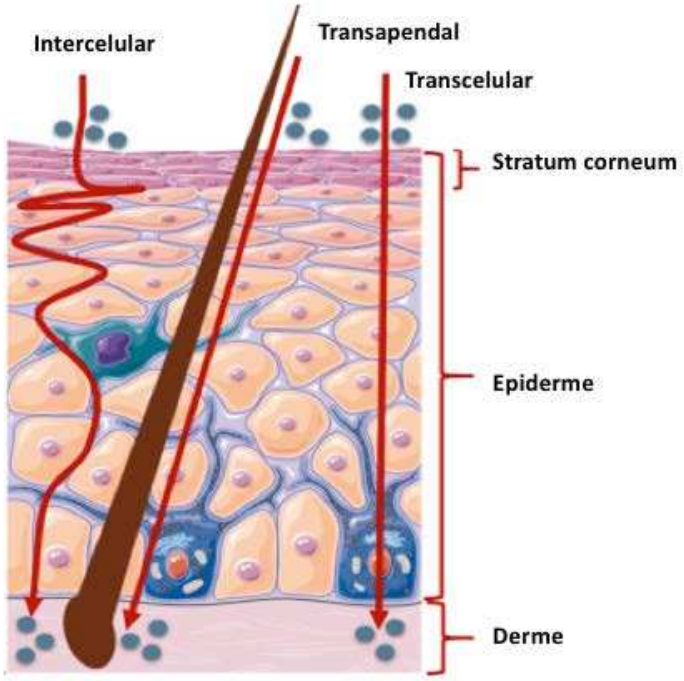


Figura 1 - Transporte de ativos através da pele. a) O stratum corneum (SC) é a camada mais externa da pele e a principal barreira para o transporte. As moléculas podem permear através das vias intercelular, transcelular e transapendal. b) A permeação através do SC é limitada principalmente pelos lipídios (argamassa), que preenchem os espaços entre os corneócitos (tijolos). c) Os lipídios que preenchem os espaços entre os corneócitos estão dispostos em uma estrutura de bílis, com caudas hidrofóbicas no interior e grupos de cabeças

polares no exterior. Os lipídios são principalmente colesterol (amarelo), ácidos graxos livres (azul), e ceramidas (verde). Adaptado de (Romanhole *et al.*, 2020).

Em termos de formulação, alguns atributos são desejáveis para manter um contato próximo e prolongado entre o fármaco e a pele afetada, ajudando na adesão do paciente e, por consequência, a eficácia clínica. Formulações adesivas, elásticas, oclusivas e duráveis são procuradas quando uma terapia tópica é necessária. As formas de dosagem tópica para aplicação na pele precisam ter propriedades mecânicas ótimas como: viscosidade aceitável (fácil remoção do recipiente), bioaderência (assegurar a retenção no local de aplicação), entrega e absorção adequadas do fármaco (Jones *et al.*, 1997; Hurler *et al.*, 2012; Alves *et al.*, 2018).

Visando terapia tópica com melhor administração de medicamentos, adesão do paciente e eficácia clínica, a busca de um sistema de administração de medicamentos com liberação modulada e propriedades mecânicas adequadas ao tratamento desejado é necessária.

3. OBJETIVOS

3.1. OBJETIVO GERAL

Estudar do comportamento físico-quimico, mecânico e de liberação de ativo de hidrogeis modificados por polímeros naturais e nanoestruturas.

3.2. OBJETIVOS ESPECÍFICOS

- Formular hidrogéis de poloxamer 407 + caseína em diferentes concentrações.
- Estudar comportamentento reológico e mecânico dos hidrogéis de poloxamer 407 + caseína em diferentes concentrações.
- Estudar biocompatibilidade dos hidrogéis de poloxamer 407 com caseína em diferentes concentrações.
- Caracterizar hidrogéis de poloxamer 407 com caseína em diferentes concentrações.
- Produzir nanopartículas de policaprolactona com terbinafina.
- Caracterizar morfológica e físico-quimicamenteas nanopartículas de PCL com terbinafina.
- Estudar citotoxicidade das nanopartículas de PCL com terbinafina
- Estudar a eficiência de encapsulação das nanopartículas de PCL com terbinafina
- Estudar a liberação de terbinafina das nanopartículas de PCL.
- Estudar a liberação das nanopartículas de PCL com terbinafina a partir do hidrogel de 18% P407 e 10% caseína.
- Estudar o comportamento reológico do hidrogel de 18% P407 e 10% caseína com nanopartículas de PCL.

4. EXECUÇÃO

Capitulo I – Bio-polymeric delivery systems for modified-release

Louise Lacalendola Tundisi; Rogério Aparecido Bataglioli; Marisa Masumi Beppu; Priscila Gava Mazzola

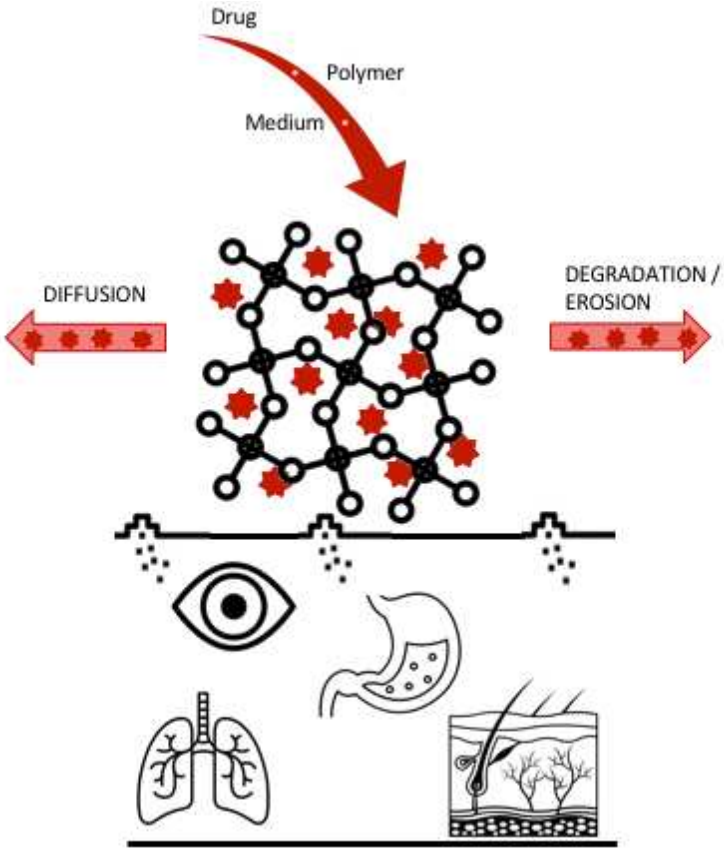
Submetido ao Journal of Controlled Release

ABSTRACT

The new generation of drug delivery systems has been designed to address challenges on drug efficiency, administration cycles, and unspecific targeting. Biopolymers have played an essential role in developing advanced therapies, granted to their macromolecular diversity, biocompatibility, bioresorbability, and tunable processing of polymeric materials. This article reviews the recent advances on (bio)polymeric drug delivery systems, emphasizing materials, dosage form, and trigger release mechanisms. The interplay between the macromolecular features (including molecular weight, cross-linking density, grafting) and the release mechanism is investigated in detail. Polymer swelling and erosion, and their role in drug release, are also analyzed for different material shapes and release media. Advances on new triggered release mechanisms, focusing on pH- and temperature-responsive materials, are also discussed. Finally, this review presents future perspectives on the drug delivery arena, highlighting the challenges and opportunities to use polymer-based materials to design targeted treatments.

Keywords: Drug Delivery Systems, Modified Release; Polymers; Biopolymers

GRAPHICAL ABSTRACT



1. Introduction

In order to maintain therapeutic levels of a drug for extended periods without administering several doses a day, drug release-controlled systems are necessary. Polymers as a material for modified drug release have undergone a near 45-years-long development [1, 2]. Not only synthetic but also natural polymers became a crucial part of the design of drug delivery devices. Due to their structural and functional versatility, polymers provide a great contribution to drug delivery systems, increasing the effectiveness of the drug, reducing therapy side effects, enhancing patient' compliance by minimizing repeated administrations, maintaining constant levels of the drug in the plasma, and also being able to protect the drug from degradation and clearance [3, 4]. Several mechanisms are used to control the release of the therapeutic agent, including changes in the pharmaceutical form of the drug delivery system (DDS), in the production method (modulation of the surface, cross-linking, layer-by-layer), in the polymeric system responsiveness to stimuli (smart-responsive polymer, already established), or all the above [5,6]. Besides pharmaceutical, other areas also take advantage of the tunable release properties of polymer-based materials to target specific applications in cosmetic, agricultural industries, and household products.

This review focuses on the recent advances of polymer-based materials for tuning system's drug release. We begin with an overview of the features of the polymeric materials and the main requirements for DDS design. The following section presents an overview of the main aspects of DDS design, including materials, shape, and release mechanisms, detailing several examples and recent advances involving synthetic and natural polymers and their combinations. The final section presents the outcome of the current technologies and highlights the future perspectives and challenges for the development of DDS with controlled release properties.

2. Bio(polymeric) material characteristics

When one thinks about polymeric DDS, an immediate thought comes to mind: what is the fate of this polymer? It is more convenient to have a polymer that does not need to be recovered after drug release. According to the Chengdu, China 2018 [7] conference, the biomaterial is defined as "a material designed to take a form that can direct the course of any therapeutic or diagnostic procedure through interactions with living systems." A biomaterial

would give this advantage as it can be either biodegraded or be directly excreted by the body after erosion.

Degradation is a chemical process, bond cleavage, while erosion is a physical process conditioned on dissolution and diffusion, depleting the material [2]. Biodegradable materials are unstable in living environments, having chemical functionalities like amide bonds, esters, and anhydride, being eliminated by natural metabolic pathways. Erosion can occur either faster on the surface or in bulk. For example, if the drug needs to be protected from water until all the polymer is gone, a surface erosion would be more beneficial, and a polymer that would have an erosion faster than water permeation is needed.

More than the body being able to dispose of the polymer, we have to think about biocompatibility as a whole. Biomaterials should also present the ability to perform the desired functions for medical therapy [7] to induce an appropriate host response in a specific application, and to interact with living systems without having any risk of injury, toxicity, or rejection by the immune system and undesirable or inappropriate local or systemic effects. When designing biocompatible materials, one must consider several factors, including inflammation, toxicity, interference on the regular physiological daily basis of the patient (i.e., lenses cannot impair patients' vision), among other aspects.

This bio (polymeric) material can be either of synthetic or natural origin, but they need to be biocompatible to be considered for DDS. Synthetic polymers tend to be more consistent from batch to batch, more predictable in their DDS, and easier to work. Natural polymers may vary in their molecular weight, and subsequently, their polydispersity among batches, affecting the drug release profile and reproductivity and can be mildly immunogenic. Alternatively, recombinant protein-based materials are an interesting alternative to reach the desired biocompatibility, while keeping low polydispersity and modularity for drug delivery applications [8].

Polymeric materials have structural versatility, which allows properties to be adapted to specific uses. In this context, by altering the chemical groups present in the macromolecular architecture of the chains and modifying bulk or surface properties, one may tailor specific interactions between the polymer and the biological environment [9]. These modulations can result in polymers with unique properties to formulate intelligent systems that can be applied to control drug release by changing how the material interacts with the environment. For example, when limited water permeation is desired, designing a polymer with

hydrophobic monomer units may limit the hydrolysis rate. It is important to acknowledge that the same polymer can be shaped into different formats (microparticles, nanoparticles, lenses, inserts, hydrogels, among others) and can be administered using various routes, from localized to systemic administration. Go into all the possible pharmaceutical formulations is beyond the scope of this review, which will focus on the essential principles for the selection and design of polymeric drug delivery systems.

3. Drug delivery systems

Designing an ideal DDS is reliant on the choice of the biomaterial. As described by [10], this choice addresses challenges related to both the polymer matrix and the drug diffusion itself. Starting with material, it is possible to design the perfect amount of crystalline phase, the porous size, or the degree of swelling. However, from a diffusion point of view, the physicochemical properties of the compound to be released are a crucial factor to reach the ideal release rate. The drug release process in polymeric systems takes place through one, or a combination, of the following mechanisms (not including triggered release): (i) drug diffusion through the polymer matrix (through a previously cross-linked mesh, pores, or by a swelled polymer layer), (ii) erosion of the polymer matrix (surface or bulk erosion) and (iii) degradation of the polymer matrix [11, 12]. The diffusion usually involves the polymer matrix swelling, which promotes its transition glassy to the rubbery state, facilitating the drug transport.

The mechanism governing drug transport through the swelled polymer matrix depends on both the rate of solvent penetrating and the drug leaving the matrix. As the water penetrates the polymer, the swelled region becomes rubbery, increasing polymer chains' mobility and creating a moving boundary between the swelled and the glassy regions inside the polymer matrix. Systems that present a fast water penetration rate relative to the drug release rate swells without immediately releasing the drug. These systems are described as diffusion controlled. Cases in which the drug diffuses out of a polymer region as soon as it swells, meaning the water penetration rate limits the drug release process, are called chain relaxation controlled systems [13]. Release mechanisms are determined by fitting the drug release data to deterministic models, such as Higuchi [14] and Ritger and Peppas [15] models, and assessing the parameters retrieved from them. For example, the traditional Ritger-Peppas, or power-law model, classifies the release mechanism according to the value of the diffusional exponent into diffusion-controlled ($n = 0.5$), both diffusion- and chain relaxation-controlled ($0.5 < n < 1$),

chain relaxation-controlled (n=1), and super case II (n>1). Other models such as Peppas and Sahlin model [16] and the phenomenological Berens and Hopfenberg model [17] also enable decoupling of each mechanism's contribution to the drug delivery process.

All these mechanisms, as cited above, depend on the polymer and the drug. We would add a third situation that calls for attention, which will be in imminent contact between the polymer and the release medium. This interaction relies on the administration route, which in turn defines if the DDS will be in contact with the skin, the gastric fluid, mucosa, eye tear, epidermis, eardrum, among others. Different routes present different retention times, pH conditions, temperatures, and other relevant factors. For example, if a DDS is applied to the skin, it will not have a swollen mechanism for drug release, and pH would be lower than the physiological.

The polymer systems discussed here were based on the experience of the authors and some of the most used materials for DDS.

3.1. Materials

3.1.1. Alginate (ALG)

Alginate is a widely abundant anionic polysaccharide obtained from brown seaweed by alkaline extraction [18], with suitable properties for biomedical applications, including biocompatibility, low toxicity, mucoadhesion, and ease hydrogel-formation [19]. From a macromolecular standpoint, alginate comprises a family of linear copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues distributed over the polymer chain as blocks containing only-G, only-M, or a mixture of both monomers (Figure 1). Several factors, including the M/G ratio, the sequence, and the molecular weight, affect the overall properties of the alginate, including its gelation and mechanical performance [20].

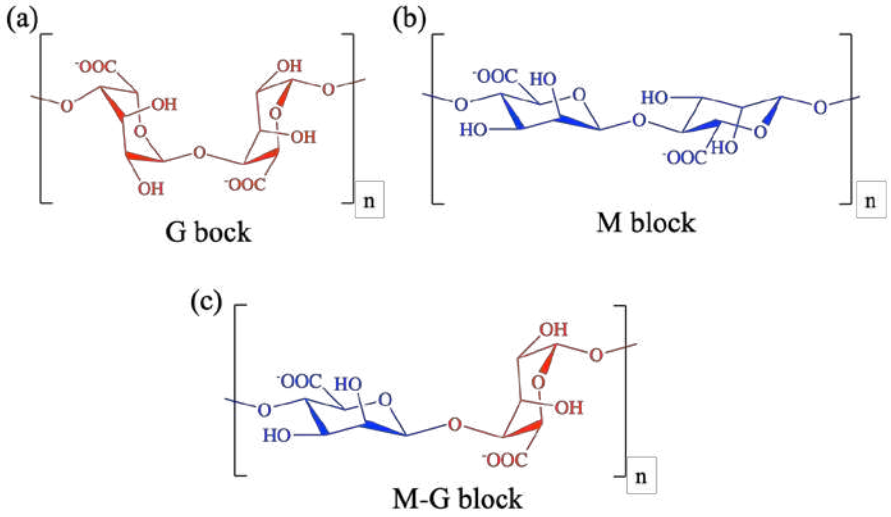


Figure 1. Schematic representation of (a) guluronic C- block, (b) mannuronic M-block, and (c) alternating G-M block present in alginate macromolecule.

Due to similarity to extracellular matrix components [21], alginate biomaterials are widely explored as hydrogels for biomedical applications, from cell scaffolding to the delivery of therapeutic agents. Ionic cross-linking is the most traditional method to form alginate hydrogels. In this method, ionic species, such as divalent cations (usually, Ca2+), are mixed with the polymer aqueous solution, leading to gel formation due to the coordination of G monomers with different polymeric chains according to an egg-box cross-linking model [22]. Although simple, this method enables little control on the gelation rate and gel homogeneity. The release of the divalent cations is another drawback, leading to gel dissolution and poor stability under physiological conditions [23]. Alternative gelation methods have been designed to create alginate-based hydrogels in combination with other polymers. Literature reports alginate hydrogels with several covalent cross-linkers, including bi-functionalized poly(ethylene glycol)-diamines (PEG), which allows the control of the elastic modulus and the gel swelling rate based on the weight fraction of PEG [24, 25]. Thermal-induced gelation of alginate and poly(N-isopropylacrylamide) mixtures [26] and cell-induced shear-thinning hydrogels via arginine-glycine-aspartate (RGD)-modified alginate and cell receptors cross-linking [27] have also emerged as alternatives for alginate hydrogel formation.

Alginate biomaterials have been explored in several formats (beads, microspheres, microcapsules, and tablets) to deliver therapeutic agents, including small molecules, proteins, and genes. One of the most exciting applications of hydrogels involves the in-situ hydrogel formation for ocular and oral drug delivery as a strategy to overcome the poor availability or

superior degradation of drugs delivered in solution. The drug release mechanism in alginate hydrogels varies depending on the alginate bioconjugation [28] the interactions between the polymer and drug molecules [29] and the release media [30]. For example, Maiti and co-workers describe the fast, diffusion-controlled release of the anti-inflammatory flurbiprofen from nano-pored-sized alginate hydrogel beads formed by Ca2+ ionic cross-linking [31]. The authors also reported the prolonged drug release profile from calcium-alginate beads partially oxidized with covalent agent adipic acid dihydrazide, limiting the polymer matrix swelling. Sriamornska and co-workers highlighted the role of swelling and erosion on the rate and mechanisms that control the drug release from alginate tablets [30]. Under acidic pH, the authors describe the non-Fickian, or both diffusion- and erosion-controlled, release mechanism of the antibiotic metronidazole from alginate tablets, according to the Korsmeyer-Peppas data fitting model. Neutral pH conditions (phosphate buffer, pH 6.8), on the other hand, favor the time-independent, zero-order release associated with the more extensive swelling of the polymer matrix under neutral conditions. Alternative methods have been developed to improve alginate hydrogels' mechanical and resistance properties by depositing a macromolecular protective shell over the gel hydrogel particle. This method involves the complexation of polycationic molecules, such as chitosan, over the alginate core hydrogel, which may be later ionically [32] or covalently [33] cross-linked, to limit hydrogel degradation and to promote the sustained release of drug molecules loaded into the polymer shell.

3.1.2. Bacterial nanocellulose (BNC)

Bacterial nanocellulose (BNC) is a natural polymer excreted as exopolysaccharide (as a pure component of bacteria’s biofilm) mainly by Gluconacetobacter xylinus, among other bacteria in the genera Gluconacetobacter, Agrobacterium Rhizobium, Pseudomonas, Sarcina, and Acetobacter. BNC is a sustainable and renewable biopolymer that has characteristics that differentiate it from traditional materials. This polymer is also known for its biocompatibility, biodegradability, low toxicity, and its morphology, as the cellulose chains in disordered (amorphous) regions contribute to the flexibility and plasticity, whereas the ordered (crystalline) regions contribute to the stiffness and elasticity of the material [34].

BNC has a similar chemical structure as plant cellulose, stabilized by intra- and interfibrillar hydrogen bonds. This polymer is synthesized in a pure form, and it does not require intensive processing to remove unwanted impurities or contaminants such as lignin, pectin, and

hemicellulose, unlike plant celluloses. During BNC biosynthesis, glucose chains are produced inside the bacterial body and extracted through small pores present in the cell envelope. By combining the glucose chains, microfibers are formed and aggregated as ribbons (nanofibers). These ribbons subsequently generate a network-like structure with cellulose fibers (CF), which have a diameter of 20-100 nm and different nanofiber networks, giving them a large surface area per unit mass. This property, combined with the hydrophilic nature of BNC, makes this polymer highly capable of absorbing water extending the network facilitating the diffusion through the matrix. Moreover, CF formation occurs at the interface to the air, and it can be molded into three-dimensional structures [34].

The scope of BNC applications can be further expanded through its association with bioactive molecules due to the BNC 3D structure. For example, incorporating antimicrobial agents into BNC membranes has been shown to produce an active packaging system [35]. As a DDS, BNC can be used in many formulations of nanoparticles, hydrogels, tablets, to name a few [36]. For example, BNC is used to control the release of bromelain, a protease that has anti-inflammatory and antimicrobial activity. Aiming for wound-healing application, BNC has mucoadhesive properties and presents a 9-fold increase in bromelain antimicrobial activity [37].

Nanostructured BNC patches were developed targeting treatment for aphthous stomatitis. BNC patches with (i) diclofenac (DCF, anti-inflammatory) and (ii) combining DCF with hyaluronic acid (DCF/HA, tissue regeneration claim, and physical barrier) were prepared. BCN/DCF/HA was planned for a dual effect, adhesion and anti-inflammatory, DDS. When the drug release is compared, BNC/DCF patch showed a slower release rate than BNC/DCF/HA, about 2-fold in 8h. These patches in contact with simulated salivary fluid presented diffusion and swelling, controlling the drug release mechanism [38].

3.1.3. Casein

Casein, a protein from bovine milk, is highly stable, non-toxic, and biodegradable. Casein is a natural polymeric surfactant and its micelles, formed in a hydrated state, are heat-stable [39]. These micelles are spontaneously formed and are held together mostly by hydrophobic interactions, and calcium-phosphate nanoclusters bound to phosphorylated serine residues of the casein side chains. They are nearly spherical, with a hydrophobic core and a hydrophilic kappa casein “hairy” layer. This layer stabilizes the micelle through intermicellar

electrostatic and steric repulsion [40](Figure 2). Casein has a strong UV absorbance, around 200-300 nm, and could serve as a shield against radiation, protecting sensitive therapeutics with no need for antioxidants. In addition, casein shows an open tertiary structure, making it easily accessible for proteolytic cleavage, making this protein an excellent smart-responsive biopolymer for stomach drug delivery [41]

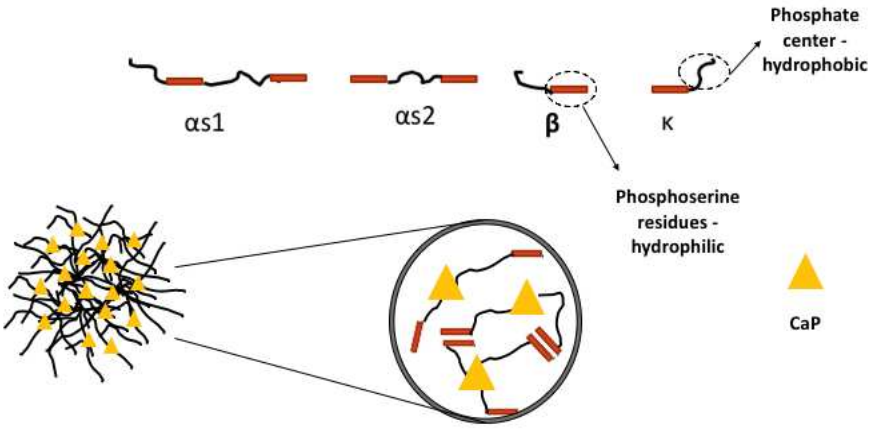


Figure 2. Schematic representation of casein structure and micelle formation. Casein micelle is composed of four phosphoproteins: αs1-casein, αs2-casein, β-casein, and κ-casein, at a molar ratio of about 4:1:4:1.3. CaP: Calcium Phosphate.

Casein micelles (CM) are natural nano-delivery systems. Having DDS based on natural food proteins bear a lot of potential for being biocompatible and biodegradable [40, 42]. CM application as a drug delivery carrier can either use natural casein or re-assembling casein in micelles [41, 42]. Natural CM can incorporate hydrophobic molecules by pH variation. When pH rises, CM expands due to electrostatic repulsion between protein monomers, making the core more accessible to hydrophobic molecules to interact. This change in the micelle structure is reversible by lowering the pH, and as the structure becomes smaller and narrower, drug molecules are entrapped [41].

CM was used to carry curcumin to cancer cells. A complex casein-curcumin was formed by hydrophobic interaction and showed the same cytotoxicity as curcumin by itself [40]. Also, Elzogby and his group developed a dual-targeted CM for hepatocellular carcinoma carrying two phytochemicals for cancer therapy. They combined multi-target therapy with the synergy of the two active compounds [43]. Casein hydrogels are formed by the cross-linking method, and they can change their mechanical and drug release properties, affecting the diffuse rate of the compound incorporated in this hydrogel. When combined with other polymers, casein may promote the formation of hydrogels with superior adhesive properties through

physicochemical interactions, including hydrogen bonds, hydrophobic interactions, metal complexation, and electrostatic effects, promoting slower drug release rates [44, 45].

3.1.4. Chitosan (CHI)

Chitosan is a random linear copolymer composed of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine residues (Figure 3). This polysaccharide is derived from chitin, the second most abundant biopolymer [46], present in the exoskeleton of crustaceans and insects. Chitosan is usually obtained by the deacetylation of chitin from crab and shrimp shells, leading to the formation of primary amine groups (pKa ~ 6.3) that confer solubility to chitosan under acidic conditions [47].

In addition to biocompatible [48] and antimicrobial properties [49-51], chitosan present features that are of biotechnological interest for the preparation of anticoagulant devices [52], incorporation in bone and dressing implants [53], and preparation of devices for the controlled release of drugs [54].

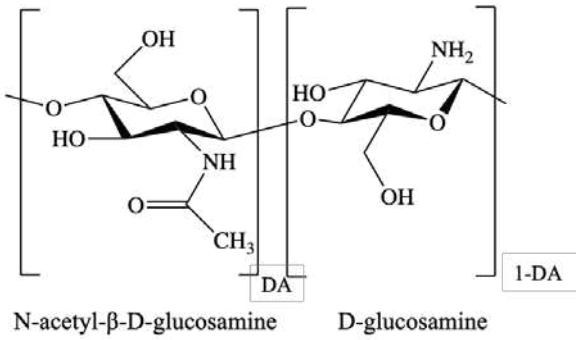


Figure 3. Schematic representation of chitosan macromolecular structure composed of N-acetyl-β-d-glucosamine and D-glucosamine residues. DA represents the degree of acetylation of chitosan, which is usually below 0.4.

Over the last decades, chitosan-based materials have been prepared over different geometries, including membranes, films, tablets, capsules, nanoparticles, microspheres, sponges, fibers, and composites. Due to its cationic character, exclusive among polysaccharides, and the reactivity of amino and hydroxyl groups, chitosan has also been explored in the formation of hydrogels based on physical and chemical cross-linking methods. The network gel may be formed by various physical interactions, including the multivalent interaction of chitosan with small anionic molecules (such as tripolyphosphate), the formation of polyelectrolyte complexes with polyanions, and secondary bonding formation with non-ionic

molecules, and hydrophobic associations (those present in thermo-responsive hydrogels). Agnihotri and co-workers mention two requirements to the formation of physical networks: (i) stronger inter-chain interactions to form semi-permanent nodes, and (ii) the formation of a polymeric matrix that enables the presence of water molecules inside the network [11]. Chemically cross-linked methods are explored as an alternative to overcome limitations on the control of the physical properties and in-vivo performance of physically-cross-linked chitosan hydrogels. Alternative methods have been used to form hydrogels, including small cross-linker agents, secondary polymerization enzyme-, and light-induced ligands to form irreversible bonds between intra- and inter-molecular chitosan chain segments. This method enables a tight control on the cross-linking density through the reaction parameters, ultimately leading to the formation of chitosan hydrogels' predictable properties.

Chitosan nanoparticles have been widely used as drug delivery carriers. The nanoparticles can be produced using various techniques, such as atomization and drying [55], coacervation/ precipitation [56], and ionotropic gelation [57-60]. Due to its relatively mild conditions from the physicochemical point of view, the ionotropic gelation technique sets it apart from the others for chitosan drug encapsulation. In this technique, the formation of nanoparticles occurs by dripping the chitosan solution into the solution of a cross-linking agent (such as tripolyphosphate), or vice versa, in which the process of cross-linking the polymer chains results in the formation of nanoparticles [61-63]. Studies also report chitosan potential for the production of micro and nanoparticles and encapsulation of various antitumor drugs, including doxorubicin [64, 65] 5-fluorouracil [66] and paclitaxel [67], among others.

The release mechanism in chitosan-based materials also depends upon aspects related to the polymeric matrix (including molecular weight, cross-linking density, particle size, and morphology), the therapeutic molecule (charge, solubility, and molecular weight), and the release medium. Several studies report the release by the diffusion of the drug through the swelled polymer matrix, also describing the occurrence of the anomalous mechanism (both diffusion- and polymer chain relaxation-controlled) for drug delivery [68-72]. For example, Delmar and Bianco-Peled report the prolonged release of the Nile red and curcumin from cross-linked chitosan hydrogels embedded with microemulsions containing these hydrophobic molecules, indicating the predominance of anomalous release mechanism for hydrogels cross-linked at pH 4.0, and the swelling controlled mechanism at pH 5.5 [69]. Soares, Sousa, Silva, Ferreira, Novo and Borges [70] describe the encapsulation of doxorubicin into both raw and quaternized chitosan nanoparticles. The authors report the burst release within the first hour,

typical for the release of hydrophilic molecules from polymeric systems surface, followed by the slow release rate with both diffusion and chain relaxation mechanisms Sedyakina and co-workers also investigated the encapsulation and release of bovine serum albumin (BSA), as a model protein drug, in chitosan microparticles prepared under various degrees of cross-linking with citric acid [72]. Compared to the non-cross-linked matrix, the ionic cross-linking reaction favored higher drug encapsulation efficiency and reduced the drug burst release when carried out at moderate levels, does not restricting the adsorption of BSA only at the surface of the microparticle. Studies also describe the erosion contribution on the drug release from chitosan-based materials [71, 73]. For example, Wen and co-workers studied the release of BSA from ionically-cross-linked fiber mat matrices, prepared via electrospinning deposition of alginate-coated chitosan nanofibers. Based on the Ritger-Peppas data fitting parameters, the authors describe the change from anomalous to the super case II (where the polymer matrix erosion is dominant for the drug release) with the increase in the solution pH from 6.8 to 7.4 in in-vitro BSA release experiments [71].

3.1.5. Hyaluronic acid (HA)

Hyaluronic acid (hyaluronan, hyaluronate, or HA) is an unbranched, non-sulfated glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid (1→3)β and N-acetyl-D-glucosamine (1→4) β units (Figure 4). This anionic polysaccharide is mostly found in the extracellular matrix (ECM) of connective tissues [74], in bacteria (particularly from Streptococci strain, for commercial interest), and larger amounts in rooster combs [75]. HA molecular weight ranges from 100,000 to 8,000,000 Da, depending on its source [76], which confers to this polysaccharide unique viscoelastic and rheological properties suitable for biomedical implants. HA-based materials have been explored for years for biomedical use, including cartilage repair [77, 78], and ophthalmologic surgery [79, 80]. Studies also report the biocompatibility of cross-linked-modified HA-biomaterials in-vivo experiments [81, 82].

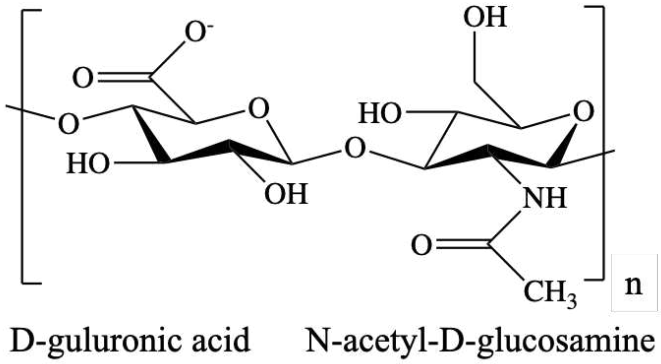


Figure 4. Schematic representation of hyaluronic acid macromolecule with D-glucuronic acid (1→3)β N-acetyl-D-glucosamine (1→4) β groups.

HA by itself is unable to form hydrogels, demanding a wide variety of covalent cross-linking reactions with HA functional groups (glucuronic carboxylic acid, primary and secondary carboxyl groups, and N-acetyl groups) to form a stable polymer network [83]. Burdick and Prestwich review various chemical reactions used for HA modification. The authors highlight various bioconjugation reactions for HA modification, including etherification, bis-epoxide cross-linking, esterification, and divinyl sulfone cross-linking reactions for hydroxyl groups functionalization [76]. The resulting cross-linked material is stable over a wide range of pH values and is more resistant under the presence of hyaluronidase.

Several studies report the application of cross-linked HA-hydrogels to deliver small drugs and proteins for therapeutic purposes [84, 85]. Lou and co-workers report the derivatization of HA with adipic dihydrazide, followed by cross-linking with PEG for hydrogel formation [86]. The resulting material swells instantly in contact with the aqueous medium, decreasing drug release rate for more hydrophobic anti-inflammatory and antibacterial drugs in neutral pH values. Alternative systems also explore the covalent attachment of drugs in thiol-modified HA hydrogels, cross-linked via PEG diacrylate. For example, Li and co-workers describe the design of cross-linked hydrogels with covalent attachment of anti-proliferative drug mitomycin C [87]. This chemotherapeutic molecule is slowly released by hydrolysis of the labile chemical bonds formed between the hydrogel and the mitomycin C, enabling this material for bladder cancer treatment.

The literature also reports injectable HA-based systems, which combine the facile injectable administration of liquid mixtures containing the polymeric matrix and the drug, followed by the in-situ gelling formation. Choh and co-workers report the production of injectable hydrogels via thiol-disulfide exchange reactions between HA pyridyl disulfide and

cross-linker-modified PEG-dithiol, presenting a surface-controlled degradation rate in the presence of hyaluronidase and erosion-controlled degradation mechanism in the presence of disulfide reducing agents [88]. Also, the release of encapsulated stromal cell-derived factor-1α was mostly diffusion-controlled, considering the lack of binding interaction between the protein and the gel and the small protein size (~8 kDa) relative to the large mesh size the hydrogel. Zhang and co-workers describe the release of BSA from modified HA hydrogels prepared under different concentrations (3%, 5%, and 7%), indicating the protein release via the diffusion through the gel layers and gel matrix degradation [89]. Additional studies also report enzymatic degradation as a strategy for drug delivery, exploring the design of HA-based hydrogels cross-linked with metalloprotease degradable proteins for the controlled release of cell-binding peptides for tissue regeneration [90][91, 92].

3.1.6. Hydroxypropyl methylcellulose (HPMC)

HPMC is a cellulose mixed ether resulting from the alkaline substitution of hydroxyl groups by methyl and hydroxypropyl groups (Figure 5). The hydrophilic degree of the HPMC is dictated by both the number of methyl (degree of substitution, DS) or hydroxypropyl ether groups (molar substitution, MS) added to the cellulose molecule. This polymer is water-soluble and transparent (Tundisi, L. et al., 2021), also presenting biocompatible and biodegradable features relevant to biomedical applications [93]. Unlike cellulose itself, the derivatization into HPMC bestows thermoplastic behavior to the material, allowing it to be extruded with the possibility of producing implants.

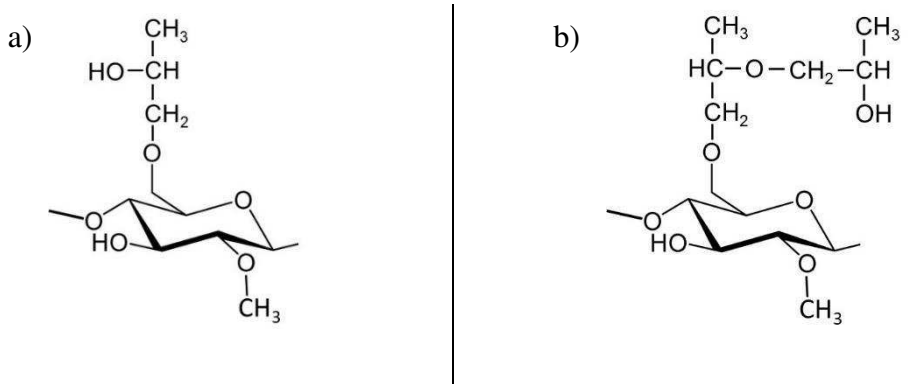


Figure 5. Representation of chemical structure of cellulose repeated units substituted with (a) DS = 1.0 and MS = 1.0 and (b) DS = 1.0 and MS = 2.0. Adapted from Tundisi, L. 2021.

Drug release from the HPMC matrix depends on the nature of the drug. When HPMC gets in contact with water or any other fluid, its upper layer gets wet, and the HPMC chains start to hydrate. This hydration rate differs on HPMC molecular weight, DS, and MS. This hydration starts forming a gel layer, leading to a gradient gel layer as it depends on diffusion. Soluble or partially soluble drugs diffuse through the gel, but insoluble drugs tend to remain aggregated until the polymer is dissolved and eroded [94].

HPMC is widely used in the pharmaceutical industry as a tablet or pallets coating agent. This polymer application usually aims for a site-controlled delivery or even a gastrointestinal (GI) pH-/time-related performance as it can have physicochemical modulations accordingly to the desired characteristic [95, 96]. Also, the interplay between rheological properties and transparency makes HPMC a unique biopolymer for ophthalmic formulations (eye drops, gels, inserts, and films [94, 97, 98]. Vancomycin-HPMC microparticles were embedded in chitosan/ glycerophosphate (GP) hydrogel to local treatment of osteomyelitis. Compared to the hydrogel on its own, the Vancomycin/HPMCs-CHI/GP decreased vancomycin's total release by 20% at 8 hours and 85% at 160 hours. This DDS presented two similar release mechanisms combined due to the formation of a double diffusion barrier. This design demands first crossing HPMC microparticle and after the chitosan hydrogel for drug release, slowing the overall vancomycin release [99].

3.1.7. Poloxamer (P407)

P407 is a well-known thermo-responsive copolymer composed of two blocks of hydrophilic ethylene oxide monomers (EO), flanking one block composed of hydrophobic propylene oxide monomers (PO) (Figure 6). Polyoxypropylene molecular mass of 4000g/mol and a 70% polyoxyethylene content. This material presents a solid-gel transition attributed to the temperature increase, leading to the dehydration of hydrophobic PO blocks [44]. The sol-gel transition temperature (Tsol-gel) is concentration-dependent and decreases as the polymer concentration increases. There is a point that the P407 concentration is so low that the sol-gel transition does not occur. The P407 is widely used to deliver peptides, small molecules, and biological molecules, mainly when controlled release is required. It is wanted for water-insoluble molecules due to its hydrophobic PO core, also capable of protecting compounds from outside factors inside the micelle [100].

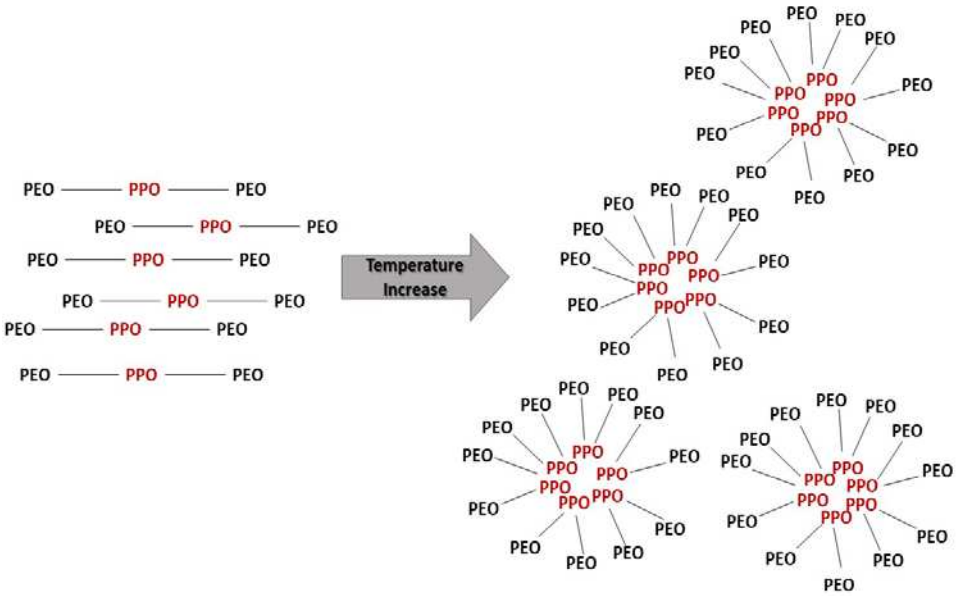


Figure 6. Schematic representation of the temperature-driven micelle formation on aqueous P407 solution. The increase in temperature favors the dehydration of the hydrophobic PO block, which promotes the gelation process.

The drug release mechanism from P407 hydrogels may be erosion-controlled, diffusion-controlled, or both. Due to its gel formation in contact with water, P407 systems may erode faster than the drug diffuses. By having a hydrophobic core, one may expect that hydrophobic drugs would diffuse slower in P407 micelles than the hydrophilic drug molecules. The hydrogel viscosity also affects the drug diffusion, which is inherent to the temperature and the polymer concentration. The higher the temperature and concentration, the higher the viscosity, and the slower the drug diffusion process [44]. P407 is an example of thermo-

responsive material with appropriate physic-mechanical and biocompatible properties that can control drug delivery, increase skin contact time, decrease local irritation, and improve patient compliance. Also, proteins and copolymerization can be added to these hydrogels to increase their mechanical and adhesive properties [44, 45, 101, 102].

3.1.8. Polycaprolactone (PCL)

Biodegradable polymers in drug delivery are highly relevant because they are biocompatible and decompose into non-toxic natural products. PCL (Figure 7) is a synthetic, biodegradable, hydrophobic, semicrystalline, and permeable polymer, which can be obtained either by ring-opening polymerization of ε-caprolactone or via ring-opening polymerization of 2-methylene-3-dioxepane. This polymer presents a glass transition temperature between 60-65 °C and a melting point of 56-65 °C [103], facilitating its processing. It has high mechanical strength, and sometimes it is blended or functionalized with other polymers to improve cellular interaction and mechanical properties, as crack resistance and adhesion, improving the physical stability of the scaffold [104-106].

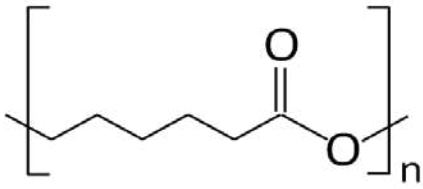


Figure 7. Representation of PCL molecule

As part of the biodegradable polymer group as PLA, and PGA, the PCL release mechanism is by degradation or sometimes diffusion. PCL permeability depends on the drug-polymer interaction. Body enzymes cannot degrade PCL as it presents ester links between the monomers. PCL hydrolysis degradation is autocatalyzed by the released carboxylic acids. Its hydrolytic degradation can be tunned relative to degradation time, which can vary from seconds to years, depending on the molecular weight, the degree of crystallinity of the polymer, and the medium [103].

PCL offers suitable alternatives for controlled drug delivery and tissue engineering. This polymer has been used to develop a biodegradable soft scaffold using 3D printing technology, taking advantage of its mechanical strength, flexibility, and low melting point. For example, PCL-based, implantable delivery systems loaded with curcumin were shaped and structured to fit the tumor-resected site of glioblastoma multiforme, a type of brain cancer [107]. PCL scaffolds also presented a slow degradation rate, around eight months. A prothesis that fulfills a structural purpose and releases drugs concurrently to treat the desired area can show that delivery systems can act beyond drug release.

PCL can also be used as a limiting membrane for drug-loaded nanofibers, aiming to reduce the dose frequency by a long-term DDS. Using PCL membrane, drug delivery is prolonged and mechanical properties are enhanced [108]. Micelles are another alternative for PCL use in drug delivery. PCL linked with PEG formed a polymeric micelle to study a more efficient and safe way to treat proliferative vitreoretinopathy. [109, 110] Ophthalmic drug delivery systems are still behind on the machinery in DDS development. PEG-b-PCL micelles decreased the cytotoxicity of dasatinib, increased its solubility by 475-fold, and provided a sustained drug release profile. Also, PCL nanoparticles appear to increase the residence of the drug on the skin by reducing permeation to deeper layers. This technology is used for sunscreen preparations, photostability, and a more local treatment avoids the drug from reaching further layers [111].

3.1.9. Poly(N-isopropylacrylamide) (PNIPAAm)

PNIPAAm is a thermo-responsive polymer, presenting different structural arrangements when the system is above or below its lower critical solution temperature (LCST). Above 32 oC, hydrophobic polymeric interactions become dominant, turning the gel opaque, whereas hydrogen bonds are formed below this temperature. Once reaching a temperature above LCST, a hydrophilic-to-hydrophobic phase transition occurs, releasing the compound once trapped in the gel matrix [4]. To tune some of its properties, PNIPAAm can be blended with other polymers or copolymerized. LCST of PNIPAAm can also be tuned by changing the hydrophilic/hydrophobic balance in the polymer chain [112-114]. The thermo-responsive

properties of PNIPAAm make it suitable for DDS, usually presenting a diffusion-dependent drug release mechanism.

Several drug delivery PNIPAAm blended with other polymers to tune the drug release profile. PNIPAAm was blended with PEG and PVA forming a hydrogel to study bromelain's release as a potential noninvasive burn treatment and topical inflammation. Both hydrogels modified the proteins release, especially when related to enzymatic activity. PVA hydrogels showed more biocompatibility than PEG hydrogels [113]. PNIPAAm-co-AAm hydrogel was studied as DDS, also exploring bromelain loading. Mucoadhesion, rheological behavior, and drug release were analyzed. Besides presenting mechanical properties compatible with biological conditions, this blend showed a controlled release after an initial burst. The optimal enzymatic activity release rate was 60 minutes at 25 oC and 37 oC [114].

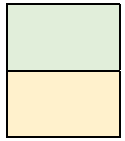
Because of its thermosensitive properties and its LCST being close to body temperature, PNIPAAm is wanted for at-site sol-gel transition. Ophthalmic drug delivery is always a challenge as it rapidly eliminates drugs release in the cornea by nasolacrimal drainage. Having a DDS that would allow inner contact with the treatment site for an extended time without impairing the eye vision can enhance the eye drug delivery efficacy. PNIPAAm-graft-PAAc formed a thin film when in contact with the corneal surface and showed a sustained release profile [115].

3.1.10. Polymer combinations

The combination of polymers or other additives is a refined strategy to tune and control DDS properties towards the desired application. Oftentimes, a polymer does not have all the characteristics needed, but along with other materials, it can present either synergetic or somatic possibilities for the delivery. Here, one must keep in mind that (i) materials improvement varies depending on the desired properties, and (ii) the increase in one desired feature may be detrimental to another one (biomechanical, thermo-responsive, adhesivity). For example, if the design of a more flexible polymer is required, increasing its stiffness will not be seen as an improvement. Table 1 presents a set of polymer combinations that have been recently reported to improve the properties of DDS.

Table 1. Advances on polymer combination explored for drug delivery applications.

	PURE	PCL	POLOXAMER 407	HPMC	ALGINATE	CHITOSAN	CASEIN	PNIPAAm	BNC	HA
PCL	Implant, nanofibers [1, 2]									
POLOXAMER 407	Hydrogels [3]	Nanoparticles [4]								
HPMC	Hydrogels [5]	Micelles [6]	Hydrogels [7]							
ALGINATE	Nanoparticles [8], Tablets [9]	Nanofibers [10]	Hydrogels [11]	Mucoadhesive films [12]						
CHITOSAN	Nanoparticles [13], microspheres[14]	Electrospun-nanofibers [15]	Nanosponges [16]	Microparticles / hydrogels [17] nanoparticles [18]	Beads [19, 20], Nanofibers (Wen 2020)					
CASEIN	Micelles [21] Hydrogels [22]	Electrospun-nanofibers [23]	Hydrogels [24]	Polymer matrices [25]	Nanoparticles [26]	Microparticles [27]				
PNIPAAm	Hydrogels [28]	Nanofibers [29]Micelles [30]	Nanoparticles [31]	Hydrogels [32]	Hydrogels [33]	Hydrogels [34]	Hydrogel particles [35]			
BNC	Hydrogel [36]	Nanoparticles [37]	Micelles and hydrogels [38]	Capsules [39]	Hydrogel membranes [40]	Patches [41]	Hydrogels [42]	Hydrogels [43]		
HA	Microspheres [44]	Electrospun scafolds [45]	Hydrogels [46]	Film [47]	Contact lenses [48]	Mesoporous silica[49]	Nanoparticles [50]	Microgels [51]	Patches [52]	
OTHER COMBINATIONS		Nanoparticles [53]	Implants [54], hydrogel [55]	Inserts [56]films [57], Pellets [58]	Hydrogel [59]	Hydrogels [60]	Hydrogel [55]	Hydrogel [61-63]	Nanoparticles [64]; Films [65]	Micelles [66]



Describes hydrogel synthesis, lacking drug delivery data

Also presents ALG in hydrogen composition

3.2. Presentations

3.2.1. Polymeric Nanoparticles (NPs)

The first inkling of what would be the nanotechnology revolution was the presentation given by Richard Feynman in 1959, entitled “There is plenty room at the bottom,” pointing to the possibility to manipulate materials at the molecule and atom scale. This almost untapped potential could control physical and chemical properties, enabling the association and modulations of materials on a molecular level [116]. Engineering and manufacturing of the materials is nanotechnology.

The continued development of NPs has the potential to provide many benefits compared to conventional DDS and formulations. Nanomedicine presents several advantages: enhanced personalization and patient compliance, effective drug delivery and treatment, reducing potential side effects by limiting drug interaction to desirable sites, increased active concentration, and bioavailability of therapeutics that could decrease the administered drug dosage, among others. NPs are versatile platforms for drug delivery, as they can be made of many materials and by different fabrication methods. They can also be decorated to reach a specific target or even trigger its release.

In general, there are four traditional methods for producing polymeric NP: nanoprecipitation, emulsification/reverse salting-out, emulsification/solvent diffusion, and solvent evaporation. Most techniques require an organic phase to dissolve the polymer and an aqueous phase (where the polymer is insoluble) to either force or maintain the polymer in the lower energetic state. The products are usually obtained as aqueous colloidal suspensions either by filtration or evaporation of the organic solvent. These methodologies are reviewed in detail by [117]

The final formulation carrying the NPs must be carefully chosen. If in suspension, surfactants can be added to allow electrostatic and steric stabilization to maintain the morphology. One may also consider the possibility of drug release during shelf life. This aspect highlights the clear trade-off between shelf life and drug release rate from nanoparticles, limiting the formulations based on nanocarriers. Lyophilization brings many advantages related to stability [94, 118, 119].

Polymeric NPs show broad drug delivery applications when based on biocompatible and biodegradable polymers. They bring the ability of drug control release,

depending on the polymer degradation kinetics. When nanosized, the surface area increases substantially, resulting in a bulk and surface eroding with similar erosion kinetics and constant rate over the desired timescale. Polymeric NP appears to increase the drug's residence on the skin by reducing permeation to deeper layers [111, 120], avoiding systematic off-target delivery, and sticking to the local delivery. It also can accumulate in hair follicles and create a high-loaded drug depot, further diffusing into viable layers on the skin [120]. These nanocarriers came with the expectation to be a game-changer for the remaining uncovered aspects between a drug's function and its interaction with biological systems. Examples include the nervous system [121] immunologic system [122], gastro intestinal tract [123].

3.2.2. Hydrogel

The first hydrogel was synthesized by Wichterle and Lím in 1960 [124]. Since then, hydrogels have been extensively explored in the medical and pharmaceutical fields. Hydrogel is defined as a polymeric network with tridimensional configurations able to swell in an aqueous environment. This material has physical properties more similar to life tissues than any other synthetic biomaterials class, attributed to its significant water content, smooth and elastic consistency, and low interfacial tension facing water or biological fluids [125, 126]. Hydrogels have tunable physical properties that allow controlled drug release designed, also offering protection for labile drugs [127].

Hydrogels have been studied for a huge variety of biomedical and biological applications, such as separation, bio-sensors, artificial muscles, mechanical valves, supports, nanoparticles, and lenses for controlled drug release [128-130]. These systems can carry the drug or even nanostructures, forming "nanocomposite hydrogels" [131, 132]. Hydrogels can be administered by different routes (topical, transdermal, ophthalmic, intravaginal), and, particularly those indicated for pharmaceutical or biomedical development purposes, should have acceptable biodegradability and biocompatibility. They are versatile, enabling the controlled release of hydrophilic, hydrophobic, and macromolecular drugs [133].

Hydrogels can come in different shapes, according to the administration route desired: lenses, microneedles, films, nanoparticles, to name a few, allowing them to fulfill several biomedical needs. For example, microneedles are minimally invasive devices that can enhance transdermal drug and vaccine delivery. Hydrogel-forming microneedles are biocompatible, easy to sterilize, deliver more drugs in each application, increase the range of transdermal drug deliverable, and sustained release [134]. Another example involves soft

contact lenses made of silicone-based hydrogels coated with layer-by-layer films to achieve a local and controlled release of two drugs. Moxifloxacin hydrochloride (MXF) and diclofenac sodium were incorporated in the hydrogel, which afterward was coated with ALG, poly-l-lysine (PLL), and HA, and cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The hydrogel itself was used to allow a higher amount of MXF loading and sustained release, and the LbL coating acted as a barrier for DCF, also improving gel surface properties [135].

Hydrogels matrices can also be either inert or "smart," meaning they respond to environmental stimuli with a change in materials conformation. Materials that change their behavior due to one or more stimuli can be used to form smart hydrogels [4]. Not all responses are related to drug release; some can show a change in mechanical properties, which may affect the drug release but is not directly related to triggering the release.

3.2.3. Films

Polymeric films have attracted attention to control the drug dosage and the release profile in pharmaceutical formulations. Different processing strategies have been investigated to prepare drug-loaded films, including casting, hot-melting extrusion, electrospinning, and 3D printing to produce free-standing films.

The casting method is the simplest and most affordable strategy for film processing since this method does not require any sophisticated equipment for film preparation. The following steps are required for film casting: polymer solution preparation, deaeration, solution pouring into a mold, drying, cutting to the final dosage, and packaging [136]. This process requires particular attention to processing, including solution dispersion and rheology, bubble entrapment, film homogeneity, and residual solvent into the final material, to reach the desired performance and meet health and environmental criteria [137]. The solvent casting method has been explored for several clinical applications, including transdermal [138] and mucoadhesive [139], corneal [140], wound dressing membranes [141], and implants [142]. Riccio and co-workers investigated betamethasone and silver sulfadiazine release from nanocomposite chitosan/nanocellulose cast films for wound dressing. The authors also report the reduction in betamethasone release rate with increasing nanocellulose content, describing the increase in drug diffusion through the polymer matrix with polyethylene glycol concentration.

Pharmaceutical films have also been prepared through hot-melt extrusion as a strategy to provide adequate drug dosage and therapeutic stability. In this method, a blend containing the thermoplastic polymer, therapeutic agents, and processing materials (such as plasticizers and oxidants) is molten and conveyed through a die, a small orifice, to mold the material into different shapes, such as granules, tablets, pellets, and films [143]. Compared to traditional methods, hot-melt extrusion offers several advantages for pharmaceutical purposes, including continuous processing, reduced health and environmental concerns due to solvent elimination, and superior drug delivery efficiency. Among the disadvantages of this method, one may include the high energy demand of the extrusion process and the limitation of drug stability due to process heating, high shearing stress, and drug recrystallization during storage [144]. In one of the first papers in the field, Aitken-Nichol and co-workers use the hot-melt extrusion method to prepare polyacrylic films for topical drug delivery applications [145]. The authors described a slower initial release rate of lidocaine from extruded polyacrylic films loaded with 5% of the drug than films prepared by casting. Montenegro-Nicolini and Morales critically review the advances in the use of hot-melt extrusion to produce orodispersible films to promote a faster pharmacological response and increase patient convenience and compliance [146]. Several studies investigate the influence of the film composition on the physicochemical and drug delivery properties of ODFs. Ajinkya and co-workers recently described the role of HPC (polymer carrier), HPMC (drug release retardant), and PEG on hot-melt extruded film properties [147]. The release profile for the anti-asthmatic drug salbutamol sulfate presents a reduction in the release rate as the amount of HPMC in the polymer blend increases, indicating an anomalous drug release profile according to the Ritger-Peppas model. In a recent study, Speer and co-workers combined both the casting and hot-melt extrusion methods to create orodispersible films with prolonged drug release properties [148].

Over the last two decades, LbL emerged as a simple, low-cost, and easily scalable approach for multilayer film deposition for drug delivery. Kirkland and Iler initially proposed this method in the 1960s [149, 150], and it became popular mid-90s, with Decher's work involving the deposition of nanostructured films on substrates from alternating deposition of oppositely charged polyelectrolytes [151]. Traditionally, the multilayer film deposition occurs through the alternating immersion of a substrate in aqueous solutions of the materials of interest, with intermediate washing steps being used to remove the material not adhered to the surface. Alternative methods were developed later, including spin-coating [152] and spraying [153].

Despite the several studies describing electrostatic-driven LbL systems, other interactions have also been explored for film deposition. Borges and Mano report a list of interactions explored for LbL film assembly [154] including covalent bonds [155], hydrophobic [156], and biological specific interactions [157], illustrating the method's versatility. Compared to other methods, the LbL method has unique advantages for the production of drug delivery systems, including the amenable processing conditions, the film-forming properties through a wide range of complementary interactions, the hierarchical control of the film nanoarchitecture – enabling one to create devices with multidrug compartmentalization, and spatiotemporal drug release profiles [158]. The method used to load therapeutic molecules into the multilayer films also varies a lot, including electrostatic adsorption [159], covalent attachment, capillary condensation [160], deposition of drug layers [161], and the addition of compartmentalized system embedding into the multilayers [162]. The electrostatic interaction between the free ionic groups from the polyelectrolyte multilayers and the drug is one of the most traditional approaches for drug loading into multilayer films [159]. When dealing with weak polyelectrolyte (i.e., macromolecules bearing pH-dependent ionizable groups), one may control the structural and drug delivery properties of multilayer films according to the assembly conditions used (notably, the polyelectrolyte solution pH and the ionic strength) [159, 163]. For example, carboxymethylcellulose/chitosan films yield the formation of thinner, rougher multilayers at higher pH values and low ionic strength (pH 6.0 with no salt added vs. pH 4.0 with 100 mM NaCl), also leading to the highest model drug loading capacity at pH 6.0 [68]. In addition, nearly all the assembly conditions investigated indicated the drug release through the anomalous, or both diffusion- and chain-relaxation, release mechanism, similar to observed in other multilayer systems [164-166]. Other studies have also described the use of polyelectrolyte multilayers of hydrolytically degradable poly (β-amino esters) [167] and therapeutic macromolecules [161]. This approach has enabled the control of the drug release rate based on film assembly parameters and building blocks macromolecular properties [168] for targeted, multidrug delivery [169]. In the last decade, several studies have also explored the use of external stimuli (such as pH, ionic strength, temperature, light, among others) to trigger the drug release from multilayer films, creating smart-responsive multilayer films [170].

3.3. Triggered and targeted DDS

There are several mechanisms to actively control the release and specific drug distribution, such as modulation of the particle surface, modification in polymeric design, use of an established stimuli-responsive polymer [5] Polymer degradation can be modulated

according to the desired degradation time, ranging from hours to years, as required by the formulation [104]. With a greater understanding of diseases among biological systems physiology, the classical modified-release has evolved into biochemical changes at the site that can serve as a trigger for drug release. Biochemical changes through the delivery route (e.g., GI tract) are intrinsically related to the physical-chemical characteristics of the polymer chosen to guide the release control mechanism. The development of stimuli-responsive particles leads to increased treatment efficacy and decreased off-target effects [5]. It is considered triggered DDS if the response behavior after the trigger is related to the drug release.

The stimulus for drug release can be physical, chemical, or biochemical, causing structural changes in the particle (degradation, erosion, diffusion). This stimulus can be either endogenous, inherent to biochemical changes caused by pathology (pH, reactive oxygen species, temperature), or exogenous, coming from external manipulation (such as heat, light, ultrasound) (see Figure 8) [171, 172]. These stimuli can either control the behavior and properties of the DDS, which may also be responsive to more than one stimuli. This diversity of existing stimuli for drug release can be exploited in search of targeted drug delivery and control of release kinetics [5, 171]. When triggered, the drug may be released, or the carrier can change in a way that binds to a specific site and becomes targeted [173].

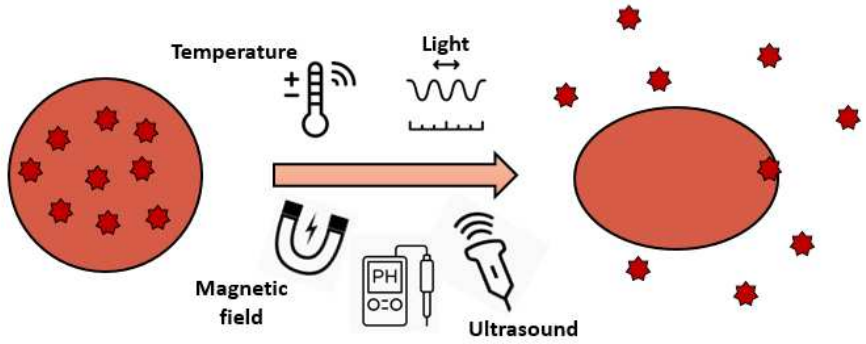


Figure 8. Exogenous stimuli for triggered drug release by temperature, ultrasound, magnetic field, pH, and light.

4. Future perspectives and conclusions

This review has provided a glimpse of the vast repertoire of polymers and designs of controlled drug delivery systems. As the struggle in this area is to deliver drugs that already

exist more efficiently, the inventive part is more about the polymer and less about the drug and the medium. If there is nothing to do about the drug, the interactions remain polymer-dependent. Aiming for a specific characteristic in the release mechanism, both the polymer chemistry and the polymer combinations can lead to advanced materials with unique properties that cannot be reached in single-component systems.

More than investing in polymer chemistry, nanocomposites is a promising field to explore. Polymer combination is included in nanocomposites, but not limited. Not only the kind of polymer, but its shape can be selected to achieve the target performance. Nanofibers, nanoparticles, nanowires… added to a DDS change their properties and be tuned for desired release mechanism and rheological characteristics. Nano-architectured films also stand out as a suitable alternative to create advanced, multi-component materials for drug delivery. Such versatility contributes to the design of multidrug systems with spatio-temporal controlled properties.

Polymers' chemistry, shapes and mixtures can be the pathway to achieve individual pharmacological needs with a DDS.

5. Author contributions

Louise Lacalendola Tundisi Conceptualization, Methodology, Validation, Writing – original draft, Project administration. Rogério Aparecido Bataglioli Conceptualization, Writing – original draft. Marisa Masumi Beppu Writing – review & editing, Supervision, Administration. Priscila Gava Mazzola Conceptualization, Resources, Writing – review & editing, Supervision, Administration, founding acquisition.

6. Declarations of competing interest

None

7. Acknowledgements

This study was supported in part by grants FAPESP 2017/10728-2 and 2018/05421-8 (to L.L.T.), FAPESP 2018/07707-6, FAPESP 2018/20560-4 (to R.A.B. and M.M.B.), and CNPQ (to P.G.M.). This study also was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001.

8. References

[1] R. Langer, J. Folkman, Polymers for the sustained release of proteins and other macromolecules, Nature, 263 (1976) 797-800.

[2] K.E. Uhrich, S.M. Cannizzaro, R.S. Langer, K.M. Shakesheff, Polymeric Systems for Controlled Drug Release, Chemical Reviews, 99 (1999) 3181-3198.

[3] S. Kim, J.-H. Kim, O. Jeon, I.C. Kwon, K. Park, Engineered polymers for advanced drug delivery, European Journal of Pharmaceutics and Biopharmaceutics, 71 (2009) 420-430.

[4] F.M. Croisfelt, L.L. Tundisi, J.A. Ataide, E. Silveira, E.B. Tambourgi, A.F. Jozala, E.M.B. Souto, P.G. Mazzola, Modified-release topical hydrogels: a ten-year review, Journal of Materials Science, (2019).

[5] N. Kamaly, B. Yameen, J. Wu, O.C. Farokhzad, Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release, Chemical reviews, 116 (2016) 2602-2663.

[6] H. Tian, Z. Tang, X. Zhuang, X. Chen, X. Jing, Biodegradable synthetic polymers: Preparation, functionalization and biomedical application, Progress in Polymer Science, 37 (2012) 237-280.

[7] L. Ghasemi-Mobarakeh, D. Kolahreez, S. Ramakrishna, D. Williams, Key terminology in biomaterials and biocompatibility, Current Opinion in Biomedical Engineering, 10 (2019) 45-50.

[8] W. Yang, Q. Hu, Y. Xu, H. Liu, L. Zhong, Antibody fragment-conjugated gemcitabine and paclitaxel-based liposome for effective therapeutic efficacy in pancreatic cancer, Materials Science and Engineering: C, 89 (2018) 328-335.

[9] C. Pichot, Surface-functionalized latexes for biotechnological applications, Current Opinion in Colloid & Interface Science, 9 (2004) 213-221.

[10] R.S. Langer, N.A. Peppas, Present and future applications of biomaterials in controlled drug delivery systems, Biomaterials, 2 (1981) 201-214.

[11] S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, Journal of Controlled Release, 100 (2004) 5-28.

[12] K.B. Sujata, E.P. Holowka, Drug Delivery, 1 ed., Springer, New York, 2014.

[13] N.A. Peppas, B. Narasimhan, Mathematical models in drug delivery: How modeling has shaped the way we design new drug delivery systems, Journal of Controlled Release, 190 (2014) 75-81.

[14] T. Higuchi, Rate of Release of Medicaments from Ointment Bases Containing Drugs in Suspension, Journal of Pharmaceutical Sciences, 50 (1961) 874-875.

[15] P.L. Ritger, N.A. Peppas, A Simple Equation for Description of Solute Release, Journal of Controlled Release, 5 (1987) 23-36.

[16] N.A. Peppas, J.J. Sahlin, A simple equation for the description of solute release. III. Coupling of diffusion and relaxation, in, 1989, pp. 169-172.

[17] A.R. Berens, H.B. Hopfenberg, Diffusion and relaxation in glassy polymer powders: 2. Separation of diffusion and relaxation parameters, Polymer, 19 (1978) 489-496.

[18] O. Smidsrød, G. Skjåk-Bræk, Alginate as immobilization matrix for cells, Trends in Biotechnology, 8 (1990) 71-78.

[19] H.H. Tønnesen, J. Karlsen, Alginate in drug delivery systems, Drug Development and Industrial Pharmacy, 28 (2002) 621-630.

[20] M. George, T.E. Abraham, Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review, Journal of Controlled Release, 114 (2006) 1-14.

[21] S.E. Sakiyama-Elbert, J.A. Hubbell, Functional biomaterials: Design of novel biomaterials, Annual Review of Materials Science, 31 (2001) 183-201.

[22] G.T. Grant, E.R. Morris, D.A. Rees, P.J.C. Smith, D. Thom, Biological interactions between polysaccharides and divalent cations: The egg-box model, FEBS Letters, 32 (1973) 195-198.

[23] K.Y. Lee, D.J. Mooney, Alginate: Properties and biomedical applications, Progress in Polymer Science (Oxford), 37 (2012) 106-126.

[24] K.Y. Lee, J.A. Rowley, P. Eiselt, E.M. Moy, K.H. Bouhadir, D.J. Mooney, Controlling mechanical and swelling properties of alginate hydrogels independently by cross-linker type and cross-linking density, Macromolecules, 33 (2000) 4291-4294.

[25] P. Eiselt, K.Y. Lee, D.J. Mooney, Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines, Macromolecules, 32 (1999) 5561-5566.

[26] D. Roy, J.N. Cambre, B.S. Sumerlin, Future perspectives and recent advances in stimuli-responsive materials, Progress in Polymer Science (Oxford), 35 (2010) 278-301.

[27] A. Al-Shamkhani, R. Duncan, Radioiodination of alginate via covalently-bound tyrosinamide allows monitoring of its fate in vivo, J Bioact Compat Pol, 10 (1995) 4-13.

[28] M. Leonard, M.R. De Boisseson, P. Hubert, F. Dalençon, E. Dellacherie, Hydrophobically modified alginate hydrogels as protein carriers with specific controlled release properties, Journal of Controlled Release, 98 (2004) 395-405.

[29] A.F. Stockwell, S.S. Davis, S.E. Walker, In vitro evaluation of alginate gel systems as sustained release drug delivery systems, Journal of Controlled Release, 3 (1986) 167-175.

[30] P. Sriamornsak, N. Thirawong, K. Korkerd, Swelling, erosion and release behavior of alginate-based matrix tablets, European Journal of Pharmaceutics and Biopharmaceutics, 66 (2007) 435-450.

[31] S. Maiti, K. Singha, S. Ray, P. Dey, B. Sa, Adipic acid dihydrazide treated partially oxidized alginate beads for sustained oral delivery of flurbiprofen Oxidized alginate beads of flurbiprofen, Pharmaceutical Development and Technology, 14 (2009) 461-470.

[32] M. Witzler, S. Vermeeren, R.O. Kolevatov, R. Haddad, M. Gericke, T. Heinze, M. Schulze, Evaluating Release Kinetics from Alginate Beads Coated with Polyelectrolyte Layers for Sustained Drug Delivery, ACS Applied Bio Materials, (2021).

[33] M. Chen, X. Zhai, Y. Pan, H. Tan, Covalent and environment-responsive biopolymer hydrogel for drug delivery and wound healing, Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, 0 (2021) 1-12.

[34] A.F. Jozala, L.C. de Lencastre-Novaes, A.M. Lopes, V. de Carvalho Santos-Ebinuma, P.G. Mazzola, A. Pessoa-Jr, D. Grotto, M. Gerenutti, M.V. Chaud, Bacterial nanocellulose production and application: a 10-year overview, Applied Microbiology and Biotechnology, 100 (2016) 2063-2072.

[35] F. Shahmohammadi Jebel, H. Almasi, Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films, Carbohydrate Polymers, 149 (2016) 8-19.

[36] S. Salimi, R. Sotudeh-Gharebagh, R. Zarghami, S.Y. Chan, K.H. Yuen, Production of Nanocellulose and Its Applications in Drug Delivery: A Critical Review, ACS Sustainable Chemistry & Engineering, 7 (2019) 15800-15827.

[37] J.A. Ataide, N.M. de Carvalho, M.d.A. Rebelo, M.V. Chaud, D. Grotto, M. Gerenutti, M. Rai, P.G. Mazzola, A.F. Jozala, Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties, Scientific Reports, 7 (2017) 18031.

[38] J.P. Carvalho, A.C. Silva, V. Bastos, H. Oliveira, R.J. Pinto, A.J. Silvestre, C. Vilela, C.S.J.N. Freire, Nanocellulose-based patches loaded with hyaluronic acid and diclofenac towards aphthous stomatitis treatment, 10 (2020) 628.

[39] A.O. Elzoghby, W.S. Abo El-Fotoh, N.A. Elgindy, Casein-based formulations as promising controlled release drug delivery systems, Journal of Controlled Release, 153 (2011) 206-216.

[40] A. Sahu, N. Kasoju, U. Bora, Fluorescence Study of the Curcumin−Casein Micelle Complexation and Its Application as a Drug Nanocarrier to Cancer Cells, Biomacromolecules, 9 (2008) 2905-2912.

[41] S. Ghasemi, S. Abbasi, Formation of natural casein micelle nanocapsule by means of pH changes and ultrasound, Food Hydrocolloids, 42 (2014) 42-47.

[42] E. Semo, E. Kesselman, D. Danino, Y.D. Livney, Casein micelle as a natural nano-capsular vehicle for nutraceuticals, Food Hydrocolloids, 21 (2007) 936-942.

[43] M.A. Abdelmoneem, M. Mahmoud, A. Zaky, M.W. Helmy, M. Sallam, J.-Y. Fang, K.A. Elkhodairy, A.O. Elzoghby, Dual-targeted casein micelles as green nanomedicine for synergistic phytotherapy of hepatocellular carcinoma, Journal of Controlled Release, 287 (2018) 78-93.

[44] L.L. Tundisi, R. Yang, L.P.P. Borelli, T. Alves, M. Mehta, M.V. Chaud, P.G. Mazzola, D.S. Kohane, Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein, Pharmaceutical Research, (2021) 1-8.

[45] J. Xu, Z. Fan, L. Duan, G. Gao, A tough, stretchable, and extensively sticky hydrogel driven by milk protein, Polymer Chemistry, 9 (2018) 2617-2624.

[46] H.Q. Mao, K. Roy, V.L. Troung-Le, K.A. Janes, K.Y. Lin, Y. Wang, J.T. August, K.W. Leong, Chitosan-DNA nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency, Journal of Controlled Release, 70 (2001) 399-421.

[47] B. Conti, T. Modena, I. Genta, P. Perugini, C. Dacarro, F. Pavanetto, Microencapsulation of cetylpyridinium chloride with a bioadhesive polymer, Proceedings of the Controlled Release Society, (1998) 822-823.

[48] G.A.F. Roberts, Chitin chemistry, The Macmil ed., Macmillan Education UK, London, 1992.

[49] P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for chitosan based antimicrobial films in food applications, Food Chemistry, 114 (2009) 1173-1182.

[50] A.A. Tayel, S. Moussa, W.F. El-Tras, D. Knittel, K. Opwis, E. Schollmeyer, Anticandidal action of fungal chitosan against Candida albicans, International Journal of Biological Macromolecules, 47 (2010) 454-457.

[51] T.B. Taketa, R.A. Bataglioli, J.B.M. Rocha Neto, M.M. Beppu, Probing axial metal distribution on biopolymer-based layer-by-layer films for antimicrobial use, Colloids and Surfaces B: Biointerfaces, 199 (2021) 111505-111505.

[52] Y. Li, J. Li, Z. Shi, Y. Wang, X. Song, L. Wang, M. Han, H. Du, C. He, W. Zhao, B. Su, C. Zhao, Anticoagulant chitosan-kappa-carrageenan composite hydrogel sorbent for simultaneous endotoxin and bacteria cleansing in septic blood, Carbohydrate Polymers, 243 (2020) 116470-116470.

[53] Y. Tsuda, H. Hattori, Y. Tanaka, M. Ishihara, S. Kishimoto, M. Amako, H. Arino, K. Nemoto, Ultraviolet light-irradiated photocrosslinkable chitosan hydrogel to prevent bone formation in both rat skull and fibula bone defects, Journal of Tissue Engineering and Regenerative Medicine, 7 (2013) 720-728.

[54] X. Tao, H. Chen, X.J. Sun, J.F. Chen, W.H. Roa, Formulation and cytotoxicity of doxorubicin loaded in self-assembled bio-polyelectrolyte microshells, 336 (2007) 376-381.

[55] A.M. Heimbuck, T.R. Priddy-Arrington, B.J. Sawyer, M.E. Caldorera-Moore, Effects of post-processing methods on chitosan-genipin hydrogel properties, Materials Science and Engineering C, 98 (2019) 612-618.

[56] D. Demir, S. Ceylan, Y. Atakav, N. Bölgen, Synthesis of silver nanoflakes on chitosan hydrogel beads and their antimicrobial potential, International Journal of Polymer Analysis and Characterization, 25 (2020) 421-430.

[57] J.A. Ataide, D.C. Geraldes, E.F. Gérios, F.M. Bissaco, L.C. Cefali, L. Oliveira-Nascimento, P.G. Mazzola, Freeze-dried chitosan nanoparticles to stabilize and deliver bromelain, Journal of Drug Delivery Science and Technology, 61 (2021).

[58] A.M. De Campos, A. Sánchez, M.J. Alonso, Chitosan nanoparticles: A new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A, International Journal of Pharmaceutics, 224 (2001) 159-168.

[59] C. Peniche, W. Argüelles-Monal, H. Peniche, N. Acosta, Chitosan: An Attractive Biocompatible Polymer for Microencapsulation, Macromolecular Bioscience, 3 (2003) 511-520.

[60] P. Sacco, S. Paoletti, M. Cok, F. Asaro, M. Abrami, M. Grassi, I. Donati, Insight into the ionotropic gelation of chitosan using tripolyphosphate and pyrophosphate as cross-linkers, International Journal of Biological Macromolecules, 92 (2016) 476-483.

[61] P. Calvo, C. Remunan-Lopez, Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers, Journal of Applied …, 63 (1997) 125-132.

[62] K.A. Janes, M.P. Fresneau, A. Marazuela, A. Fabra, M.J. Alonso, Chitosan nanoparticles as delivery systems for doxorubicin, Journal of Controlled Release, 73 (2001) 255-267.

[63] A. Vila, A. Sánchez, M. Tobío, P. Calvo, M.J. Alonso, Design of biodegradable particles for protein delivery, Journal of Controlled Release, 78 (2002) 15-24.

[64] R.M. Saeed, I. Dmour, M.O. Taha, Stable Chitosan-Based Nanoparticles Using Polyphosphoric Acid or Hexametaphosphate for Tandem Ionotropic/Covalent Crosslinking and Subsequent Investigation as Novel Vehicles for Drug Delivery, Frontiers in Bioengineering and Biotechnology, 8 (2020) 1-21.

[65] J. Zhao, X. Wang, X. Deng, K. Liu, Chitosan-based nanoparticles for controlled release of hydrophobic and hydrophilic drugs, Bioinspired, Biomimetic and Nanobiomaterials, 10 (2021) 98-106.

[66] A. Fabiano, R. Bizzarri, Y. Zambito, Thermosensitive hydrogel based on chitosan and its derivatives containing medicated nanoparticles for transcorneal administration of 5-fluorouracil, International Journal of Nanomedicine, 12 (2017) 633-643.

[67] Q. Qian, S. Niu, G.R. Williams, J. Wu, X. Zhang, L.M. Zhu, Peptide functionalized dual-responsive chitosan nanoparticles for controlled drug delivery to breast cancer cells, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 564 (2019) 122-130.

[68] R.A. Bataglioli, T.B. Taketa, J.B.M.R. Neto, L.M. Lopes, C.A.R. Costa, M.M. Beppu, Analysis of pH and salt concentration on structural and model-drug delivery properties of polysaccharide-based multilayered films, Thin Solid Films, 685 (2019) 312-320.

[69] K. Delmar, H. Bianco-Peled, Composite chitosan hydrogels for extended release of hydrophobic drugs, Carbohydrate Polymers, 136 (2016) 570-580.

[70] P.I.P. Soares, A.I. Sousa, J.C. Silva, I.M.M. Ferreira, C.M.M. Novo, J.P. Borges, Chitosan-based nanoparticles as drug delivery systems for doxorubicin: Optimization and modelling, Carbohydrate Polymers, 147 (2016) 304-312.

[71] P. Wen, Y. Wen, X. Huang, M.H. Zong, H. Wu, Preparation and characterization of protein-loaded electrospun fiber mat and its release kinetics, Journal of Agricultural and Food Chemistry, 65 (2017) 4786-4796.

[72] N. Sedyakina, A. Kuskov, K. Velonia, N. Feldman, S. Lutsenko, G. Avramenko, Modulation of entrapment efficiency and in vitro release properties of BSA-loaded

chitosan microparticles cross-linked with citric acid as a potential protein-drug delivery system, Materials, 13 (2020).

[73] I. Orienti, K. Aiedeh, E. Gianasi, V. Bertasi, V. Zecchi, Indomethacin loaded chitosan microspheres. Correlation between the erosion process and release kinetics, Journal of Microencapsulation, 13 (1996) 463-472.

[74] T.C. Laurent, The chemistry, biology and medical applications of hyaluronan and its derivatives, (1998) 341-341.

[75] G. Kogan, L. Šoltés, R. Stern, P. Gemeiner, Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications, Biotechnology Letters, 29 (2007) 17-25.

[76] J.A. Burdick, G.D. Prestwich, Hyaluronic acid hydrogels for biomedical applications, Advanced Materials, 23 (2011) 41-56.

[77] C.-W. Ha, Y.-B. Park, J.-Y. Chung, Y.-G. Park, Cartilage Repair Using Composites of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells and Hyaluronic Acid Hydrogel in a Minipig Model, STEM CELLS Translational Medicine, 4 (2015) 1044-1051.

[78] I.A.D. Mancini, S. Schmidt, H. Brommer, B. Pouran, S. Schäfer, J. Tessmar, A. Mensinga, M.H.P. Van Rijen, J. Groll, T. Blunk, R. Levato, J. Malda, P.R. Van Weeren, A composite hydrogel-3D printed thermoplast osteochondral anchor as example for a zonal approach to cartilage repair: In vivo performance in a long-term equine model, Biofabrication, 12 (2020).

[79] E.N. El Rayes, SUPRA CHOROIDAL BUCKLING IN MANAGING MYOPIC VITREORETINAL INTERFACE DISORDERS, Retina, 34 (2014) 129-135.

[80] F. Sauvage, J.C. Fraire, K. Remaut, J. Sebag, K. Peynshaert, M. Harrington, F.J. Van de Velde, R. Xiong, M.-J. Tassignon, T. Brans, K. Braeckmans, S.C. De Smedt, Photoablation of Human Vitreous Opacities by Light-Induced Vapor Nanobubbles, ACS Nano, 13 (2019) 8401-8416.

[81] Y. Hao, J. He, X. Ma, L. Feng, M. Zhu, Y. Zhai, Y. Liu, P. Ni, G. Cheng, A fully degradable and photocrosslinked polysaccharide-polyphosphate hydrogel for tissue engineering, Carbohydrate Polymers, 225 (2019) 115257-115257.

[82] B.M. Hong, G.L. Hong, M.A. Gwak, K.H. Kim, J.E. Jeong, J.Y. Jung, S.A. Park, W.H. Park, Self-crosslinkable hyaluronate-based hydrogels as a soft tissue filler, International Journal of Biological Macromolecules, 185 (2021) 98-110.

[83] T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, Polysaccharide hydrogels for modified release formulations, Journal of Controlled Release, 119 (2007) 5-24.

[84] W. Boot, T. Schmid, M. D'Este, O. Guillaume, A. Foster, L. Decosterd, R.G. Richards, D. Eglin, S. Zeiter, T.F. Moriarty, A Hyaluronic Acid Hydrogel Loaded with Gentamicin and Vancomycin Successfully Eradicates Chronic Methicillin-Resistant Staphylococcus aureus Orthopedic Infection in a Sheep Model, Antimicrobial Agents and Chemotherapy, 65 (2021) e01840-01820.

[85] M.R. Todeschi, R.M. El Backly, O.P. Varghese, J. Hilborn, R. Cancedda, M. Mastrogiacomo, Host cell recruitment patterns by bone morphogenetic protein-2 releasing hyaluronic acid hydrogels in a mouse subcutaneous environment, Regenerative Medicine, 12 (2017) 525-539.

[86] Y. Luo, K.R. Kirker, G.D. Prestwich, Cross-linked hyaluronic acid hydrogel films: New biomaterials for drug delivery, Journal of Controlled Release, 69 (2000) 169-184.

[87] H. Li, Y. Liu, X. Zheng, Z. Shu, S.D. Gray, G.D. Prestwich, Synthesis and biological evaluation of a cross-linked hyaluronan-mitomycin C hydrogel, Biomacromolecules, 5 (2004) 895-902.

[88] S.Y. Choh, D. Cross, C. Wang, Facile synthesis and characterization of disulfide-cross-linked hyaluronic acid hydrogels for protein delivery and cell encapsulation, Biomacromolecules, 12 (2011) 1126-1136.

[89] R. Zhang, Z. Huang, M. Xue, J. Yang, T. Tan, Detailed characterization of an injectable hyaluronic acid- polyaspartylhydrazide hydrogel for protein delivery, Carbohydrate Polymers, 85 (2011) 717-725.

[90] J.L. Holloway, H. Ma, R. Rai, J.A. Burdick, Modulating hydrogel crosslink density and degradation to control bone morphogenetic protein delivery and in vivo bone formation, Journal of Controlled Release, 191 (2014) 63-70.

[91] J. Kim, I.S. Kim, T.H. Cho, H.C. Kim, S.J. Yoon, J. Choi, Y. Park, K. Sun, S.J. Hwang, In vivo evaluation of MMP sensitive high-molecular weight HA-based hydrogels for bone tissue engineering, Journal of Biomedical Materials Research - Part A, 95 (2010) 673-681.

[92] J. Kim, Y. Park, G. Tae, K.B. Lee, S.J. Hwang, I.S. Kim, I. Noh, K. Sun, Synthesis and characterization of matrix metalloprotease sensitive-low molecular weight hyaluronic acid based hydrogels, Journal of Materials Science: Materials in Medicine, 19 (2008) 3311-3318.

[93] K. Deshmukh, M. Basheer Ahamed, R.R. Deshmukh, S.K. Khadheer Pasha, P.R. Bhagat, K. Chidambaram, 3 - Biopolymer Composites With High Dielectric Performance: Interface Engineering, in: K.K. Sadasivuni, D. Ponnamma, J. Kim, J.J. Cabibihan, M.A. AlMaadeed (Eds.) Biopolymer Composites in Electronics, Elsevier, 2017, pp. 27-128.

[94] L.L. Tundisi, G.B. Mostaço, P.C. Carricondo, D.F.S. Petri, Hydroxypropyl methylcellulose: Physicochemical properties and ocular drug delivery formulations, European Journal of Pharmaceutical Sciences, 159 (2021) 105736.

[95] M.E. Sangalli, A. Maroni, A. Foppoli, L. Zema, F. Giordano, A. Gazzaniga, Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: a study on process parameters and in vitro performances, Eur J Pharm Sci, 22 (2004) 469-476.

[96] M. Foroughi-Dahr, R. Sotudeh-Gharebagh, N. Mostoufi, Effect of operation conditions on coating of pharmaceutical pellets with a film of HPMC/PEG in a Wurster coater, Powder Technology, 354 (2019) 804-814.

[97] M. Tighsazzadeh, J.C. Mitchell, J.S. Boateng, Development and evaluation of performance characteristics of timolol-loaded composite ocular films as potential delivery platforms for treatment of glaucoma, International Journal of Pharmaceutics, 566 (2019) 111-125.

[98] V.K. Ravindran, S. Repala, S. Subadhra, A.K. Appapurapu, Chick chorioallantoic membrane model for in ovo evaluation of timolol maleate-brimonidine tartrate ocular inserts, Drug Delivery, 21 (2014) 307-314.

[99] M. Mahmoudian, F.J.P.i.b. Ganji, Vancomycin-loaded HPMC microparticles embedded within injectable thermosensitive chitosan hydrogels, 6 (2017) 49-56.

[100] G. Dumortier, J.L. Grossiord, F. Agnely, J.C. Chaumeil, A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics, Pharmaceutical Research, 23 (2006) 2709-2728.

[101] R. Yang, V. Sabharwal, O.S. Okonkwo, N. Shlykova, R. Tong, L.Y. Lin, W. Wang, S. Guo, J.J. Rosowski, S.I. Pelton, D.S. Kohane, Treatment of otitis media by transtympanic delivery of antibiotics, Science Translational Medicine, 8 (2016) 356ra120.

[102] M.S. Gil, J. Cho, T. Thambi, V.H. Giang Phan, I. Kwon, D.S. Lee, Bioengineered robust hybrid hydrogels enrich the stability and efficacy of biological drugs, Journal of Controlled Release, 267 (2017) 119-132.

[103] M. Labet, W. Thielemans, Synthesis of polycaprolactone: a review, Chemical Society Reviews, 38 (2009) 3484-3504.

[104] A.N. Zelikin, C. Ehrhardt, A.M. Healy, Materials and methods for delivery of biological drugs, Nat Chem, 8 (2016) 997-1007.

[105] M. Rashtchian, A. Hivechi, S.H. Bahrami, P.B. Milan, S. Simorgh, Fabricating alginate/poly(caprolactone) nanofibers with enhanced bio-mechanical properties via cellulose nanocrystal incorporation, Carbohydrate Polymers, 233 (2020) 115873.

[106] V.R. Sinha, K. Bansal, R. Kaushik, R. Kumria, A. Trehan, Poly-ϵ-caprolactone microspheres and nanospheres: an overview, International Journal of Pharmaceutics, 278 (2004) 1-23.

[107] R. Li, Y. Song, P. Fouladian, M. Arafat, R. Chung, J. Kohlhagen, S. Garg, Three-Dimensional Printing of Curcumin-Loaded Biodegradable and Flexible Scaffold for Intracranial Therapy of Glioblastoma Multiforme, 13 (2021) 471.

[108] M. Ahmad Wsoo, S. Izwan Abd Razak, S. Shahir, H. Ahmed Abdullah Al-Moalemi, M. Rafiq Abdul Kadir, N. Hasraf Mat Nayan, Development of prolonged drug delivery system using electrospun cellulose acetate/polycaprolactone nanofibers: Future subcutaneous implantation, n/a.

[109] Q. Li, X. Qian, H.Y. Li, K.L. Lai, Q. Gao, W.Y.T. Lee, Safety assessment of polymeric micelles as an ophthalmic drug delivery system for intravitreal administration of dasatinib, International Journal of Pharmaceutics, 596 (2021) 120226.

[110] Q. Li, K.L. Lai, P.S. Chan, S.C. Leung, H.Y. Li, Y. Fang, K.K.W. To, C.H.J. Choi, Q.Y. Gao, T.W.Y. Lee, Micellar delivery of dasatinib for the inhibition of pathologic cellular processes of the retinal pigment epithelium, Colloids and Surfaces B: Biointerfaces, 140 (2016) 278-286.

[111] A.F. Ourique, A. Melero, C.d.B.d. Silva, U.F. Schaefer, A.R. Pohlmann, S.S. Guterres, C.-M. Lehr, K.-H. Kostka, R.C.R. Beck, Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine, European Journal of Pharmaceutics and Biopharmaceutics, 79 (2011) 95-101.

[112] S.E. Muttaqien, T. Nomoto, X. Dou, H. Takemoto, M. Matsui, N. Nishiyama, Photodynamic therapy using LCST polymers exerting pH-responsive isothermal phase transition, Journal of Controlled Release, 328 (2020) 608-616.

[113] J.C. Coco, L.L. Tundisi, É.M.d. Santos, A.L.M. Fava, T.F. Alves, J.A. Ataide, M.V. Chaud, P.G. Mazzola, PVA-CO-AAM and peg-co-aam hydrogels as bromelain carriers, Journal of Drug Delivery Science and Technology, 63 (2021) 102483.

[114] F.M. Croisfelt, J.A. Ataide, L.L. Tundisi, L.C. Cefali, M.d.A. Rebelo, J.L.D. Sánchez, T.G. da Costa, R. Lima, A.F. Jozala, M.V. Chaud, M.A. d'Ávila, E.B. Tambourgi, E.

Silveira, P.G. Mazzola, Characterization of PNIPAAm-co-AAm hydrogels for modified release of bromelain, European Polymer Journal, 105 (2018) 48-54.

[115] A. Prasannan, H.-C. Tsai, G.-H. Hsiue, Formulation and evaluation of epinephrine-loaded poly(acrylic acid-co-N-isopropylacrylamide) gel for sustained ophthalmic drug delivery, Reactive and Functional Polymers, 124 (2018) 40-47.

[116] O.C. Farokhzad, R. Langer, Impact of Nanotechnology on Drug Delivery, ACS Nano, 3 (2009) 16-20.

[117] A. Zielińska, F. Carreiró, A.M. Oliveira, A. Neves, B. Pires, D.N. Venkatesh, A. Durazzo, M. Lucarini, P. Eder, A.M. Silva, A. Santini, E.B. Souto, Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology, Molecules, 25 (2020).

[118] C.I.C. Crucho, M.T. Barros, Polymeric nanoparticles: A study on the preparation variables and characterization methods, Materials Science and Engineering: C, 80 (2017) 771-784.

[119] J.A. Ataide, D.C. Geraldes, E.F. Gérios, F.M. Bissaco, L.C. Cefali, L. Oliveira-Nascimento, P.G. Mazzola, Freeze-dried chitosan nanoparticles to stabilize and deliver bromelain, Journal of Drug Delivery Science and Technology, 61 (2021) 102225.

[120] R. Alvarez-Román, A. Naik, Y.N. Kalia, R.H. Guy, H. Fessi, Skin penetration and distribution of polymeric nanoparticles, Journal of Controlled Release, 99 (2004) 53-62.

[121] A. Sorrentino, A. Cataldo, R. Curatolo, P. Tagliatesta, L. Mosca, S. Bellucci, Novel optimized biopolymer-based nanoparticles for nose-to-brain delivery in the treatment of depressive diseases, RSC Advances, 10 (2020) 28941-28949.

[122] K. Jin, Z. Luo, B. Zhang, Z. Pang, Biomimetic nanoparticles for inflammation targeting, Acta Pharm Sin B, 8 (2018) 23-33.

[123] Y. Fang, Q. Wang, X. Lin, X. Jin, D. Yang, S. Gao, X. Wang, M. Yang, K. Shi, Gastrointestinal Responsive Polymeric Nanoparticles for Oral Delivery of Insulin: Optimized Preparation, Characterization, and In Vivo Evaluation, Journal of Pharmaceutical Sciences, 108 (2019) 2994-3002.

[124] O. Wichterle, D. LÍM, Hydrophilic Gels for Biological Use, Nature, 185 (1960) 117-118.

[125] M. Hamidi, A. Azadi, P. Rafiei, Hydrogel nanoparticles in drug delivery, Advanced Drug Delivery Reviews, 60 (2008) 1638-1649.

[126] J. Kálal, Water sensitive chemically crosslinked gels, in: C.A. Finch (Ed.) Chemistry and Technology of Water Soluble Polymers, Springer, New York, 1983.

[127] R. Narayanaswamy, V.P. Torchilin, Hydrogels and Their Applications in Targeted Drug Delivery, Molecules, 24 (2019).

[128] W. Lee, T.G. Lee, W.-G. Koh, Grafting of Poly(acrylic acid) on the Poly(ethylene glycol) Hidrogel Using Surface-initiated Photopolymerization for Covalent Immobilization of Collagen, Journal of Industrial Engineering and Chemistry, 13 (2007) 1195-1200.

[129] J. Tang, J. Sun, F. Cui, T. Zhang, X. Liu, Z. He, Self-Emulsifying Drug Delivery Systems for Improving Oral Absorption of Ginkgo Biloba Extracts, Drug Delivery, 15 (2008) 477-484.

[130] W. Xu, X. Wang, N. Sandler, S. Willför, C. Xu, Three-Dimensional Printing of Wood-Derived Biopolymers: A Review Focused on Biomedical Applications, ACS Sustainable Chemistry & Engineering, 6 (2018) 5663-5680.

[131] G.D. Cha, W.H. Lee, C. Lim, M.K. Choi, D.-H. Kim, Materials engineering, processing, and device application of hydrogel nanocomposites, Nanoscale, 12 (2020) 10456-10473.

[132] C.A. Dreiss, Hydrogel design strategies for drug delivery, Current Opinion in Colloid & Interface Science, 48 (2020) 1-17.

[133] S. Peers, A. Montembault, C. Ladavière, Chitosan hydrogels for sustained drug delivery, Journal of Controlled Release, 326 (2020) 150-163.

[134] R.F. Donnelly, T.R.R. Singh, M.J. Garland, K. Migalska, R. Majithiya, C.M. McCrudden, P.L. Kole, T.M.T. Mahmood, H.O. McCarthy, A.D.J.A.f.m. Woolfson, Hydrogel‐forming microneedle arrays for enhanced transdermal drug delivery, 22 (2012) 4879-4890.

[135] D. Silva, H.C. de Sousa, M.H. Gil, L.F. Santos, R.A. Amaral, J.A. Saraiva, M. Salema-Oom, C. Alvarez-Lorenzo, A.P. Serro, B. Saramago, Imprinted hydrogels with LbL coating for dual drug release from soft contact lenses materials, Materials Science and Engineering: C, 120 (2021) 111687.

[136] J.O. Morales, J.T. McConville, Manufacture and characterization of mucoadhesive buccal films, European Journal of Pharmaceutics and Biopharmaceutics, 77 (2011) 187-199.

[137] S.D. Barnhart, Thin film oral dosage forms, in, 2008, pp. 209-216.

[138] I. Karakurt, K. Ozaltin, E. Vargun, L. Kucerova, P. Suly, E. Harea, A. Minařík, K. Štěpánková, M. Lehocky, P. Humpolícek, A. Vesel, M. Mozetic, Controlled release

of enrofloxacin by vanillin-crosslinked chitosan-polyvinyl alcohol blends, Materials Science and Engineering C, 126 (2021).

[139] B.H. Alrimawi, M.Y. Chan, X.Y. Ooi, S.Y. Chan, C.F. Goh, The interplay between drug and sorbitol contents determines the mechanical and swelling properties of potential rice starch films for buccal drug delivery, Polymers, 13 (2021) 1-15.

[140] C. Cubayachi, C.N. Lemos, F. Pereira, K. Dias, R.D. Herculano, O. de Freitas, R.F.V. Lopez, Silk fibroin films stabilizes and releases bioactive insulin for the treatment of corneal wounds, European Polymer Journal, 118 (2019) 502-513.

[141] B.V.F. Riccio, A.B. Klosowski, E. Prestes, T.B. de Sousa, L.C. de Assunção Morais, B.M. Lemes, F.L. Beltrame, P.M. Campos, P.C. Ferrari, Chitosan/nanocellulose-based bionanocomposite films for controlled betamethasone and silver sulfadiazine delivery, Journal of Applied Polymer Science, 138 (2021) 1-13.

[142] W.G. Sanders, P.C. Hogrebe, D.W. Grainger, A.K. Cheung, C.M. Terry, A biodegradable perivascular wrap for controlled, local and directed drug delivery, Journal of Controlled Release, 161 (2012) 81-89.

[143] M.A. Repka, S.K. Battu, S.B. Upadhye, S. Thumma, M.M. Crowley, F. Zhang, C. Martin, J.W. McGinity, Pharmaceutical applications of hot-melt extrusion: Part II, Drug Development and Industrial Pharmacy, 33 (2007) 1043-1057.

[144] M. Maniruzzaman, J.S. Boateng, M.J. Snowden, D. Douroumis, A Review of hot-melt extrusion: Process technology to pharmaceutical products, ISRN Pharmaceutics, 2012 (2012) 436763-436763.

[145] C. Aitken-nichol, F. Zhang, J.W. McGinity, Hot melt extrusion of acrylic films, Pharmaceutical Research, 13 (1996) 804-808.

[146] M. Montenegro-Nicolini, J.O. Morales, Overview and future potential of buccal mucoadhesive films as drug delivery systems for biologics, AAPS PharmSciTech, 18 (2017) 3-14.

[147] A.M. Bhagurkar, M. Darji, P. Lakhani, P. Thipsay, S. Bandari, M.A. Repka, Effects of formulation composition on the characteristics of mucoadhesive films prepared by hot-melt extrusion technology, Journal of Pharmacy and Pharmacology, 71 (2019) 293-305.

[148] I. Speer, M. Preis, J. Breitkreutz, Prolonged drug release properties for orodispersible films by combining hot-melt extrusion and solvent casting methods, European Journal of Pharmaceutics and Biopharmaceutics, 129 (2018) 66-73.

[149] R.K. Iler, Multilayers of colloidal particles, Journal of Colloid And Interface Science, 21 (1966) 569-594.

[150] J.J. Kirkland, Porous thin-layer modified glass bead supports for gas liquid chromatography, Analytical Chemistry, 37 (1965) 1458-1461.

[151] G. Decher, Fuzzy Nanoassemblies : Toward Layered Polymeric Multicomposites, Science, 277 (1997) 1232-1237.

[152] J. Cho, K. Char, J.D. Hong, K.B. Lee, Fabrication of highly ordered multilayer films using a spin self-assembly method, Advanced Materials, 13 (2001) 1076-1078.

[153] J.B. Schlenoff, S.T. Dubas, T. Farhat, Sprayed polyelectrolyte multilayers, Langmuir, 16 (2000) 9968-9969.

[154] J. Borges, J.F. Mano, Molecular interactions driving the layer-by-layer assembly of multilayers, Chemical Reviews, 114 (2014) 8883-8942.

[155] S.R. Puniredd, M.P. Srinivasan, Covalent molecular assembly of oligoimide ultrathin films in supercritical and liquid solvent media, Langmuir, 21 (2005) 7812-7822.

[156] X. Wang, H.J. Kim, P. Xu, A. Matsumoto, D.L. Kaplan, Biomaterial coatings by stepwise deposition of silk fibroin, Langmuir, 21 (2005) 11335-11341.

[157] T. Crouzier, C.H. Beckwitt, K. Ribbeck, Mucin multilayers assembled through sugar-lectin interactions, Biomacromolecules, 13 (2012) 3401-3408.

[158] P.T. Hammond, Polyelectrolyte multilayered nanoparticles: Using nanolayers for controlled and targeted systemic release, Nanomedicine : nanotechnology, biology, and medicine, 7 (2012) 619-622.

[159] A.J. Chung, M.F. Rubner, Methods of loading and releasing low molecular weight cationic molecules in weak polyelectrolyte multilayer films, Langmuir, 18 (2002) 1176-1183.

[160] M.C. Berg, L. Zhai, R.E. Cohen, M.F. Rubner, Controlled drug release from porous polyelectrolyte multilayers, Biomacromolecules, 7 (2006) 357-364.

[161] K.C. Wood, J.Q. Boedicker, D.M. Lynn, P.T. Hammond, Tunable drug release from hydrolytically degradable layer-by-layer thin films, Langmuir, 21 (2005) 1603-1609.

[162] R. Polak, R.M. Lim, M.M. Beppu, R.N.M. Pitombo, R.E. Cohen, M.F. Rubner, Liposome-loaded cell backpacks, Advanced healthcare materials, 4 (2015) 2832-2841.

[163] S.S. Shiratori, M.F. Rubner, pH-Dependent Thickness Behavior of Sequentially Adsorbed Layers of Weak Polyelectrolytes, Macromolecules, 33 (2000) 4213-4219.

[164] F. Cuomo, A. Ceglie, M. Piludu, M.G. Miguel, B. Lindman, F. Lopez, Loading and protection of hydrophilic molecules into liposome-templated polyelectrolyte nanocapsules, Langmuir, 30 (2014) 7993-7999.

[165] T.S. Anirudhan, C.S. Vasantha, A.V. Sasidharan, Layer-by-layer assembly of hyaluronic acid/carboxymethylchitosan polyelectrolytes on the surface of aminated mesoporous silica for the oral delivery of 5-fluorouracil, European Polymer Journal, 93 (2017) 572-589.

[166] J.B.M. Rocha Neto, G.G. Lima, A. Fiamingo, L.G.L. Germiniani, T.B. Taketa, R.A. Bataglioli, G.A.T. da Silveira, J.V.L. da Silva, S.P. Campana-Filho, O.N. Oliveira, M.M. Beppu, Controlling antimicrobial activity and drug loading capacity of chitosan-based layer-by-layer films, International Journal of Biological Macromolecules, 172 (2021) 154-161.

[167] D.M. Lynn, R. Langer, Degradable poly(beta-amino esters): Synthesis, characterization, and self-assembly with plasmid DNA, (2000) 10761-10768.

[168] R.C. Smith, A. Leung, B.S. Kim, P.T. Hammond, Hydrophobic effects in the critical destabilization and release dynamics of degradable multilayer films, Chemistry of Materials, 21 (2009) 1108-1115.

[169] J. Min, K.Y. Choi, E.C. Dreaden, R.F. Padera, R.D. Braatz, M. Spector, P.T. Hammond, Designer Dual Therapy Nanolayered Implant Coatings Eradicate Biofilms and Accelerate Bone Tissue Repair, ACS Nano, 10 (2016) 4441-4450.

[170] B.M. Wohl, J.F.J. Engbersen, Responsive layer-by-layer materials for drug delivery, Journal of Controlled Release, 158 (2012) 2-14.

[171] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery, Nat Mater, 12 (2013) 991-1003.

[172] V.P. Torchilin, Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery, Nature Reviews Drug Discovery, 13 (2014) 813-827.

[173] Y. Wang, D.S. Kohane, External triggering and triggered targeting strategies for drug delivery, Nature Reviews Materials, 2 (2017) 17020.

9. Table References

1. Ahmad Wsoo, M., et al., Development of prolonged drug delivery system using electrospun cellulose acetate/polycaprolactone nanofibers: Future subcutaneous implantation. n/a(n/a).

2. Li, R., et al., Three-Dimensional Printing of Curcumin-Loaded Biodegradable and Flexible Scaffold for Intracranial Therapy of Glioblastoma Multiforme. 2021. 13(4): p. 471.

3. Russo, J., J. Fiegel, and N.K. Brogden, Rheological and drug delivery characteristics of poloxamer-based diclofenac sodium formulations for chronic wound site analgesia. Pharmaceutics, 2020. 12(12): p. 1-18.

4. Saqib, M., et al., Amphotericin b loaded polymeric nanoparticles for treatment of leishmania infections. Nanomaterials, 2020. 10(6).

5. Vanti, G., et al., Hydroxypropyl methylcellulose hydrogel of berberine chloride-loaded escinosomes: Dermal absorption and biocompatibility. International Journal of Biological Macromolecules, 2020. 164: p. 232-241.

6. Lu, A., et al., Synthesis and Self-Assembly of Hydroxypropyl Methyl Cellulose-block-Poly(ϵ-caprolactone) Copolymers as Nanocarriers of Lipophilic Drugs. ACS Applied Nano Materials, 2020. 3(5): p. 4367-4375.

7. Jiang, Q., P. Zhang, and J. Li, Elucidation of Colloid Performances of Thermosensitive In Situ–Forming Ophthalmic Gel Formed by Poloxamer 407 for Loading Drugs. Journal of Pharmaceutical Sciences, 2020. 109(5): p. 1703-1713.

8. Sorrentino, A., et al., Novel optimized biopolymer-based nanoparticles for nose-to-brain delivery in the treatment of depressive diseases. RSC Advances, 2020. 10(48): p. 28941-28949.

9. Sriamornsak, P., N. Thirawong, and K. Korkerd, Swelling, erosion and release behavior of alginate-based matrix tablets. European Journal of Pharmaceutics and Biopharmaceutics, 2007. 66(3): p. 435-450.

10. Rashtchian, M., et al., Fabricating alginate/poly(caprolactone) nanofibers with enhanced bio-mechanical properties via cellulose nanocrystal incorporation. Carbohydrate Polymers, 2020. 233: p. 115873.

11. Popescu, I., et al., Alginate/poloxamer hydrogel obtained by thiol-acrylate photopolymerization for the alleviation of the inflammatory response of human keratinocytes. International Journal of Biological Macromolecules, 2021. 180: p. 418-431.

12. Pamlényi, K., et al., Formulation and optimization of sodium alginate polymer film as a buccal mucoadhesive drug delivery system containing cetirizine dihydrochloride. Pharmaceutics, 2021. 13(5).

13. Ataide, J.A., et al., Effect of Polysaccharide Sources on the Physicochemical Properties of Bromelain-Chitosan Nanoparticles. Polymers, 2019. 11(10): p. 1681.

14. Chandy, T., G.S. Das, and G.H.R. Rao, 5-Fluorouracil-loaded chitosan coated polylactic acid microspheres as biodegradable drug carriers for cerebral tumours. Journal of Microencapsulation, 2000. 17(5): p. 625-638.

15. Fahimirad, S., et al., Wound healing performance of PCL/chitosan based electrospun nanofiber electrosprayed with curcumin loaded chitosan nanoparticles. Carbohydrate Polymers, 2021. 259: p. 117640-117640.

16. Lee, J.S., et al., A novel chitosan nanosponge as a vehicle for transepidermal drug delivery. Pharmaceutics, 2021. 13(9): p. 1-11.

17. Mahmoudian, M. and F.J.P.i.b. Ganji, Vancomycin-loaded HPMC microparticles embedded within injectable thermosensitive chitosan hydrogels. 2017. 6(1): p. 49-56.

18. Jafari, H., et al., Characterization of pH-sensitive chitosan/hydroxypropyl methylcellulose composite nanoparticles for delivery of melatonin in cancer therapy. Materials Letters, 2021. 282: p. 128818.

19. Anal, A.K. and W.F. Stevens, Chitosan-alginate multilayer beads for controlled release of ampicillin. International Journal of Pharmaceutics, 2005. 290(1-2): p. 45-54.

20. Mi, F.L., H.W. Sung, and S.S. Shyu, Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent. Carbohydrate Polymers, 2002. 48(1): p. 61-72.

21. Abdelmoneem, M.A., et al., Dual-targeted casein micelles as green nanomedicine for synergistic phytotherapy of hepatocellular carcinoma. Journal of Controlled Release, 2018. 287: p. 78-93.

22. Khodaverdi, E., et al., Casein-based hydrogel carrying insulin: preparation, in vitro evaluation and in vivo assessment. Journal of Pharmaceutical Investigation, 2019. 49(6): p. 635-641.

23. Cao, Z., et al., Fabrication and characterization of PCL/CaCO3 electrospun composite membrane for bone repair. RSC Advances, 2016. 6(13): p. 10641-10649.

24. Tundisi, L.L., et al., Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein. Pharmaceutical Research, 2021: p. 1-8.

25. Gubbins, R.H., C.M. O'Driscoll, and O.I. Corrigan, The effects of casein on diclofenac release from hydroxypropylmethylcellulose (HPMC) compacts. International Journal of Pharmaceutics, 2003. 260(1): p. 69-76.

26. Niaz, T., et al., Alginate-caseinate based pH-responsive nano-coacervates to combat resistant bacterial biofilms in oral cavity. International Journal of Biological Macromolecules, 2020. 156: p. 1366-1380.

27. Zhang, X., et al., Chitosan/casein based microparticles with a bilayer shell-core structure for oral delivery of nattokinase. Food and Function, 2020. 11(12): p. 10799-10816.

28. Malektaj, H., R. Imani, and M.H. Siadati, Study of injectable PNIPAAm hydrogels containing niosomal angiogenetic drug delivery system for potential cardiac tissue regeneration. Biomedical Materials (Bristol), 2021. 16(4).

29. Gholami, S., et al., Poly(Caprolactone)-Poly(N-Isopropyl Acrylamide)-Fe3O4 Magnetic Nanofibrous Structure with Stimuli Responsive Drug Release. Macromolecular Materials and Engineering, 2020. 305(9): p. 1-9.

30. Chen, C.Y., Y.C. Wang, and C.C. Hung, In vitro dual-modality chemo-photodynamic therapy via stimuli-triggered polymeric micelles. Reactive and Functional Polymers, 2016. 98: p. 56-64.

31. Alsmadi, N.A., et al., Effects of surfactants on properties of polymer-coated magnetic nanoparticles for drug delivery application. Journal of Nanoparticle Research, 2011. 13(12): p. 7177-7186.

32. Xiao, Y.L., et al., Preparation and characterization of thermo-sensitive hydroxypropylmethyl cellulose/poly (N-isopropylacrylamide) hydrogel. Advanced Materials Research, 2011. 194-196: p. 773-776.

33. Dumitriu, R.P., et al., Biocompatible and biodegradable alginate/poly(N-isopropylacrylamide) hydrogels for sustained theophylline release. Journal of Applied Polymer Science, 2014. 131(17): p. 8939-8954.

34. Cao, Y., et al., Poly(N-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. Journal of Controlled Release, 2007. 120(3): p. 186-194.

35. Cao, Z.F., et al., Preparation and properties of a dually responsive hydrogels based on polyampholyte for oral delivery of drugs. Polymer Bulletin, 2013. 70(10): p. 2675-2689.

36. Ataide, J.A., et al., Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties. Scientific Reports, 2017. 7(1): p. 18031.

37. Numata, Y., L. Mazzarino, and R. Borsali, A slow-release system of bacterial cellulose gel and nanoparticles for hydrophobic active ingredients. International Journal of Pharmaceutics, 2015. 486(1-2): p. 217-225.

38. Alkhatib, Y., et al., Controlled extended octenidine release from a bacterial nanocellulose/Poloxamer hybrid system. European Journal of Pharmaceutics and Biopharmaceutics, 2017. 112: p. 164-176.

39. Ullah, H., et al., Fabrication, characterization and evaluation of bacterial cellulose-based capsule shells for oral drug delivery. Cellulose, 2017. 24(3): p. 1445-1454.

40. Yuan, Q., et al., Biomimetic nanofibrous hybrid hydrogel membranes with sustained growth factor release for guided bone regeneration. Biomaterials Science, 2021. 9(4): p. 1256-1271.

41. Cacicedo, M.L., et al., Chitosan-bacterial cellulose patch of ciprofloxacin for wound dressing: Preparation and characterization studies. International Journal of Biological Macromolecules, 2020. 147: p. 1136-1145.

42. Patwa, R., et al., Effect of iron-oxide nanoparticles impregnated bacterial cellulose on overall properties of alginate/casein hydrogels: Potential injectable biomaterial for wound healing applications. Polymers, 2020. 12(11): p. 1-21.

43. Pandey, M., N. Mohamad, and M.C.I.M. Amin, Bacterial cellulose/acrylamide pH-sensitive smart hydrogel: Development, characterization, and toxicity studies in ICR mice model. Molecular Pharmaceutics, 2014. 11(10): p. 3596-3608.

44. Hwang, S.M., et al., Delivery of ofloxacin to the lung and alveolar macrophages via hyaluronan microspheres for the treatment of tuberculosis. Journal of Controlled Release, 2008. 129(2): p. 100-106.

45. Janmohammadi, M., M.S. Nourbakhsh, and S. Bonakdar, Electrospun Skin Tissue Engineering Scaffolds Based on Polycaprolactone/Hyaluronic Acid/L-ascorbic Acid. Fibers and Polymers, 2021. 22(1): p. 19-29.

46. Yang, R., et al., Modified poloxamer 407 and hyaluronic acid thermosensitive hydrogel-encapsulated keratinocyte growth factor 2 improves knee osteoarthritis in rats. Materials and Design, 2021. 210: p. 110086-110086.

47. Tighsazzadeh, M., J.C. Mitchell, and J.S. Boateng, Development and evaluation of performance characteristics of timolol-loaded composite ocular films as potential

delivery platforms for treatment of glaucoma. International Journal of Pharmaceutics, 2019. 566: p. 111-125.

48. Silva, D., et al., Imprinted hydrogels with LbL coating for dual drug release from soft contact lenses materials. Materials Science and Engineering: C, 2021. 120: p. 111687.

49. Anirudhan, T.S., C.S. Vasantha, and A.V. Sasidharan, Layer-by-layer assembly of hyaluronic acid/carboxymethylchitosan polyelectrolytes on the surface of aminated mesoporous silica for the oral delivery of 5-fluorouracil. European Polymer Journal, 2017. 93(March): p. 572-589.

50. Lv, Y., et al., Targeting intracellular MMPs efficiently inhibits tumor metastasis and angiogenesis. Theranostics, 2018. 8(10): p. 2830-2845.

51. Wu, Y., et al., Enhanced and sustained topical ocular delivery of cyclosporine a in thermosensitive hyaluronic acid-based in situ forming microgels. International Journal of Nanomedicine, 2013. 8: p. 3587-3601.

52. Carvalho, J.P., et al., Nanocellulose-based patches loaded with hyaluronic acid and diclofenac towards aphthous stomatitis treatment. 2020. 10(4): p. 628.

53. Li, Q., et al., Micellar delivery of dasatinib for the inhibition of pathologic cellular processes of the retinal pigment epithelium. Colloids and Surfaces B: Biointerfaces, 2016. 140: p. 278-286.

54. Hamoudi-Ben Yelles, M.C., et al., PLGA implants: How Poloxamer/PEO addition slows down or accelerates polymer degradation and drug release. Journal of Controlled Release, 2017. 253: p. 19-29.

55. Xu, J., et al., A tough, stretchable, and extensively sticky hydrogel driven by milk protein. Polymer Chemistry, 2018. 9(19): p. 2617-2624.

56. Ravindran, V.K., et al., Chick chorioallantoic membrane model for in ovo evaluation of timolol maleate-brimonidine tartrate ocular inserts. Drug Delivery, 2014. 21(4): p. 307-314.

57. Bhagurkar, A.M., et al., Effects of formulation composition on the characteristics of mucoadhesive films prepared by hot-melt extrusion technology. Journal of Pharmacy and Pharmacology, 2019. 71(3): p. 293-305.

58. Foroughi-Dahr, M., R. Sotudeh-Gharebagh, and N. Mostoufi, Effect of operation conditions on coating of pharmaceutical pellets with a film of HPMC/PEG in a Wurster coater. Powder Technology, 2019. 354: p. 804-814.

59. Eiselt, P., K.Y. Lee, and D.J. Mooney, Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines. Macromolecules, 1999. 32(17): p. 5561-5566.

60. Rasool, A., S. Ata, and A. Islam, Stimuli responsive biopolymer (chitosan) based blend hydrogels for wound healing application. Carbohydrate Polymers, 2019. 203(March 2018): p. 423-429.

61. Coco, J.C., et al., PVA-CO-AAM and peg-co-aam hydrogels as bromelain carriers. Journal of Drug Delivery Science and Technology, 2021. 63: p. 102483.

62. Croisfelt, F.M., et al., Characterization of PNIPAAm-co-AAm hydrogels for modified release of bromelain. European Polymer Journal, 2018. 105: p. 48-54.

63. Prasannan, A., H.-C. Tsai, and G.-H. Hsiue, Formulation and evaluation of epinephrine-loaded poly(acrylic acid-co-N-isopropylacrylamide) gel for sustained ophthalmic drug delivery. Reactive and Functional Polymers, 2018. 124: p. 40-47.

64. Shahmohammadi Jebel, F. and H. Almasi, Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. Carbohydrate Polymers, 2016. 149: p. 8-19.

65. Jipa, I.M., A. Stoica-Guzun, and M. Stroescu, Controlled release of sorbic acid from bacterial cellulose based mono and multilayer antimicrobial films. LWT - Food Science and Technology, 2012. 47(2): p. 400-406.

66. Yan, K., et al., Fabrication of hyaluronic acid-based micelles with glutathione-responsiveness for targeted anticancer drug delivery. Journal of Colloid and Interface Science, 2022. 606: p. 1586-1596.

Capitulo II – Nanotechnology as a tool to overcome macromolecules delivery issues

Louise Lacalendola Tundisi; Janaína Artem Ataide; Juliana S. R. Costa; Diego F. Coêlho; Raquel Bester Liszbinski; Andre Moreni Lopes; Laura Oliveira-Nascimento; Marcelo Bispo de Jesus; Angela Faustino Jozala; Carsten Ehrhardt; Priscila Gava Mazzola
Submetido em: Journal of Nanoparticle Research

Abstract

Nanocarriers can deliver drugs to tiny areas within the body and potentially become the bridge to fulfil the gaps between a drug function and its interaction with biological systems, such as human physiology. Nanotechnology's untapped potential comes from its capacity to manipulate materials, providing control over physical-chemical properties and overcoming drug-associated problems, *e.g.* poor solubility or bioavailability. Most protein drugs are delivered by parenteral routes, each one with its own challenges and specificities. Short biological half-life, large size and molecular weight, poor permeability through biological membranes, and structural instability leading to low bioavailability are some of the problems faced by biotech protein drugs. Some used strategies to overcome these problems, center either in altering the macromolecule itself or changing the formulation, in which nanotechnology emerges as a promising strategy. Nanoparticulated system should be carefully chosen and consider protein loading efficiency, final properties of nano-systems, and production conditions that prevent protein instability. Moving from bench to bedside is still one of the major bottlenecks to nanomedicine, and toxicological issues are amongst the most challenging to be overcome. This paper provides an overview of approaches that have been taking to delivery biotech drugs, the nanotechnology novelty involved, its toxicological issues and regulation.

Keywords: Nanobiotechnology; Drug Delivery Systems; Nanotoxicity; Regulation; Protein

INTRODUCTION

While the origin of biotechnology precedes both nanotechnology and the systemic delivery of biological drugs by a few decades, the very nature of targeting an active compound to specific sites bound researchers to study the potential of synergetic benefits from using the advantages of each of those fields.

Biotechnology developed quickly and outgrew from brewing [1] and production of simple molecules (such as lactic acid or acetone [2]) into the notorious penicillin [3] and other socially useful products [4], and finally culminated with the birth of genetic engineering [5].

Nanotechnology was conceptually conceived by Richard Feynman in his 1959's lecture "There's plenty of room at the bottom" [6], when he pointed it as an important field for future scientific researches to promote advances in a wide range of possibilities, including but not limited to engineering, chemistry, physics, medicine, and biology. It's almost untapped potential comes from its capacity to manipulate materials at the molecule and atom scale, which provides control over physical and chemical properties by creating molecular-scale structure which can be combined and form larger functional structures [7].

The capability to engineer materials with the desired physic-chemical properties represent the main contribution of nanotechnology to drug delivery systems (DDS): the ability to overcome drug-associated problems, e.g. poor solubility or poor bioavailability. These specially structured carriers can control precisely the release of drugs and/or limit the drug-interaction to desirable sites, which subsequently reduce unwanted side effects on other tissues[8].

Thereafter, the world witnessed a development of sophisticated tools able to manipulate atomic, molecular, genetic, and cellular processes to create structured nanocarriers smaller than 1 micrometre [9]. A nanocarrier is a synthetic or natural, normally biodegradable device made of polymers [10], lipids [11], phospholipids [12] or organometallic compounds [13]. These nanocarriers can deliver drugs to tiny areas within the body [14] and potentially become the bridge to fulfil the gaps between a drug function and its interaction with biological systems, such as human physiology [15].

2. BIOTECHNOLOGY AND DRUG DELIVERY

Effective drug delivery requires a certain amount of active substance reaching its target. Figure 1 illustrates the administration routes claimed by commercial biotech protein drugs and experimental ones. If the intended route requires absorption, most protein drugs cannot simply diffuse through barriers due to high molecular radius or hydrophilicity. In addition, mucosal and topical milieu can trap the molecule (mucous) or degrade it (enzymes, pH). Therefore, most protein drugs are delivered by parenteral routes, each one with its own

challenges and specificities. A detailed discussion of commercial formulations, including dosage form and administration route frequency, has been recently done [16].

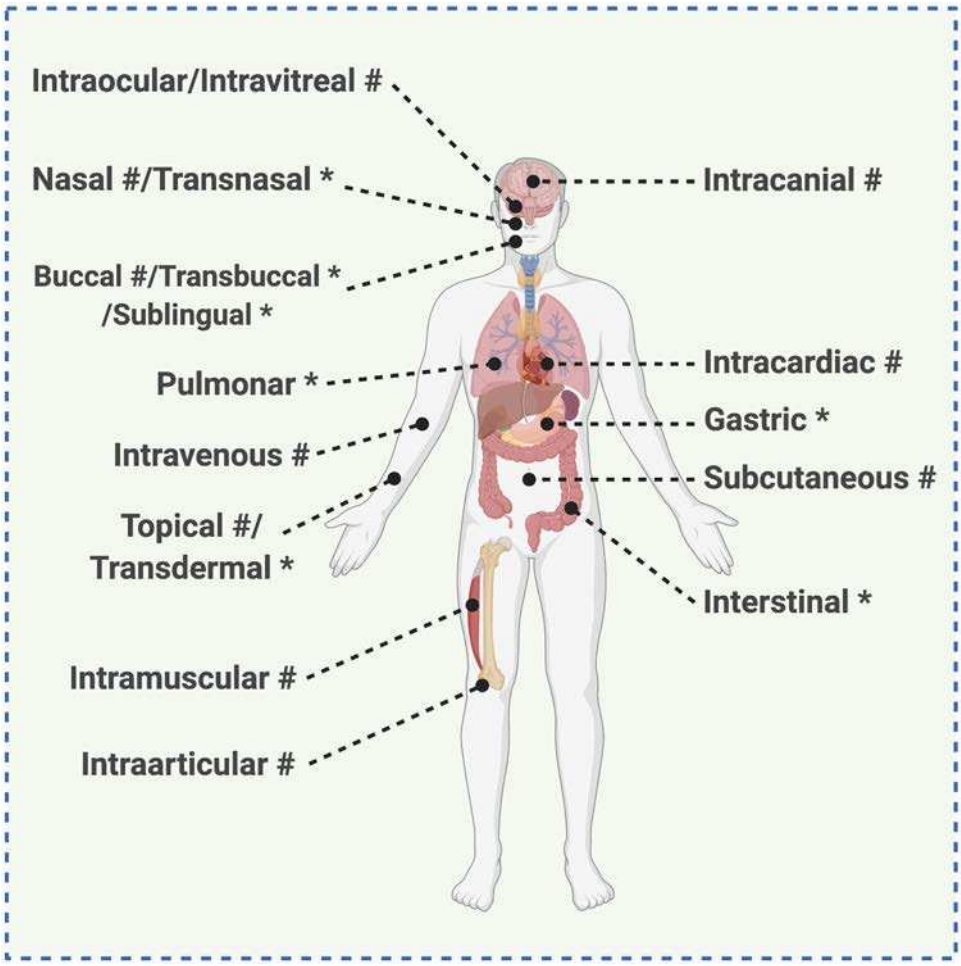


Figure 1. Anatomical indication of administration and absorption routes for biotech drugs, according to medicine claim [17]. #administration routes/anatomical application and *absorption routes. The exact anatomical site may vary for intravenous, subcutaneous, intramuscular and topical/transdermal applications. Human figure created by freepik and adapted with leg intern structures.

Noteworthy, there are ways to deliver drugs in situ during surgeries that will not be discussed here. For example, intraosseous/intramedullary administration of morphogenetic proteins embedded in nails, which are used in trials to accelerate bone recovery of tibial fractures [18].

2.1. Parenteral Drug Delivery

Parenteral route usage happens mainly when therapy needs a rapid effect, under oral administration impairment and unconsciousness, or if drugs are poorly absorbed/stable. The downside of parenterals relates to the pain associated with injections and the health professional requirement for most dosage applications [19]. Another relevant purpose is to direct therapy. Accordingly, intracranial injection can deliver antibody drugs to brain tumors without their systemic spread or blockage by blood brain barrier, both challenges of systemic routes [20]. Intra-articular injections of hydrolyzed collagen enhanced knee osteoarthritis traditional treatment [21]. Noteworthy, the application site may be too permeable or too dense; the first case promotes systemic distribution, whereas the second case do not allow drug full dosage application. As a solution to enhanced drug local dosage, the intravitreal solution of hyaluronidase breaks endogenous hyaluronic acid, facilitating the infusion of other drugs in the eye [17]. If the site is too permeable or vascularized, nano and microparticles can help with drug permanence, as discussed in the following topics. As these parenterals vary in anatomical application, formulation requirements vary and should be considered case by case.

The most used parenteral routes are intravenous, subcutaneous and intramuscular. Intravenous application leads to 100% bioavailability, but has strict particle, pH, viscosity and osmolarity ranges, demanding sterile solutions or suspensions as dosage forms. Particles may fail to permeate the endothelium or be phagocyted by blood monocytes according to their size, shape, surface and mechanical properties [22]. Molecule physiological clearance and immunogenicity can change with covalent attachment to polyethylene glycol-based polymers, like with several others. For instance, PEGylation of asparaginase enhanced protein circulation time, decreased dose strength and immunogenicity, and increased storage stability (allowing distribution of solutions instead of freeze-dried medicines, Oncaspar®) [17]. As a downside, repeated administration induces anti-PEG antibodies and consequent rapid drug clearance and/or allergy symptoms in some patients [23]. Commercial innovations for intravenous application relies mostly on excipients to enhance storage stability [16] and PEGylation refinement (linkers, directed modification and production process optimization [24].

Subcutaneous injections stand out among self-administration parenterals, especially due to insulin therapy. Patients reported the use of injection pens as a discrete and practical way for treatment. Even in hospitals, this route offers cost, time and contamination risk reduction, when compared to intravenous. There are already several monoclonal antibodies given by subcutaneous injections, but it is not certain that all molecules could be administered

this way. The drug is expected to present a decreased plasma maximum peak and may be more immunogenic than when administered as an infusion. Immunogenicity increase, as reported for subcutaneous trastuzumab, may come from the lymphatic system drug distribution, which is a common path for large molecules (>20 kDa) [25]. Other constrains refers to application of volumes up to 1.5 mL, isotonic and at neutral pH, to avoid pain and local damage [26]. On the good side, it favors application of depot formulations by implants or pellets, like human growth hormone or Insulin detemir. The depots can offer therapy for days, months or even years, increasing patient adherence to treatment and therapy control [27].

2.2. Non-Parenteral Drug Delivery

Non-parenteral routes with biotech drugs works mainly locally, due to poor and erratic absorption through physiological barriers. The oral route has the added challenge of the degradation milieu in the gastro-intestinal tract. Therefore, oral presentations aimed local action, like sacrosidase and pancrelipases [17]. Conventional topical DDS, such as ointments, creams or gels, mainly release the drug onto the skin surface, like the hemostatic agent thrombin alfa [17]. Skin permeation may happen according to the physicochemical properties of the drug; if the size and solubility are unfavorable, only limited uptake by the skin will occur [28].

Expressive efforts have been devoted to the development of various approaches to overcome physiological barriers. The outcomes include the development of a large repertoire of physical techniques and penetration enhancer compounds that facilitate drug penetration. Penetration enhancers, in general, promote drug diffusion by disturbing the structure of the stratum corneum and/or deeper layers [29-30]. Penetration enhancers in experimental research focus mainly on nanocarriers, as discussed further. Although some transdermal systems achieve systemic distribution, such as transdermal patches, none is approved for biological medicines so far, mostly due to low absorption in experimental reports. Physical methods can decrease the molecule size impact due to skin transitory disturbance, such as the painless microneedles. Insulin and other biologicals cross the skin this way, but human trials are still to determine the efficacy of such dosage form [31]. Other physical methods need an auxiliary equipment, like ultrasound and injectors, which exclude self-administration option and may induce pain [32]. It is important to note that insulin is in the borderline of peptide-protein in size terms, and therefore, even if the experimental non-parenteral dosage forms reach efficacy in insulin delivery, it might not work for antibodies and other large protein molecules.

Transmucosal drug delivery of large molecules requires mucosal retention, release from the delivery system in a sustained pattern and access of the drug to the drug-transport machinery in the epithelial cells or to the lymphatic/blood system [33-34]. In order to optimize the transport of these macromolecules across mucosal barriers, one of the approaches concerns nanoparticulate carriers, also discussed further in this work [35]. To deepen concepts concerning this promising route, we will focus on pulmonary delivery in the following topic.

2.2.1. Pulmonary drug delivery

Pulmonary delivery of biopharmaceuticals, specifically insulin, was reported soon after the hormone's discovery in 1924 [36]. Nonetheless, major advances in systemic delivery of biological drugs by inhalation have been seen in the past 20 years. Problems due to an insufficient understanding of the physiological fundamentals of aerosol inhalation and insufficient inhaler technology, as lack of dosing accuracy, efficiency and reproducibility, have been overcome [37]. Similarly to other non-invasive administration routes, most data are available for the inhalation of insulin [38]. Two inhaled insulins have been approved by the FDA – Afrezza® and Exubera®. However, the latter was withdrawn for economic reasons only months after its launch. One other product (i.e. AER-501) has recently completed several clinical phase 1/2a studies. In addition, an overabundance of other biomolecules has been tested in pre-clinical aerosol inhalation studies [41]. Albeit the mechanisms of absorption are not always fully understood, extremely rapid absorption and relatively high bioavailability are credited to alveoli high surface area, a dense network of capillaries and comparatively low levels of proteolytic enzymes, even for larger biopharmaceuticals up to 40 kDa [37, 42-43]. The relative bioavailability for Afrezza vs. Insulin lispro (based on comparison of area under the curve (AUC) over 6 h) is about 33%, which is approximately double the value reported for Exubera [44]. In some cases, the therapeutic efficacy of inhaled bio-drugs is limited by the rapid absorption of the drug, which may be overcome using advanced delivery systems to provide sustained drug release [45].

For the successful delivery of biological drugs via inhalation, there is a need of formulations with an appropriate inhalable form with sufficient stability and aerodynamic properties is critical 46. Peptides or proteins can be dissolved in a liquid, suspended within nano- or microparticles, liposomes or micelles, or formulated into a dry powder, and then inhaled using one of three main type of devices used for inhalation, namely pressurized metered dose inhalers (pMDIs), nebulizers, and dry powder inhalers (DPIs). It is generally accepted that

aerosol particles must present favorable aerodynamic diameter. On their flying way to reach terminal bronchi and alveoli, particles should escape from mouth or throat impact, which lead to particle swallowing and can happen with particles with more than 6 µm of aerodynamic diameter; and from exhalation on the next breath which happens with particles lower than 1 µm [47-48]. Aerosol particles smaller than 5 µm are able to clear the oropharyngeal impaction barrier, while particles smaller than 3 µm reach final destination [37, 47, 49-50]. Nanoparticles (NPs) can be aggregated to the microparticle size (1-5 µm) [51-52] or be embedded in an inert dissolvable matrix [53-55] such that release of individual NPs occurs on administration.

In the case of pMDIs, the therapeutic agent is dissolved or suspended in a non-polar liquefied propellant (i.e. a hydrofluroalkane, HFA), which provides the pressure and forces the liquid formulation out of the container through a fine orifice. For delivery of biological drugs, denaturation of the drug in the presence of HFA is a significant limitation, as is denaturation at the large air–liquid interface [56]. Successful formulations, therefore, use drugs co-formulated with a stabilizing excipient [57-58] or loaded into a polymeric microparticle [59-60] or microemulsion [61]. However, no commercial pMDI containing a macromolecule has come to the market as yet.

For nebulization, an active agent can be formulated as an aqueous solution or suspension which is converted into an aerosol by one of three types of nebulizer - jet nebulizers, ultrasonic nebulizers, and mesh nebulizers [62]. Limiting factors for nebulization are the limited stability of the drug in an aqueous solution and denaturation in response to the shear stress exerted during nebulization. The latter problem is exacerbated by the fact that, in the case of jet and ultrasonic nebulizers, 99% of the droplets generated are recycled back into the reservoir to be nebulized during the next dosing [63]. Furthermore, aerosols tend to be heterogeneous in terms of their particle size, which can result in poor drug delivery to the lower respiratory tract [64]. On the upside, to aid successful drug delivery, liposomes as drug carriers with a potential for controlled release and capacity to enhance stability of the bio/active material, may be delivered using nebulizers [65]. Human deoxyribonuclease (rhDNase, Pulmozyme®) was the first recombinant protein to be delivered via the pulmonary route in the form of a nebulized solution and is still the most widely used mucoactive therapy in patients with cystic fibrosis.

Where the protein/peptide to be used for pulmonary delivery is a low dose, high potency material, it may be delivered as a DPI formulation. Inert excipients (sugars, polyols, amino acids or organic salts) can be used to increase the volume of powder loaded and delivered from the DPI device and also to enhance stability and protect the protein structure in the final

formulation. Two hypotheses are described for the mechanism of stabilization – the glassy immobilization [66] and the water replacement hypothesis [67]. The former hypothesis states that stabilization is achieved by formation of an amorphous glass, whereby molecular motions and ensuing structural changes are minimized [68]. While the water replacement hypothesis, attributes stabilization to the formation of hydrogen bonds between the drug and excipient, replacing those between the sensitive component (biologic) and water, and thus maintaining the structural integrity of proteins [69]. The inhaled insulin products, Exubera and Afrezza, which are referred to above, are DPI formulations.

2.3. Delivery of Proteins and Peptides

Macromolecules, such as proteins and peptides, represent an opportunity for the development of new drugs [70], and progress in biotechnology field had increased the number of clinical useful ones [71]. Proteins and other macromolecules offer advantages as site target, specific mechanism of action and high potency [72]. However, these molecules' delivery suffers certain challenges as short biological half-life, large size and molecular weight, poor permeability through biological membranes, and structural instability, leading to low bioavailability [73-74]. Besides the activity reduction, proteins structural instability may also alter proteins immunogenicity [71, 75]. Even though it is associated with discomfort and pain, parental route is widely used for macromolecules delivery, once it allows maximum bioavailability [74,76-78].

In this scenario, efforts have been made to design a versatile therapeutic protein delivery system to overcome macromolecules formulation challenges. Addition of enhancers as sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC) and even the development of indigestible self-oriented system have been studied and proved as successful strategies to transform injectable proteins into oral dosage forms [79-80]. Other used strategies center either in changing the macromolecule itself (for example, mutations in protein structures or attachment of other molecules) or changing the formulation [16, 72], in which nanotechnology emerges as a promising strategy 73, 81-84. Nanotechnology and other used tools aim to overcome challenges imposed by macromolecules during formulation, supply chain (including transportation, storage and shelf life), and clinical practice (reduce dosing frequency, ease application, decrease adverse events, and increase patients compliance) [78, 83, 85].

Using nanotechnology to deliver macromolecules has been examined and considered a strategy well accepted over decades. A review study in 2018 pointed [54] nanotechnology-based products approved by FDA, of which around 25% have macromolecules as active ingredients [85]. However achieving a successful system that reach market remains challenging, and there are many initiatives currently in progress in terms of clinical trials [85-86]. One of the reasons for this may be technical difficulties encountered during formulation process, including protein denaturation during formulation, low encapsulation efficiency, burst or incomplete release, and formulation complexity [86].

Unlike low-molecular weight actives, proteins have secondary, tertiary and even quaternary structures, which are closely related with their efficacy. Thus, their structure should be maintained through all delivery systems formulation steps and also while they are released from those systems, being of primary importance and very challenging [71, 87].

3. NANOTECHNOLOGY AND DRUG DELIVERY

Nanoparticulated system should be carefully chosen and consider protein loading efficiency, production conditions, which should be mild to prevent protein instability, and final properties of nano-systems. Main features, advantages, drawbacks and recent developments of protein-loaded nanostructures have been reviewed by Pachioni-Vasconcelos, et al. [88].

Bromelain, a proteolytic enzyme found in plants from Bromeliaceae family, imposes the same challenges as other proteins [89-90]. Despite challenges faced, bromelain has been applied in pharmaceutical nanotechnology field, playing different roles as reviewed by Ataide, et al. [91]. With chitosan NPs, for example, bromelain was used as surface modifier and encapsulated as active ingredient [91-92].

3.1. Nanotechnology for Targeting and Triggering Drug Delivery

There is a search for decreasing off target effects, higher treatment effectiveness, better patient compliance and an independent control off its release or not. Delivery for local effect or local administration are appealing when there is a desire to avoid the side effects [93].

Depending on the disease, the local application can be difficult requiring systemic administration even though, a local effect must be achieved. In order to not act on the whole body it is important to have a target control for the drug to accumulate at a desired location [93], and delivery systems in the nano-range can be specific or target-designed.

When we address conventional controlled release systems, a potential downside is the monotonic release achieves an effect in a known manner that cannot be adjusted according to the necessity of the patient. Cases like diabetes or chronic pain, the ability to switch drug delivery on and off at patient's will would be beneficial [94].

The development of stimulus-responsive or triggering particles leads to an increase in treatment efficacy and a decrease in off-target effects [95]. The stimulus for drug release may be physical, chemical or biochemical, causing structural changes in the particle (degradation, erosion, diffusion). This stimulus can be both endogenous, inherent to biochemical variations caused by pathology (reactive oxygen species, pH, temperature), and exogenous, from external action (heat, ultrasound, light, electrical and magnetic fields) [94, 96]. This diversity of stimuli for drug release can be explored for triggered, targeted drug delivery and control of release kinetics [94-96].

There are several mechanisms to control the release of the active ingredient, such as modulation of the particle surface, modification in the polymeric design, use of a stimulus-responsive polymer already established, among others [95,97]. Polymers degradation can be modulated according to the desired degradation rate, ranging from hours to years, as required by formulation [37,98]. These modulations can be done in order to tune drug release profile or direct its delivery and these modifications can come from material modification: blending polymers, changing its molecular weight (MW), surface functionalization as PEGylation or other coating or adding surface functional groups (e.g., -SH, -NH2, -COOH), changing surface charge, adding target ligand (e.g., antibody, peptide, and aptamer) [99].

Considering the relevance of biomolecules drug delivery and the potential of nanostructures as its carrier, we focus on systems that allow biomolecules encapsulation and, therefore, protection against degradation and immunogenicity, namely liposomes, polymeric NPs, and solid lipid NPs. In this sense, several modifications can be developed on the surface of these nanostructures, aiming greater specificity, stealth, activity, among other approaches, as can be seen in Figure 2.

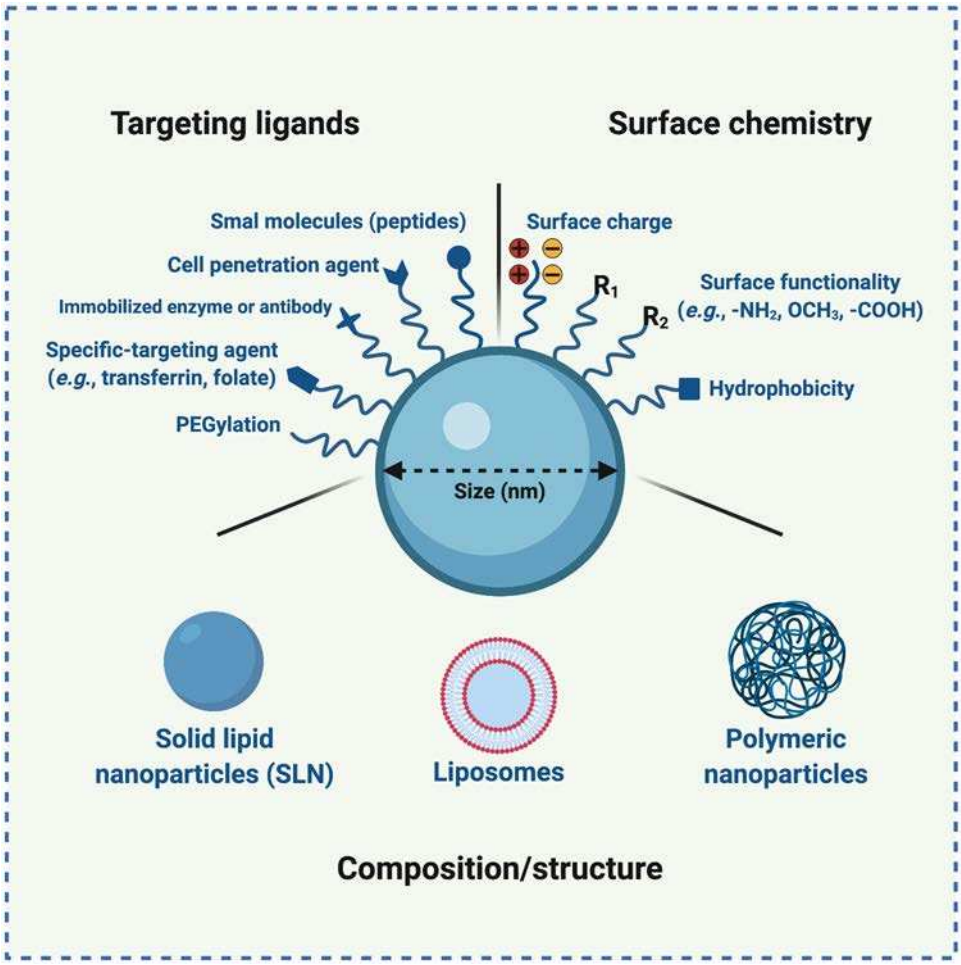


Figure 2. Schematic diagram of solid lipid nanoparticles (SLN), liposomes, and polymeric NPs, which can be decorated with multi-types agents and surface-targeting molecules. Multifunctional NPs for drug delivery combine a specific set of targeting agent with NPs for imaging, a cell-penetrating agent, a stabilizing polymer to ensure biocompatibility, a stimulus-sensitive element for drug release and the therapeutic compound.

3.1.1. Liposomes

The composition of liposomes is similar to cell membranes. Consisting in one or more phospholipid bilayers, with amphiphilic molecules, hydrophobic tail and hydrophilic head, self-assembling in a vesicle in the presence of water. Their size can vary from 20nm to several μm in diameter, being able to carry either hydrophilic or lipophilic drugs based on the affinity of different parts of the vesicle, aqueous core or bilayer membrane respectively [100]. The main component of a liposome is phospholipid and the type of phospholipid used can affect characteristics as zeta potential, encapsulation efficiency and even vesicle size. It is also really

important the presence of sterol molecules to guarantee liposome stability, affecting its fluidity, vesicle size and even the polydispersity index (PDI) [101-102]. Liposome's bilayer composition can be modulated to reach desirable properties from different particle size to a long-circulation liposome.

There are four generations of liposomes. The first generation, also called the conventional liposome, are formed by either anionic, neutral or cationic phospholipids. This generation showed some disadvantages, as fast clearance from the bloodstream by binding with proteins or macrophages uptakes and undergo through chemical degradation or aggregation, that the next generations pursued to overcome. Second generation are the Stealth liposomes and the stimuli-responsive liposomes. Stealth liposomes are liposomes with polymer coating aiming a prolonged circulatory half-life. Third generation liposomes are ligand-targeted liposomes and the PEGylated ligand-target liposomes. Finally, the last generation comprises the theranostic liposomes[103], which are made of targeting ligands/stimuli-responsive lipids, phospholipids, imaging agents and therapeutic components having both therapeutic and diagnostic functions in one, including controlled drug release [104].

In the biological delivery field, liposome structure was used to form a virosome in the Inflexal® V, a vaccine for Influenza viruses, that mimics the structure of the native influenza virus [105].

Using antibody fragment-conjugate to decorate the particle, Yang, et al. 106 made a ligand-targeted liposome which enhanced the cellular uptake in pancreatic cancer cells, and can induce significant apoptosis of cancer cells. Also aiming cancer cells, a transferrin-targeted liposome was reported to delivery resveratrol for the treatment of glioblastoma. Transferrin-targeted resveratrol-loaded liposome enhanced therapeutic effects comparing with a non-targeted one [107].

The stimuli-responsive liposomes have been used for many proposes. Cullion, et al. [108] developed liposomes for local anesthesia using ultrasound as a trigger for on-demand release. Also looking for a better control on duration, timing and degree to match the patients need, a photo trigger has been used as stimulus for anesthetics release [109-110].

As a drug delivery system, liposomes are used for a controlled release but it is important to take in account its short half-life, its low biological and physical stability compared

to polymeric particles mainly because phospholipid oxidation and its high cost production [102, 111].

3.1.2. Polymeric nanoparticles

The search for advances in new polymeric materials and the development of drug-delivery systems has been stimulated aiming a new approach in the treatments focusing in effectiveness and patient compliance. Biodegradable polymeric particles are one of the possible alternatives for biodegradable polymers use. Production of stable particles is fundamental for drug delivery, which regulates therapeutic effects, biological activity of the encapsulated drug, rate of drug release and degradation time [37, 112].

Polymers physicochemical properties can be modulated according to the desired degradation rate, ranging from hours to years, to tune drug release profile or direct its delivery as required by formulation [37, 78, 98, 113].

NPs surface chemistry dictates their interaction with biological systems [114]. Therefore, surface modification strategy, using designed molecular ligands, produces unique NPs, modulating these systems biological behavior, providing targeting capabilities, improving their stability, being an alternative to overcome challenges in pharmaceutical area [114-117].

The use of biodegradable polymers in drug-delivery is of great relevance because they are biocompatible and decompose into non-toxic natural products [118].

Aiming to selectively neutralize and capture Interleukin-6 (IL-6), a crucial pro-inflammatory mediator in arthritic joints, Lima, et al. [119] developed a natural biodegradable chitosan-hyaluronic acid NP biofunctionalized with anti-IL-6.

3.1.3. Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) were introduced in 1991 as an alternative system of bioactive loading, in order to offer an option against traditional methods such as liposomes and nano and micro polymeric particles [120]. The high surface area, high carrying capacity of

bioactive and nanometric dimensions are unique properties that make the method especially attractive due to its potential for improvement of drugs, nutraceuticals and other materials [121].

SLN are colloidal carriers composed of one or more lipids of physiological origin, dispersed in water or in an aqueous solution containing surfactants. The substitution of liquid lipids by those in the solid state allows disadvantages associated with oil droplets to be eliminated, improving bioavailability and reducing variation in the plasmatic profile of the bioactive [122], combining virtually all the advantages presented by polymeric NPs, liposomes and lipid emulsions.

Among these advantages, it stands out its versatility of application; better control of the kinetic behavior of the encapsulated bioactive; high long-term stability; simplicity of production, especially when compared to the polymer particles; and relative ease of scale-up of existing production methods [123-125].

The physicochemical characteristics of SLN depend on the preparation method chosen, but generally they are based on the fragmentation of a lipid matrix coated with a non-toxic surfactant [126] using high pressure homogenization [127]; ultra-sonication [128]; supercritical fluids [129]; solvent evaporation [130], etc.

Oliveira, et al. [131] developed a paclitaxel-loaded magnetic SLN. This particle is triggered by magnetic hyperthermia, increasing the temperature of the particle's core (made of magnetite – Fe3O4) releasing the drug. It is a temperature-dependent release, triggered by magnetic field. This result could lead to a tunable paclitaxel delivery system and improve cancer treatment by increasing the drug's bioavailability. A targeted SLN coated with hyaluronic acid (HA) was developed to delivery glucocorticoid prednisolone (PD) to inflamed joints in rheumatoid arthritis (RA). HA binds to hyaluronic receptor CD44, which is over expressed on inflamed joints [132].

3.1.4. Novel nanocarriers

Naturally, the synthetic polymers used on the early DDS were gradually replaced by their biotechnological counterparts, such as chitosan [133], starch [134] and fibroin [135], due to its non-toxicity, low immunogenicity, biocompatibility and quick biodegradability [136].

These biopolymers, as discussed earlier in this manuscript, can be conformed into nanostructures that allow the existence of a wide range of highly customized drug delivered systems, despite a some well-known limitations [137]. However, their combination with liposomes originate a fairly novel drug delivery system known as lipid-polymer hybrid nanoparticles (LPHNs), which has more benefits as compared to liposomes and NPs [138].

The structure of LPHNs varies significantly, but can be described as a multi-layered particle in which both lipoidal layer and polymeric layer confer biocompatibility, high loading efficiency [139] and can provide stability by reducing outward diffusion from the encapsulated drug [140]. While the inner layers are linked through Van der Walls forces, hydrophobic interactions and several other noncovalent forces, the outer layer is made up of – or coated with – a water-soluble polymer which enhances the steric stabilization and prolongs in vivo circulation time [141], as illustrated in Figure 3. As a result, LPHNs got rapidly involved in robust drug delivery platforms due its highly structural stability, storage stability and exceptional controlled release, outperforming other nanocarrier systems [139].

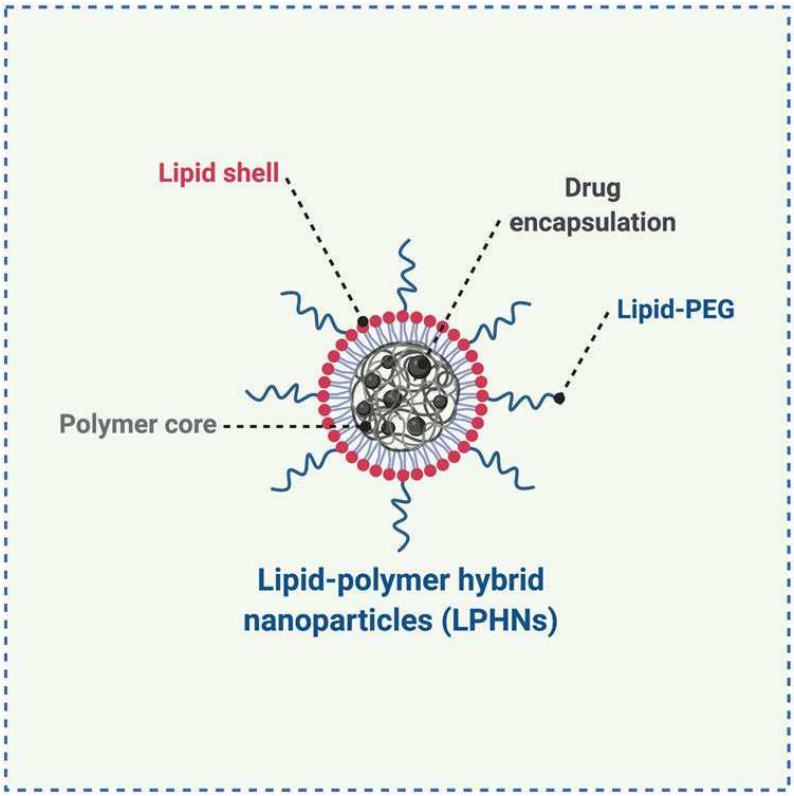


Figure 3. Schematic illustration shows the formulation of lipid-polymer hybrid nanoparticles (LPHNs). These NPs comprise a hydrophobic core (e.g., polylactic-co-glycolic acid) or PLGA), a hydrophilic PEG shell, and a lipid (e.g., lecithin) monolayer at the interface of the hydrophobic core and the hydrophilic shell (based on Dave, et al. 139).

One early-stage proof-of-concept studied the concurrent treatment of chemotherapy and radiotherapy, developing small LPHNs named ChemoRad co-encapsulating chemotherapy. These NPs had docetaxel in the PLGA core and radiotherapy agents (indium-111 or yttrium-90) chelated to a 1,2-dimyristoyl-sn-glycero-3-phosphoethanolaminediethylene-triamine-penta acetate (DMPE–DTPA) in the lipid shell [142]. Their report shows that there was an increase of cytotoxicity against prostate cancer cells and the radioactive isotopes had no negative effect towards encapsulation or controlled released of the drug.

Doxorubicin-loaded LPHNs were produced by the self-assembling modified precipitation method and had its controlled delivery evaluated, as well as its therapeutic and physicochemical properties. Results indicated better safety and both enhanced antitumor effect and cell uptake, which may improve therapeutic effects in the tumour microenvironment [143].

The development of novel NPs structures and their production methods are then followed by investigations regarding its application, which benefit from a myriad of compounds suitable for its production, as well as the drugs and treatments that makes the best use of its inherent properties. Consider the benefits of an active targeting delivery system, differing from its passive counterpart by not depending on the physical properties of the particles and/or target tissue, and which can be engineered to deliver drugs to their specific targets by antibodies, peptides and other specific ligands [144].

In short, these particles are engineered to change membrane permeability or its selectivity when under a specific wavelength. Trabulo, et al. [145] modified the surface of poly(ethylene oxide)-poly(D,L-lactic acid) NPs by creating a photocleavable caging group with high photocleavage efficiency at 400nm. The caging group was removed by the photo-activated bond cleavage and allow the peptide-coated surface to readily bind to nearby cells.

3.2. Nanotechnology and Drug Use Safety

Nanotechnology field has presented a potential strategy to overcome challenges that act in precision medicine, one of them works with the possibility to reduce the toxic effect of pharmaceuticals and in the specific treatment of several serious diseases (such as cancer, diabetes, among others). In the last years, the nanotechnology safety received wide attention of scientists leading to technological revolution. In addition, it has provided a great opportunity in

a variety of conventional research areas, such as chemistry, physics, pharmacy, engineering, biotechnology and mainly in biomedicine and health sciences, as can be seen in Figure 4. In this sense, this approach has allowed combining platforms focused on solutions to challenges with open questions and issues still poorly understood [146-148].

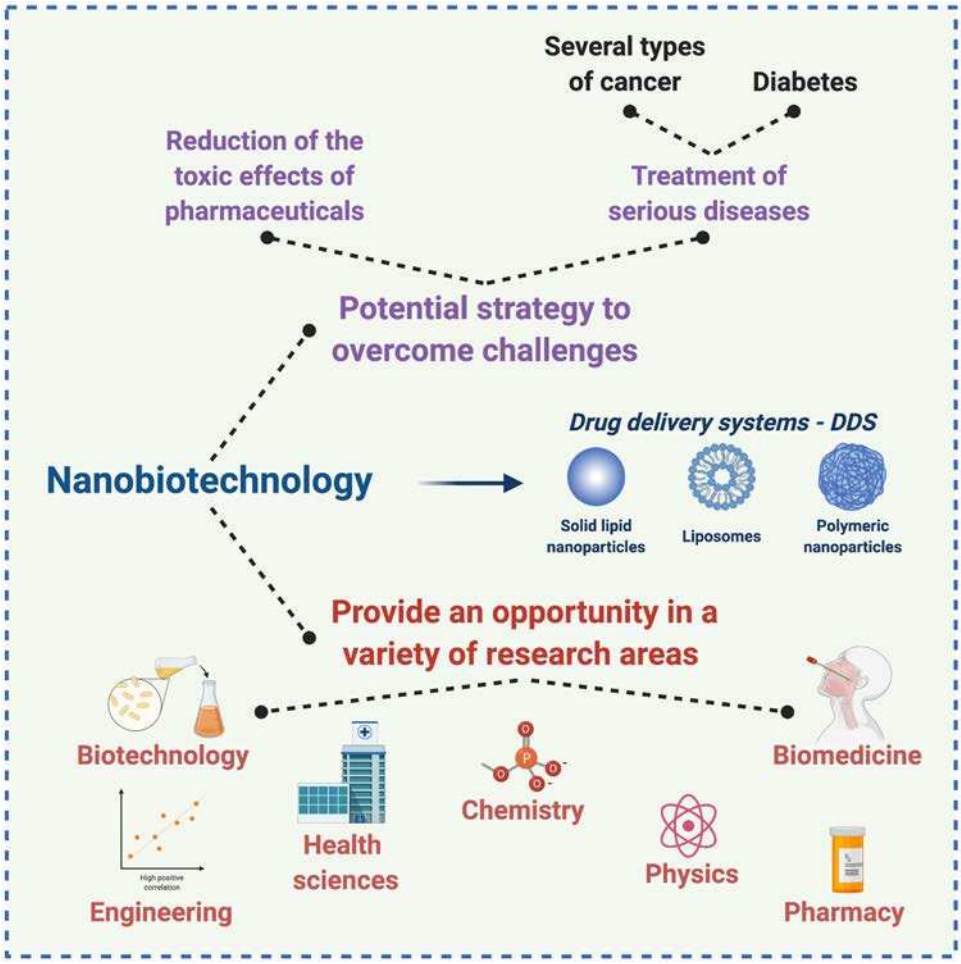


Figure 4. Schematic illustration of the nanobiotechnology potential and its relationship with different research areas.

The relevant nanodevices and engineered nanomaterials (NMs) at the atomic, molecular and macromolecular nanoscale level have been developed and conceived. The vastness of NMs includes nanoimaging agents, theranostics, and nanopharmaceuticals, triggered to an advance of innovation in treatment/prevention, as well as imaging/illness diagnosis [149-151].

The safety of substances and products placed on the market in the European Union (EU) is regulated by various legislative acts, even if the legislation does not explicitly mention

NMs. In addition, the legislation on cosmetic products (#1223/2009), consumer food information (#1169/20119), active and intelligent materials and articles (#450/2009), plastic food contact materials and articles (#10/2011) and Biocidal Products (#528/2012) explicitly address NMs [152-153]. These legal acts introduce specific provisions for NMs, and some require labeling (listing) of ingredients that are in the nanoform and, for cosmetics, a register of products containing NMs.

Despite a strong demand to promote the NMs, their specific characteristics have raised substantial considerations for regulatory and manufacturing activities. Moreover, there has been a widespread lack of specific procedures to characterize the NMs at biological, physiological, and physicochemical points, which in several situations may have been responsible for their failure in the late clinical trial phases. For this reason, the regulatory requirement issue for the nanostructures has been gradually challenged by key issues, which is a chance to provide clearer direction for their progress and expansion [154-156].

3.2.1. Large-scale process for nanotechnology

In recent years the nanoscience has attracted considerable attention, as it expects the impact that nanostructured materials can have on improving the quality of life and preserving the environment [157-159].

The synthesis utilizing different sources can generated a great variety of NPs with different properties, as highlighted in Table 1. Due to different properties and applications area, the advance of nanoscience is expected to lead to an industrial revolution, also being a tool that stimulate not only the exploration of new phenomena and new theories, becoming the new impulse of economic growth in this century. Through the years and with intense research effort, there are different ways to enhanced a nanotechnology process with great variety of NPs, one them is approaching the laboratory nanotechnology and their use in large-scale process for different applications [160-163].

Table 1. Nanoparticles (NPs) generated with different properties obtained from different sources.

Different sources	NPs with different properties	References
Inorganic or organic compounds	Antimicrobial	(Lead e Wilkinson, 2006; Heiligtag e Niederberger, 2013; Ravichandran *et al.*, 2019)
	Antioxidant	
	Photocatalytic	
	Drug delivery and other properties	

Though the large-scale nanotechnology has been a growing challenge, requiring precise control of all manufacturing processes and procedures, because the NPs should offer a uniform size, long shelf-life, without agglomeration. Moreover, the process should be reproducible and sustainable to deal considerably with the commercialization [165-166].

Paliwal, et al. [167] summarized features and limitations of various methods of NP production as high-pressure homogenization, microemulsion, nanocrystallization, membrane extrusion, supercritical fluid technology, microfluidizer and others.

On the basis of literature supporting this review, numerous methods have been developed in order to produce NPs in large-scale, however, the choice of methodology to produce NPs depend on the type of material applied such as polymer, lipid, and metal; market demands; sustainability, eco-friendly, profitability and costs [160, 168-170].

3.2.2. Regulatory requirements for nanotechnology

A considerable number of NMs approved for the biomedical sector have emerged in recent years; however, the lack of specific general protocols for the characterization of these products and for preclinical development has become an additional obstacle in clinical practice. Regulatory agencies need to approach in order to achieve a more collaborative work, as well as global regulatory trends need to be defined and standardized. Despite this several important

steps have already been taken in the last 5-10 years. As an option, the approaches employed for the development of established or conventional medicinal products have been regularly altered to assess the compatibility and toxicity/safety of nanostructures [156, 171-172].

According to [173] there are global efforts to regulate and address the safety and production use/handling of nanotechnology and NMs either by non-binding or by legislation guidance and recommendations. Working teams have been formed by regulatory agencies from the USA (FDA), Japan (Pharmaceutical and Medical Devices Agency - PMDA), and European Union (EMA), and worked in collaboration. In parallel, major pharmaceutical corporations have also augmented their attention in clinical development and proof of concept of these materials. Overall, these aspects will contribute to clearer methods for identifying the efficacy and safety of novel nanotechnology-based treatments and medicines. However, at the present time there is no material of legislation completely devoted to regulation of NMs [174]. Present legislation has been considered by many countries specific and sufficient enough to regulate nanotechnology/NMs; nevertheless, minor changes have been suggested by several stakeholders, including the European Parliament, and non-governmental organizations [175-178].

More specifically, significant regulatory issues to be considered for NMs include a formal definition of the term "nanomaterial", specific information requirements for risk management, provisions, and risk assessment, authorization or registration procedures to raise the traceability and transparency on the commercial employment, for instance by notifying or labelling to a register for materials consisting of NMs [175, 179-180]. Moreover, as well highlighted by [175]: "NMs have to be categorized for hazardous properties if they display the corresponding properties according to the Regulation on Classification, Labelling and Packaging (CLP). Products containing NMs or hazardous substances depending on the concentration limit and concentration have to be labelled", based on [181].

In general aspects, one of the obstacles inherent the regulation of NMs is clearly associated to their specific characteristics. Following the large extent of evidence gathered for polymeric micelles, polymer therapeutics (including other polymeric systems), liposomes, and in view of the regulatory issues connected to their development and design, the clinical employ of these complex and sophisticated nanostructures is strongly based on extensive characterization, assessment, and understanding of main properties [156]. Their properties can, in fact, be easily changed, not only by small alterations in manufacturing procedures but also by minimal changes in raw materials. Thus, even though these changes might culminate in limited

modifications in the biological properties, structure, and biodistribution patterns may be considerably altered [150]. Moreover, as scientists usually incorporate or encapsulate pharmaceuticals or biopharmaceuticals, targeting molecules, as well as imaging and tracking compounds to NMs, robust techniques and new quality control tests have to be established and developed in order to better characterize and monitor not only their physicochemical features, such as size and polydispersity index (PDI), charge and morphology, but also to evaluate their behavior, as drug release, protein binding, specific cellular uptake, metabolism analysis, among others. These methods would further allow connecting the modifications with a consequent effect on biocompatibility, biological properties, and therapeutic effect and with overall physicochemical alterations [156, 182-183].

It is essential to control and identify the critical points during each manufacturing process. This case is another obstacle in the clinical and development translation of NMs. Major questions associated with their manufacturing face current pharmaceutical innovation at production facilities, challenging their scale-up possibility, especially, due to the considerable variety of properties of new materials/biomaterials. Thus, through of process analytical technologies will ensure an on-line/at-line quality assessment approach applying concepts of quality-by-design. Hence, anticipating and knowing the most critical steps of production facilitates the employment of automated methodologies to determine problems as they happen in line [184-185].

In addition, prior to the marketing of NMs, it will be vital to carry out pharmacoeconomic evaluations to show the additional economic and social value of these new products when correlated to established or conventional treatments. Important indicators such as the costs associated with future consecutive hospitalizations or the increase in life expectancy should also be taken into account in the concept and design of new NMs [184, 186]. It also should be consider in this step, the nanostructures potential toxicity and immunological deleterious effects [187-189].

Based on considerations mentioned above, we believe as also highlighted by [190-191] that the three scientific fronts: nanotechnology, nanotoxicology and nanomedicine are the corresponding areas of studies aimed at the enhancement of human life: nanotechnology has a bright future with several approaches in many fields. Nanotoxicity and nanomedicine provide for the required safety assessment of nano‐enabled products, as well as will develop applications for diagnostic, novel therapeutic, and preventive measures, respectively. Great

achievements based on nanotechnology and nanomedicine await us in the near future. Although many challenges still exist in the order to recognizing and get it right and avoiding the potential risks related to these new discoveries, where nanotoxicology will play a key role. Therefore, successful development on this theme both in the present and in the future will take place through a multidisciplinary team approach with physicians, bioengineers and pharmacists that must interact and work in collaboration.

3.3. Nanoparticle Toxicity in Therapeutic Delivery

Moving from bench to bed is one of the significant obstacles to nanomedicine nowadays. The translational nanomedicine faces many hurdles; toxicological issues are among the most challenging to be overcome [192]. Ironically, to date, the main advantage nanomedicine had achieved is a reduction of the toxicity of drugs [193-195], rather than improving the therapeutic efficacy. Moreover, recently, two magnetic resonance imaging (MRI) contrast agents (Feruglose and Resovist) containing NPs have been withdrawn from the market due to safety concerns. Therefore, among challenges and uncertainty, the development of nanomedicines needs more efficient ways for assessing toxicity and models that more accurately resemble the in vivo environment [192]. Even though from all the adversities, during the last years the number of nanomedicines that entered the market increased, reaching more than hundred [196-198]. Improving the toxicological issues is for sure one of the bricks in paving the way for the establishment of nanomedicine, helping to meet urgent clinical needs.

It is well established that the side effects triggered by nanomedicines result from an interplay between the physicochemical characteristics and the biological environment [199-201]. Parameters such as composition, size, shape, and surface charge are essential for determining nano-cell interactions [202-204], internalization pathways [205], intracellular biopersistence [206], to thus determine the toxic potential of nanomaterials [207-208]. After intravenous administration, NPs reach the vascular interstitium and endothelium cells [209-210]. In the interstitium, NPs promptly interact with the proteins of the serum forming a “crown of biomolecules” - corona protein dependent on the physicochemical characteristics of NP and this interaction will determine their biological identity as well as the toxicological profile [211-213]. Hence, for injectable formulations, it is essential to evaluate platelet aggregation [214] and agglomeration of particles [215] to avoid fine capillary obstruction [216]. Finally, the intracellular biopersistence of NP, increased circulating time, and interacting with tissues [217] can affect various organs such as liver, lungs, kidneys and even cross the blood-brain barrier [218-219] promoting particle

accumulation by disrupting the orchestrated functioning of metabolism [207]. These side effects are only a brief overview of the impact of therapeutic delivery systems in vivo.

During the past years, many types of nanomaterials have been developed, improved, or modified to achieve more efficient and less toxic DDS, including polymeric, lipidic, and metallic NPs [220-221]. Many toxic effects are linked to the production or functionalization of the NPs such as sterility and endotoxin contamination, residual manufacturing components, the biocompatibility of components, batch-to-batch consistency, NP in vivo biodegradability and biodistribution, and drug release rates; which have been reviewed elsewhere [222]. Surfactants, emulsifiers, and detergents at high concentrations improve shelf stability but generate toxic effects on cell membranes and also deserve to be carefully considered during the optimization phase of development [223-224]. Next, we will briefly discuss the singularities about the toxic potential of the main categories of DDS.

Polymeric NPs produced from biodegradable polymers, whether synthetic, natural or combinations of both are intensely described as drug carriers and demonstrated being safe in both in vitro and in vivo applications [225-226]. Widely used synthetic polymers such as poly-D,L-lactide-co-glycolide (PLGA), used in many FDA-approved drug products, due to the breakdown in lactic acid and glycolic acid [227-228]. Likewise, ester poly-ε-caprolactone (PCL) can be hydrolyzed at physiological pH allowing the degradation [229]. Natural polymers, in general, present low toxicity and have low immunogenicity. For example, the high availability of functional groups present in gelatin allows a wide range of chemical modifications to carry hydrophilic drugs [230], and human serum albumin (HSA) is useful for delivering hydrophobic drugs, with the advantage of binding to active tumor cell receptors [231-232]. Chitosan, a cationic polysaccharide with mucoadhesive properties, is capable of permeating cellular junctions for greater distribution of drugs [233-234]. Arguably, the non-toxic profile of natural polymers can be associated with low cellular uptake, biodegradability, and biocompatible monomers [235]. Nevertheless, attention must be paid to the polymeric degradation that in biological environment could lead to toxic effects such as reactive oxygen species and TNFα production, in particular synthetic polymers [235].

Lipidic nanoparticles, including liposomes, SLN, and nanostructured lipid carriers (NLC) are widely regarded as low toxic and biodegradable [236-238]. Conversely, cationic NPs that are compromising of the lysosomal membrane, used in drug or gene delivery, could also lead to the release of proteolytic enzymes, such as cathepsins, into the cytoplasm. Consequently,

these enzymes could damage mitochondria, leading to activation of apoptotic proteins (e.g., caspases 9, 3 and 7) and ultimately to cell death [222]. Liposomes and micelles are non-toxic at low concentrations; however, at higher doses they could lead to undesirable effects, probably related to cationic surface loading [239]. In vivo, the administration of lipid carriers does not seem to have toxic effects; nevertheless, genotoxic effects have been found in several studies since the liposomes can interact with biomembranes [240-241], altering the intracellular environment, generating oxidative stress. Additionally, inflammatory signaling molecules such as IL-6, CCL2, and CXCL2 were activated, indicating systemic disturbance [239]. In some cases, subtle effects may not lead to cell death, and these alterations can remain unnoticed. Nonetheless, these alterations can be particularly important for some tissues, such as disturbing in neuronal exocytosis (e.g., brain delivery), or chronic effects (e.g., long-term exposure), and alterations in cell signaling that are related to chronic diseases. Therefore, lipid nanoparticles should also be carefully examined for these minor effects, even at low concentrations.

Beyond the most common side effects described before, some shortcomings still need to be tackled and can be the focus of future research. For intravenous administration, much attention has been paid to the impact of nanomaterials in the reticuloendothelial system (kidney, liver, lung, and spleen) because they are responsible for the most significant part of the clearance of NPs [242]. But even retaining small percentage of nanomaterials, other organs can have considerable toxicity or also lead to a systemic response. Albeit being considerably less toxic, biodegradable NPs (e.g., lipid and some polymeric NPs) have not been properly investigated regarding long-term exposition, biopersistence, and repeated administration. Additionally, we have a very limited knowledge about the interaction of nanomaterials and other therapeutic molecules (e.g., drugs, genes, antibodies) and how the administration of a nanomedicine could interfere on their therapeutic effects. These aspects are crucial for regulatory agencies taking decision, another hurdle faced by translational nanomedicine discussed above.

Some alternatives have been employed to decrease or manage the toxicity NPs for therapeutic purpose. The most commonly employed is the PEGylation, commonly used for increasing the circulating time, but care should be taken when using PEG because many studies have demonstrated that it can triggers immunogenic response, compromising the current treatment and the future use of applications containing PEG [243].

Although some nanomaterials have little toxic effects, it is rare the nanomaterial that has no impact in vivo. Therefore, one strategy would be taking advantage of transient or minor side effects for therapeutic purposes. Many nanomaterials are described to induce an immune response, which could be used to enhance the immune response against cancer (i.e., immunotherapy) [244-245]. Recently, few strategies have been developed to control the toxic effects of NPs, resembling an antidote; this could be used as a last resort, and more strategies like these are desired for controlling therapeutic, occupational, accidental or self-inflicted intoxication related with nanomaterials [246]. Hence, there is no straight forward solution for the side effects, and solutions for managing should be tailored for each type of formulation.

To reduce the use of animals testing and achieve a better prediction on the toxic effects in vivo, more relevant in vitro models must be used and improved. One alternative is the use of 3D models, which were developed to overcome the limitations of monolayer cell culture (Figure 5), because they better resemble in vivo conditions for the delivery efficiency of molecules (i.e., drugs, genes, proteins) [247]. This model has been successfully employed to investigate the toxicity of NP towards blood vessels [248]; breast tumor connected to hepatic organoid for pharmacological metabolism [249]; paracrine signals of stem cells for neuronal regeneration [250]; and mimic in vivo administration of neurotropic NPs [251]. Thus, the use of more relevant models can be determinant in helping translational nanomedicine overcoming the toxicity limitation.

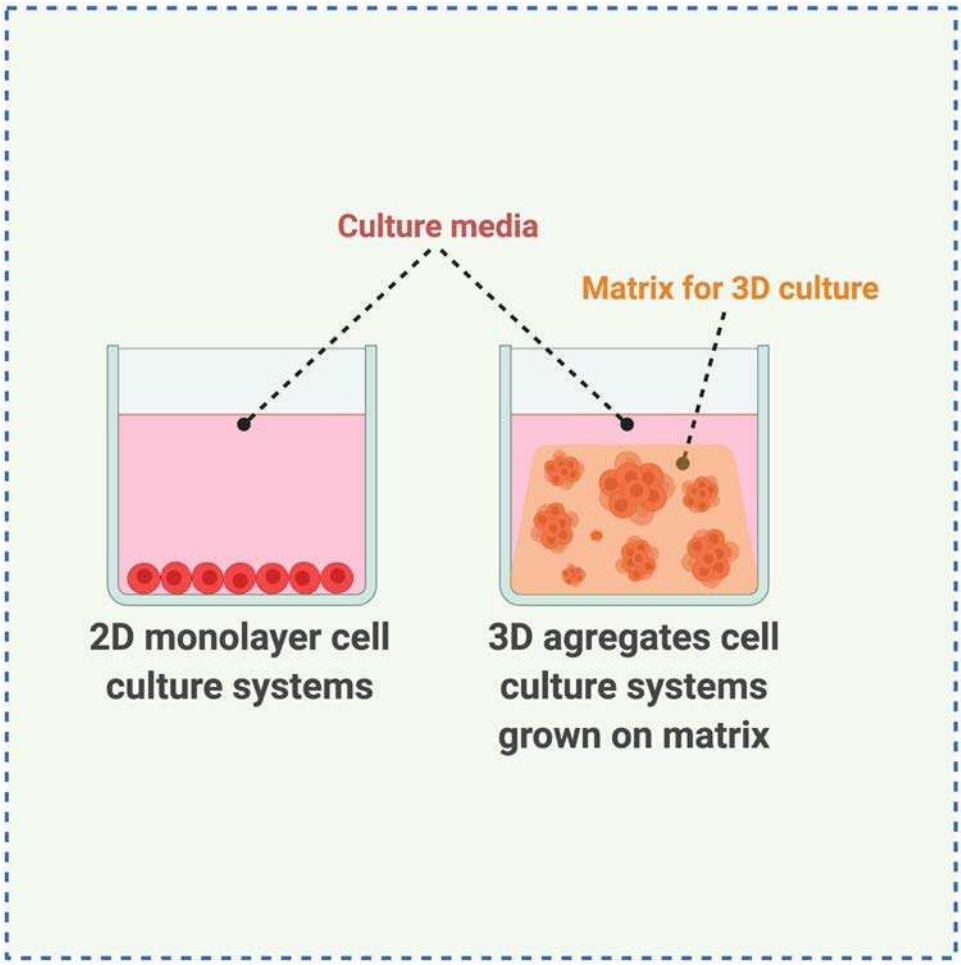


Figure 5. Schematic diagrams of the traditional two-dimensional (2D) monolayer cell culture systems and typical three-dimensional (3D) cell culture systems: cell aggregates embedded and grown on matrix (based on Chaicharoenaudomrung, et al. [252]).

4. LATEST DEVELOPMENT AND PERSPECTIVES

There are countless challenges to overcome, particularly given the number of obstacles in the process to make nanocarriers suitable for the treatment of patients. These problems are often so depending on the structure and physical-chemical properties of the carrier that problems are bound to differ in nature, ranging from poor drug solubility [253] to the design of production lines for large scale production [254]. Nevertheless, the pharmaceutical industry has clearly recognized the benefits brought by nanotechnology and its potential to expand the current drug markets, consisting of a strategic tool to repack – and reassess – classic drugs and provide the means to compete with generics after patent expirations.

The combination of a biotechnologically-produced drug with a nanosized smart delivery system can provide the appropriate conditions to reduce, or even eliminate, problems specifically related to a molecule, e.g. conformation issues or non-specific interactions [255-256]. These problems often manifest with such intensity that a drug clinic application might be even disregarded, even with a possibility of positive results for patients. Nano delivery of proteins and peptides is the future trend to overcome these challenges. Lipossomes, polymeric NPs, and NLS are broadly studied in the field of biomolecules release, because of their possibility of a more specific target approach. These structures are getting more innovative approaches, as PEGlated liposomes.

LPHN, for exemple, turned out to be a robust drug delivery platform. The combination of its inner lipoidal layer and outer polymeric layer, presented a more stable, more control release and more prolong in vivo circulation than other nanocarriers.

An effective integration with biotechnology still requires a procedural methodology to guide the selection of delivery system based on limitations of a given drug and the desired pharmacokinetics properties. Beyond the technological progresses in the delivery of drugs, nanotechnology has provided a short-term solution to enhance the efficacy of known bioactive molecules and has shed light to the path for the development of Closed-Loop monitoring devices. Realistically, one would not be able to accurately prognose the development of the nearly established integration between nanotechnology and biotechnology neither its implication on future DDS.

The regulatory requirements are essential to stablish the nanotechnology process. It´s a complicated and challenge task and it has to be further investigated as every new NP and biodrug is another unknown field of toxicological possibilities. To reach a successful and safe nanotechnology development process it´s important to work together with other areas and add knowledge to each other's studies.

Nonetheless, it seems there is still "plenty of room at the bottom" to allow the continuous development of DDS for the decades to come.

5. ACKNOWLEDEGMENTS

The authors would like to acknowledge FAPESP (2017/10728-2, 2018/06475-4, 2018/07707-6, 2017/10789-1, 2018/10799-0), CNPq and CAPES (Finance Code 001) for financial support. Our figures were created with BioRender.

6. DATA AVAILABILITY

Not applicable.

7. REFERENCES

1. Thackray, A., Private science: Biotechnology and the rise of the molecular sciences. University of Pennsylvania Press: 1998.

2. Elvers, B.; Hawkins, S.; Russey, W., Ullmann's encyclopedia of industrial chemistry. Wiley Online Library: 1989.

3. Gordon, J. J.; Grenfell, E.; et al., Methods of penicillin production in submerged culture on a pilot-plant scale. J Gen Microbiol 1947, 1 (2), 187-202.

4. Fiechter, A.; Beppu, T.; Beyeler, W., History of modern biotechnology. Springer: 2000.

5. Grace, E. S., Biotechnology unzipped: Promises and realities. Joseph Henry Press: 2006.

6. Feynman, R. P., There's plenty of room at the bottom. Engineering and science 1960, 23 (5), 22-36.

7. Rocco, M., National Science Foundation, Official who oversees the nanotechnology initiative, vol. 285. Scientific American 2001, 3.

8. Solomon, A. O.; Young, C. U.; Ogechukwu, O. A.; Chukwunonyelum, I.; Ugochi, J. E., Making Drugs Safer: Improving Drug Delivery and Reducing Side-Effect of Drugs on the Human Biochemical System. Journal of Pharmaceutics & Drug Delivery Research 2017, 2015.

9. Jeevanandam, J.; Barhoum, A.; Chan, Y. S.; Dufresne, A.; Danquah, M. K., Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 2018, 9, 1050-1074.

10. Kabanov, A. V.; Batrakova, E. V., Polymer nanomaterials for drug delivery across the blood brain barrier. In Neuroimmune Pharmacology, Springer: 2017; pp 847-868.

11. Nasrollahi, S. A.; Hassanzade, H.; Moradi, A.; Sabouri, M.; Samadi, A.; Kashani, M. N.; Firooz, A., Safety Assessment of Tretinoin Loaded Nano Emulsion and Nanostructured Lipid Carriers: A Non-invasive Trial on Human Volunteers. Curr Drug Deliv 2017, 14 (4), 575-580.

12. Singh, R. P.; Gangadharappa, H. V.; Mruthunjaya, K., Phospholipids: Unique carriers for drug delivery systems. Journal of Drug Delivery Science and Technology 2017, 39, 166-179.

13. Srivastava, R., Theoretical Insight into the Medicinal World of Organometallics: Macro versus Nano. In Recent Progress in Organometallic Chemistry, InTech: 2017.

14. Roco, M.; Williams, R.; Alivisatos, P., Biological, medical and health applications.“Nanotechnology Research Directions,” Chap. 8. Kluwer Academic Publishers, Boston: 2000.

15. Brambilla, D.; Luciani, P.; Leroux, J.-C., Breakthrough discoveries in drug delivery technologies: The next 30years. Journal of Controlled Release 2014, 190, 9-14.

16. Geraldes, D. C.; Beraldo-de-Araújo, V. L.; Pardo, B. O. P.; Pessoa Junior, A.; Stephano, M. A.; de Oliveira-Nascimento, L., Protein drug delivery: current dosage form profile and formulation strategies. Journal of Drug Targeting 2019, 1-17.

17. Wishart, D. S.; Feunang, Y. D.; Guo, A. C.; Lo, E. J.; Marcu, A.; Grant, J. R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; Assempour, N.; Iynkkaran, I.; Liu, Y.; Maciejewski, A.; Gale, N.; Wilson, A.; Chin, L.; Cummings, R.; Le, D.; Pon, A.; Knox, C.; Wilson, M., DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 2018, 46 (D1), D1074-d1082.

18. Poon, B.; Kha, T.; Tran, S.; Dass, C. R., Bone morphogenetic protein-2 and bone therapy: successes and pitfalls. Journal of Pharmacy and Pharmacology 2016, 68 (2), 139-147.

19. Jimenez, N.; Bradford, H.; Seidel, K. D.; Sousa, M.; Lynn, A. M., A comparison of a needle-free injection system for local anesthesia versus EMLA for intravenous catheter insertion in the pediatric patient. Anesthesia and analgesia 2006, 102 (2), 411-4.

20. Razpotnik, R.; Novak, N.; Čurin Šerbec, V.; Rajcevic, U., Targeting Malignant Brain Tumors with Antibodies. Front Immunol 2017, 8, 1181-1181.

21. De Luca, P.; Colombini, A.; Carimati, G.; Beggio, M.; de Girolamo, L.; Volpi, P., Intra-Articular Injection of Hydrolyzed Collagen to Treat Symptoms of Knee Osteoarthritis. A Functional In Vitro Investigation and a Pilot Retrospective Clinical Study. J Clin Med 2019, 8 (7), 975.

22. Ye, H.; Shen, Z.; Yu, L.; Wei, M.; Li, Y., Manipulating nanoparticle transport within blood flow through external forces: an exemplar of mechanics in nanomedicine. Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences 2018, 474 (2211), 20170845.

23. Kloos, R.; van der Sluis, I. M.; Mastrobattista, E.; Hennink, W.; Pieters, R.; Verhoef, J.-J., Acute lymphoblastic leukaemia patients treated with PEGasparaginase develop antibodies to PEG and the succinate linker. British Journal of Haematology n/a (n/a).

24. Santos, J. H. P. M.; Torres-Obreque, K. M.; Meneguetti, G. P.; Amaro, B. P.; Rangel-Yagui, C. O., Protein PEGylation for the design of biobetters: from reaction to purification processes. Brazilian Journal of Pharmaceutical Sciences 2018, 54.

25. Bittner, B.; Richter, W.; Schmidt, J., Subcutaneous Administration of Biotherapeutics: An Overview of Current Challenges and Opportunities. BioDrugs 2018, 32 (5), 425-440.

26. Usach, I.; Martinez, R.; Festini, T.; Peris, J.-E., Subcutaneous Injection of Drugs: Literature Review of Factors Influencing Pain Sensation at the Injection Site. Advances in Therapy 2019, 36 (11), 2986-2996.

27. Creque, H. M.; Langer, R.; Folkman, J., One Month of Sustained Release of Insulin from a Polymer Implant. Diabetes 1980, 29 (1), 37-40.

28. Foldvari, M., Non-invasive administration of drugs through the skin: challenges in delivery system design. Pharmaceutical science & technology today 2000, 3 (12), 417-425.

29. Karande, P.; Mitragotri, S., Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochimica et Biophysica Acta (BBA) - Biomembranes 2009, 1788 (11), 2362-2373.

30. Trommer, H.; Neubert, R. H., Overcoming the stratum corneum: the modulation of skin penetration. A review. Skin Pharmacol Physiol 2006, 19 (2), 106-21.

31. Jin, X.; Zhu, D. D.; Chen, B. Z.; Ashfaq, M.; Guo, X. D., Insulin delivery systems combined with microneedle technology. Advanced Drug Delivery Reviews 2018, 127, 119-137.

32. Mitragotri, S., Current status and future prospects of needle-free liquid jet injectors. Nature Reviews Drug Discovery 2006, 5 (7), 543-548.

33. Zhang, H.; Zhang, J.; Streisand, J. B., Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. Clinical pharmacokinetics 2002, 41 (9), 661-80.

34. Lee, V., Mucosal Drug Delivery. Journal of the National Cancer Institute. Monographs 2001, 2001, 41-4.

35. Prego, C.; García, M.; Torres, D.; Alonso, M. J., Transmucosal macromolecular drug delivery. J Control Release 2005, 101 (1-3), 151-62.

36. Heubner, W.; de Jongh, S. E.; Laquer, E., Über Inhalation von Insulin. Klinische Wochenschrift 1924, 3 (51), 2342-2343.

37. Zelikin, A. N.; Ehrhardt, C.; Healy, A. M., Materials and methods for delivery of biological drugs. Nat Chem 2016, 8 (11), 997-1007.

38. de Kruijf, W.; Ehrhardt, C., Inhalation delivery of complex drugs—the next steps. Current Opinion in Pharmacology 2017, 36, 52-57.

39. ClinicalTrials.gov [Internet]-Dance Biopharm Inc.Identifier NCT02713841, Pharmacologic Response and Safety of Inhaled Insulin in Type 1 Diabetes (Samba-01). https://clinicaltrials.gov/ct2/show/NCT02713841 (accessed July 8th).

40. ClinicalTrials.gov [Internet]-Dance Biopharm Inc.Identifier NCT02716610, Dose-Response and Variability of Inhaled Insulin in Type 2 Diabetes (Samba-02). https://clinicaltrials.gov/ct2/show/study/NCT02716610?term=samba&draw=2&rank=3 (accessed July 8th).

41. Siekmeier, R. S., G., Treatment of Systemic Diseases by Inhalation of Biomolecule Aerosols. J Physiol Pharmacol 2009, 60, 15-26.

42. Patton, J. S.; Brain, J. D.; Davies, L. A.; Fiegel, J.; Gumbleton, M.; Kim, K.-J.; Sakagami, M.; Vanbever, R.; Ehrhardt, C., The Particle has Landed—Characterizing the Fate of Inhaled Pharmaceuticals. Journal of Aerosol Medicine and Pulmonary Drug Delivery 2010, 23 (S2), S-71-S-87.

43. Siekmeier, R. S., G., Systemic Treatment by Inhalation of Macromolecules - Principles, Problems, and Examples. J Physiol Pharmacol 2008, 59, 53-79.

44. F.D.A. Endocrinologic and metabolic drugs advisory committee meeting. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM390864.pdf. (accessed 19/11).

45. Loira-Pastoriza, C.; Todoroff, J.; Vanbever, R., Delivery strategies for sustained drug release in the lungs. Advanced Drug Delivery Reviews 2014, 75, 81-91.

46. Forbes, B.; Asgharian, B.; Dailey, L. A.; Ferguson, D.; Gerde, P.; Gumbleton, M.; Gustavsson, L.; Hardy, C.; Hassall, D.; Jones, R.; Lock, R.; Maas, J.; McGovern, T.; Pitcairn, G. R.; Somers, G.; Wolff, R. K., Challenges in inhaled product development and opportunities for open innovation. Advanced drug delivery reviews 2011, 63 (1-2), 69-87.

47. Chow, A. H. L.; Tong, H. H. Y.; Chattopadhyay, P.; Shekunov, B. Y., Particle engineering for pulmonary drug delivery. Pharmaceutical research 2007, 24 (3), 411-437.

48. Edwards, D. A.; Ben-Jebria, A.; Langer, R., Recent advances in pulmonary drug delivery using large, porous inhaled particles. Journal of Applied Physiology 1998, 85 (2), 379-385.

49. Pilcer, G.; Amighi, K., Formulation strategy and use of excipients in pulmonary drug delivery. International Journal of Pharmaceutics 2010, 392 (1), 1-19.

50. Johnson, K. A., Preparation of peptide and protein powders for inhalation. Advanced drug delivery reviews 1997, 26 (1), 3-15.

51. Tsapis, N.; Bennett, D.; Jackson, B.; Weitz, D. A.; Edwards, D. A., Trojan particles: Large porous carriers of nanoparticles for drug delivery. Proceedings of the National Academy of Sciences 2002, 99 (19), 12001.

52. Sung, J. C.; Pulliam, B. L.; Edwards, D. A., Nanoparticles for drug delivery to the lungs. Trends in biotechnology 2007, 25 (12), 563-570.

53. Li, X.; Anton, N.; Ta, T. M. C.; Zhao, M.; Messaddeq, N.; Vandamme, T. F., Microencapsulation of nanoemulsions: novel Trojan particles for bioactive lipid molecule delivery. International journal of nanomedicine 2011, 6, 1313-1325.

54. McBride, A. A.; Price, D. N.; Lamoureux, L. R.; Elmaoued, A. A.; Vargas, J. M.; Adolphi, N. L.; Muttil, P., Preparation and Characterization of Novel Magnetic Nano-in-Microparticles for Site-Specific Pulmonary Drug Delivery. Molecular Pharmaceutics 2013, 10 (10), 3574-3581.

55. Tewes, F.; Ehrhardt, C.; Healy, A. M., Superparamagnetic iron oxide nanoparticles (SPIONs)-loaded Trojan microparticles for targeted aerosol delivery to the lung. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 2014, 86 (1), 98-104.

56. Banga, A. K., Therapeutic Peptides and Proteins. Formulation, Processing, and Delivery Systems. Third ed.; CRC Press: Boca Raton, 2015.

57. Liao, Y.-H.; Brown, M. B.; Jones, S. A.; Nazir, T.; Martin, G. P., The effects of polyvinyl alcohol on the in vitro stability and delivery of spray-dried protein particles from surfactant-free HFA 134a-based pressurised metered dose inhalers. International Journal of Pharmaceutics 2005, 304 (1), 29-39.

58. Jones, S. A.; Martin, G. P.; Brown, M. B., Stabilisation of deoxyribonuclease in hydrofluoroalkanes using miscible vinyl polymers. J Control Release 2006, 115 (1), 1-8.

59. Cocks, E.; Somavarapu, S.; Alpar, O.; Greenleaf, D., Influence of Suspension Stabilisers on the Delivery of Protein-Loaded Porous Poly (DL-Lactide-co-Glycolide) (PLGA) Microparticles via Pressurised Metered Dose Inhaler (pMDI). Pharmaceutical Research 2014, 31 (8), 2000-2009.

60. Conti, D. S.; Bharatwaj, B.; Brewer, D.; da Rocha, S. R. P., Propellant-based inhalers for the non-invasive delivery of genes via oral inhalation. Journal of Controlled Release 2012, 157 (3), 406-417.

61. Shan, Z.; Tan, Y.; Qin, L.; Li, G.; Pan, X.; Wang, Z.; Yu, X.; Wang, Q.; Wu, C., Formulation and evaluation of novel reverse microemulsions containing salmon calcitonin in hydrofluoroalkane propellants. International Journal of Pharmaceutics 2014, 466 (1), 390-399.

62. Ari, A., Jet, Ultrasonic, and Mesh Nebulizers: An Evaluation of Nebulizers for Better Clinical Outcomes. Eurasian Journal of Pulmonology 2014, 16 (1), 1-7.

63. Gupta, S.; Moussy, F.; Dalby, R. N.; Miekka, S. I.; Bruley, D. F., Pulmonary Delivery of Human Protein C and Factor IX. In Oxygen Transport to Tissue XVIII, Nemoto, E. M.; LaManna, J. C.; Cooper, C.; Delpy, D.; Groebe, K.; Hunt, T. K.; Keipert, P.; Mayevsky, A.; Pittman, R. N.; Rumsey, W. L.; Vaupel, P.; Wilson, D. F., Eds. Springer US: Boston, MA, 1997; pp 429-435.

64. Agu, R. U.; Ugwoke, M. I.; Armand, M.; Kinget, R.; Verbeke, N., The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir Res 2001, 2 (4), 198-209.

65. Lange, C. F.; Hancock, R. E. W.; Samuel, J.; Finlay, W. H., In Vitro Aerosol Delivery and Regional Airway Surface Liquid Concentration of a Liposomal Cationic Peptide. Journal of Pharmaceutical Sciences 2001, 90 (10), 1647-1657.

66. Franks, F., Hatley, R. H. M. & Mathias, S. F., Materials Science and the Production of Shelf-Stable Biologicals. Biopharm-Technol 1991, 38-42.

67. Carpenter, J. F.; Crowe, J. H., The mechanism of cryoprotection of proteins by solutes. Cryobiology 1988, 25 (3), 244-255.

68. Lee, G., Pharmaceutical Biotechnology. In Rational Design of Stable Protein Formulations, Manning, J. F. C. M. C., Ed. Springer US: 2002; Vol. 13, pp 135-158

69. Arakawa, T.; Prestrelski, S. J.; Kenney, W. C.; Carpenter, J. F., Factors affecting short-term and long-term stabilities of proteins. Advanced Drug Delivery Reviews 2001, 46 (1), 307-326.

70. Agrahari, V.; Agrahari, V.; Mitra, A. K., Nanocarrier fabrication and macromolecule drug delivery: challenges and opportunities. Therapeutic Delivery 2016, 7 (4), 257-278.

71. Mishra, N.; Goyal, A. K.; Khatri, K.; Vaidya, B.; Paliwal, R.; Rai, S.; Mehta, A.; Tiwari, S.; Vyas, S.; Vyas, S. P., Biodegradable polymer based particulate carrier (s) for the delivery of proteins and peptides. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 2008, 7 (4), 240-251.

72. Pisal, D. S.; Kosloski, M. P.; Balu-Iyer, S. V., Delivery of therapeutic proteins. Journal of pharmaceutical sciences 2010, 99 (6), 2557-2575.

73. Baseer, A.; Koenneke, A.; Zapp, J.; Khan, S. A.; Schneider, M., Design and Characterization of Surface-Crosslinked Gelatin Nanoparticles for the Delivery of Hydrophilic Macromolecular Drugs. Macromolecular Chemistry and Physics 2019, 220 (18), 1900260.

74. Dragan, E. S.; Dinu, M. V., Polysaccharides constructed hydrogels as vehicles for proteins and peptides. A review. Carbohydrate Polymers 2019, 225, 115210.

75. Sanchez-Ruiz, J. M.; Makhatadze, G. I., To charge or not to charge? Trends in Biotechnology 2001, 19 (4), 132-135.

76. Pawar, V. K.; Meher, J. G.; Singh, Y.; Chaurasia, M.; Surendar Reddy, B.; Chourasia, M. K., Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: Strategies and industrial perspectives. Journal of Controlled Release 2014, 196, 168-183.

77. Wang, J.; Yadav, V.; Smart, A. L.; Tajiri, S.; Basit, A. W., Toward Oral Delivery of Biopharmaceuticals: An Assessment of the Gastrointestinal Stability of 17 Peptide Drugs. Molecular Pharmaceutics 2015, 12 (3), 966-973.

78. Mundargi, R. C.; Babu, V. R.; Rangaswamy, V.; Patel, P.; Aminabhavi, T. M., Nano/micro technologies for delivering macromolecular therapeutics using poly(d,l-lactide-co-glycolide) and its derivatives. Journal of Controlled Release 2008, 125 (3), 193-209.

79. Abramson, A.; Caffarel-Salvador, E.; Khang, M.; Dellal, D.; Silverstein, D.; Gao, Y.; Frederiksen, M. R.; Vegge, A.; Hubálek, F.; Water, J. J.; Friderichsen, A. V.; Fels, J.; Kirk, R. K.; Cleveland, C.; Collins, J.; Tamang, S.; Hayward, A.; Landh, T.; Buckley, S. T.; Roxhed, N.; Rahbek, U.; Langer, R.; Traverso, G., An ingestible self-orienting system for oral delivery of macromolecules. Science (New York, N.Y.) 2019, 363 (6427), 611-615.

80. Buckley, S. T.; Bækdal, T. A.; Vegge, A.; Maarbjerg, S. J.; Pyke, C.; Ahnfelt-Rønne, J.; Madsen, K. G.; Schéele, S. G.; Alanentalo, T.; Kirk, R. K.; Pedersen, B. L.; Skyggebjerg, R. B.; Benie, A. J.; Strauss, H. M.; Wahlund, P. O.; Bjerregaard, S.; Farkas, E.; Fekete, C.; Søndergaard, F. L.; Borregaard, J.; Hartoft-Nielsen, M. L.; Knudsen, L. B., Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist. Sci Transl Med 2018, 10 (467).

81. Lemarchand, C.; Gref, R.; Couvreur, P., Polysaccharide-decorated nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics 2004, 58 (2), 327-341.

82. Le Droumaguet, B.; Nicolas, J.; Brambilla, D.; Mura, S.; Maksimenko, A.; De Kimpe, L.; Salvati, E.; Zona, C.; Airoldi, C.; Canovi, M.; Gobbi, M.; Magali, N.; La Ferla, B.; Nicotra, F.; Scheper, W.; Flores, O.; Masserini, M.; Andrieux, K.; Couvreur, P., Versatile and Efficient Targeting Using a Single Nanoparticulate Platform: Application to Cancer and Alzheimer's Disease. ACS Nano 2012, 6 (7), 5866-5879.

83. Lopes, C. M.; Martins-Lopes, P.; Souto, E. B., Nanoparticulate carriers (NPC) for oral pharmaceutics and nutraceutics. Pharmazie 2010, 65 (2), 75-82.

84. Liu, H.-J.; Xu, P., Smart Mesoporous Silica Nanoparticles for Protein Delivery. Nanomaterials (Basel) 2019, 9 (4), 511.

85. Patra, J. K.; Das, G.; Fraceto, L. F.; Campos, E. V. R.; Rodriguez-Torres, M. d. P.; Acosta-Torres, L. S.; Diaz-Torres, L. A.; Grillo, R.; Swamy, M. K.; Sharma, S.; Habtemariam, S.; Shin, H.-S., Nano based drug delivery systems: recent developments and future prospects. Journal of Nanobiotechnology 2018, 16 (1), 71.

86. Wu, F.; Jin, T., Polymer-Based Sustained-Release Dosage Forms for Protein Drugs, Challenges, and Recent Advances. AAPS PharmSciTech 2008, 9 (4), 1218-1229.

87. Yang, S.; Yuan, W.; Jin, T., Formulating protein therapeutics into particulate forms. Expert Opinion on Drug Delivery 2009, 6 (10), 1123-1133.

88. Pachioni-Vasconcelos, J. d. A.; Lopes, A. M.; Apolinario, A. C.; Valenzuela-Oses, J. K.; Costa, J. S. R.; Nascimento, L. d. O.; Pessoa, A.; Barbosa, L. R. S.; Rangel-Yagui, C. d. O., Nanostructures for protein drug delivery. Biomaterials Science 2016, 4 (2), 205-218.

89. Ataide, J. A.; Cefali, L. C.; Croisfelt, F. M.; Shimojo, A. A. M.; Oliveira‐Nascimento, L.; Mazzola, P. G., Natural actives for wound healing: A review. Phytotherapy Research 2018, 32 (0), 11.

90. de Lencastre Novaes, L. C.; Jozala, A. F.; Lopes, A. M.; de Carvalho Santos-Ebinuma, V.; Mazzola, P. G.; Pessoa Junior, A., Stability, purification, and applications of bromelain: A review. Biotechnology progress 2016, 32 (1), 5-13.

91. Ataide, J. A.; Gérios, E. F.; Mazzola, P. G.; Souto, E. B., Bromelain-loaded nanoparticles: A comprehensive review of the state of the art. Advances in Colloid and Interface Science 2018, 254, 48-55.

92. Ataide, J. A.; Gerios, E. F.; Cefali, L. C.; Fernandes, A. R.; Teixeira, M. D. C.; Ferreira, N. R.; Tambourgi, E. B.; Jozala, A. F.; Chaud, M. V.; Oliveira-Nascimento, L.; Mazzola, P. G.; Souto, E. B., Effect of Polysaccharide Sources on the Physicochemical Properties of Bromelain-Chitosan Nanoparticles. Polymers 2019, 11 (10).

93. Ji, T.; Kohane, D. S., Nanoscale systems for local drug delivery. Nano Today 2019, 100765.

94. Wang, Y.; Kohane, D. S., External triggering and triggered targeting strategies for drug delivery. Nature Reviews Materials 2017, 2, 17020.

95. Kamaly, N.; Yameen, B.; Wu, J.; Farokhzad, O. C., Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release. Chemical Reviews 2016, 116 (4), 2602-2663.

96. Mura, S.; Nicolas, J.; Couvreur, P., Stimuli-responsive nanocarriers for drug delivery. Nat Mater 2013, 12 (11), 991-1003.

97. Tian, H.; Tang, Z.; Zhuang, X.; Chen, X.; Jing, X., Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. Progress in Polymer Science 2012, 37 (2), 237-280.

98. Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M., Polymeric Systems for Controlled Drug Release. Chem. Rev. 1999, 99 (11), 3181-3198.

99. Sun, T.; Zhang, Y. S.; Pang, B.; Hyun, D. C.; Yang, M.; Xia, Y., Engineered Nanoparticles for Drug Delivery in Cancer Therapy. Angewandte Chemie International Edition 2014, 53 (46), 12320-12364.

100. Li, M.; Du, C.; Guo, N.; Teng, Y.; Meng, X.; Sun, H.; Li, S.; Yu, P.; Galons, H., Composition design and medical application of liposomes. European Journal of Medicinal Chemistry 2019, 164, 640-653.

101. Tai, K.; Liu, F.; He, X.; Ma, P.; Mao, L.; Gao, Y.; Yuan, F., The effect of sterol derivatives on properties of soybean and egg yolk lecithin liposomes: Stability, structure and membrane characteristics. Food Research International 2018, 109, 24-34.

102. Daraee, H.; Etemadi, A.; Kouhi, M.; Alimirzalu, S.; Akbarzadeh, A., Application of liposomes in medicine and drug delivery. Artificial Cells, Nanomedicine, and Biotechnology 2016, 44 (1), 381-391.

103. Islam Shishir, M. R.; Karim, N.; Gowd, V.; Zheng, X.; Chen, W., Liposomal delivery of natural product: A promising approach in health research. Trends Food Sci Tech 2019, 85, 177-200.

104. Sercombe, L.; Veerati, T.; Moheimani, F.; Wu, S. Y.; Sood, A. K.; Hua, S., Advances and Challenges of Liposome Assisted Drug Delivery. Frontiers in Pharmacology 2015, 6, 286.

105. Herzog, C.; Hartmann, K.; Künzi, V.; Kürsteiner, O.; Mischler, R.; Lazar, H.; Glück, R., Eleven years of Inflexal® V—a virosomal adjuvanted influenza vaccine. Vaccine 2009, 27 (33), 4381-4387.

106. Yang, W.; Hu, Q.; Xu, Y.; Liu, H.; Zhong, L., Antibody fragment-conjugated gemcitabine and paclitaxel-based liposome for effective therapeutic efficacy in pancreatic cancer. Materials Science and Engineering: C 2018, 89, 328-335.

107. Jhaveri, A.; Deshpande, P.; Pattni, B.; Torchilin, V., Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma. Journal of Controlled Release 2018, 277, 89-101.

108. Cullion, K.; Rwei, A. Y.; Kohane, D. S., Ultrasound-triggered liposomes for on-demand local anesthesia. Therapeutic Delivery 2017, 9 (1), 5-8.

109. Rwei, A. Y.; Wang, B. Y.; Ji, T.; Zhan, C.; Kohane, D. S., Enhanced Triggering of Local Anesthetic Particles by Photosensitization and Photothermal Effect Using a Common Wavelength. Nano Letters 2017, 17 (11), 7138-7145.

110. Rwei, A. Y.; Zhan, C.; Wang, B.; Kohane, D. S., Multiply repeatable and adjustable on-demand phototriggered local anesthesia. Journal of Controlled Release 2017, 251, 68-74.

111. Date, A. A.; Joshi, M. D.; Patravale, V. B., Parasitic diseases: Liposomes and polymeric nanoparticles versus lipid nanoparticles. Advanced Drug Delivery Reviews 2007, 59 (6), 505-521.

112. Huda, M. K.; Das, P. P.; Saikia, P. J.; Baruah, S. D., Synthesis of poly(n-octadecyl methacrylate-co-2-hydroxyethyl methacrylate) copolymer and their utilization as polymeric stabilizer in the preparation of PCL microspheres. Polymer Bulletin 2017, 74 (5), 1661-1676.

113. Patel, A.; Patel, M.; Yang, X.; Mitra, A. K., Recent advances in protein and Peptide drug delivery: a special emphasis on polymeric nanoparticles. Protein Pept Lett 2014, 21 (11), 1102-1120.

114. Mizuhara, T.; Moyano, D. F.; Rotello, V. M., Using the power of organic synthesis for engineering the interactions of nanoparticles with biological systems. Nano Today 2016, 11 (1), 31-40.

115. Affonso de Oliveira, J. F.; Scheffer, F. R.; Landis, R. F.; Teixeira Neto, É.; Rotello, V. M.; Cardoso, M. B., Dual Functionalization of Nanoparticles for Generating Corona-Free and Noncytotoxic Silica Nanoparticles. ACS Applied Materials & Interfaces 2018, 10 (49), 41917-41923.

116. Saha, K.; Rahimi, M.; Yazdani, M.; Kim, S. T.; Moyano, D. F.; Hou, S.; Das, R.; Mout, R.; Rezaee, F.; Mahmoudi, M.; Rotello, V. M., Regulation of Macrophage Recognition through the Interplay of Nanoparticle Surface Functionality and Protein Corona. ACS Nano 2016, 10 (4), 4421-4430.

117. Guerrini, L.; Alvarez-Puebla, R. A.; Pazos-Perez, N., Surface Modifications of Nanoparticles for Stability in Biological Fluids. Materials (Basel) 2018, 11 (7), 1154.

118. Ulery, B. D.; Nair, L. S.; Laurencin, C. T., Biomedical Applications of Biodegradable Polymers. J Polym Sci B Polym Phys 2011, 49 (12), 832-864.

119. Lima, A. C.; Cunha, C.; Carvalho, A.; Ferreira, H.; Neves, N. M., Interleukin-6 Neutralization by Antibodies Immobilized at the Surface of Polymeric Nanoparticles as a Therapeutic Strategy for Arthritic Diseases. ACS Applied Materials & Interfaces 2018, 10 (16), 13839-13850.

120. Eldem, T.; Speiser, P.; Hincal, A., Optimization of spray-dried and-congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. Pharmaceutical research 1991, 8 (1), 47-54.

121. Mukherjee, S.; Ray, S.; Thakur, R., Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian journal of pharmaceutical sciences 2009, 71 (4), 349.

122. Ekambaram, P.; Sathali, A. A. H.; Priyanka, K., Solid lipid nanoparticles: a review. Sci Rev Chem Commun 2012, 2 (1), 80-102.

123. Galindo-Pérez, M. J.; Quintanar-Guerrero, D.; Cornejo-Villegas, M. d. l. Á.; Zambrano-Zaragoza, M. d. l. L., Optimization of the emulsification-diffusion method using ultrasound to prepare nanocapsules of different food-core oils. LWT 2018, 87, 333-341.

124. Schubert, M. A.; Müller-Goymann, C. C., Solvent injection as a new approach for manufacturing lipid nanoparticles--evaluation of the method and process parameters. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 2003, 55 (1), 125-131.

125. Bayón-Cordero, L.; Alkorta, I.; Arana, L., Application of Solid Lipid Nanoparticles to Improve the Efficiency of Anticancer Drugs. Nanomaterials (Basel) 2019, 9 (3), 474.

126. Müller, R. H.; MaÈder, K.; Gohla, S., Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. European journal of pharmaceutics and biopharmaceutics 2000, 50 (1), 161-177.

127. Mehrad, B.; Ravanfar, R.; Licker, J.; Regenstein, J. M.; Abbaspourrad, A., Enhancing the physicochemical stability of β-carotene solid lipid nanoparticle (SLNP) using whey protein isolate. Food Research International 2018, 105, 962-969.

128. Kumar, R.; Singh, A.; Garg, N.; Siril, P. F., Solid lipid nanoparticles for the controlled delivery of poorly water soluble non-steroidal anti-inflammatory drugs. Ultrasonics sonochemistry 2018, 40, 686-696.

129. Chattopadhyay, P.; Shekunov, B. Y.; Yim, D.; Cipolla, D.; Boyd, B.; Farr, S., Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system. Advanced drug delivery reviews 2007, 59 (6), 444-453.

130. Rishitha, N.; Muthuraman, A., Therapeutic evaluation of solid lipid nanoparticle of quercetin in pentylenetetrazole induced cognitive impairment of zebrafish. Life sciences 2018, 199, 80-87.

131. Oliveira, R. R.; Carrião, M. S.; Pacheco, M. T.; Branquinho, L. C.; de Souza, A. L. R.; Bakuzis, A. F.; Lima, E. M., Triggered release of paclitaxel from magnetic solid lipid nanoparticles by magnetic hyperthermia. Materials Science and Engineering: C 2018, 92, 547-553.

132. Zhou, M.; Hou, J.; Zhong, Z.; Hao, N.; Lin, Y.; Li, C., Targeted delivery of hyaluronic acid-coated solid lipid nanoparticles for rheumatoid arthritis therapy. Drug Delivery 2018, 25 (1), 716-722.

133. Yu, S.; Xu, X.; Feng, J.; Liu, M.; Hu, K., Chitosan and chitosan coating nanoparticles for the treatment of brain disease. International journal of pharmaceutics 2019, 560, 282-293.

134. Apostolidis, E.; Mandala, I., Modification of resistant starch nanoparticles using high-pressure homogenization treatment. Food Hydrocolloids 2020, 105677.

135. Gianak, O.; Kyzas, G. Z.; Samanidou, V. F.; Deliyanni, E. A., A review for the synthesis of silk fibroin nanoparticles with different techniques and their ability to be used for drug delivery. Current Analytical Chemistry 2019, 15 (4), 339-348.

136. Nallasamy, P.; Ramalingam, T.; Thajuddin, N.; Shanmuganathan, R.; Pugazhendhi, A.; Natarajan, S., Polyherbal drug loaded starch nanoparticles as promising drug delivery system: Antimicrobial, antibiofilm and neuroprotective studies. Process Biochemistry 2020.

137. Patel, M.; Souto, E. B.; Singh, K. K., Advances in brain drug targeting and delivery: limitations and challenges of solid lipid nanoparticles. Expert opinion on drug delivery 2013, 10 (7), 889-905.

138. Li, Q.; Cai, T.; Huang, Y.; Xia, X.; Cole, S. P.; Cai, Y., A review of the structure, preparation, and application of NLCs, PNPs, and PLNs. Nanomaterials 2017, 7 (6), 122.

139. Dave, V.; Tak, K.; Sohgaura, A.; Gupta, A.; Sadhu, V.; Reddy, K. R., Lipid-polymer hybrid nanoparticles: Synthesis strategies and biomedical applications. Journal of Microbiological Methods 2019, 160, 130-142.

140. Du, M.; Ouyang, Y.; Meng, F.; Zhang, X.; Ma, Q.; Zhuang, Y.; Liu, H.; Pang, M.; Cai, T.; Cai, Y., Polymer-lipid hybrid nanoparticles: A novel drug delivery system for enhancing the activity of Psoralen against breast cancer. International Journal of Pharmaceutics 2019, 561, 274-282.

141. Chan, J. M.; Zhang, L.; Yuet, K. P.; Liao, G.; Rhee, J.-W.; Langer, R.; Farokhzad, O. C., PLGA–lecithin–PEG core–shell nanoparticles for controlled drug delivery. Biomaterials 2009, 30 (8), 1627-1634.

142. Wang, A. Z.; Yuet, K.; Zhang, L.; Gu, F. X.; Huynh-Le, M.; Radovic-Moreno, A. F.; Kantoff, P. W.; Bander, N. H.; Langer, R.; Farokhzad, O. C., ChemoRad nanoparticles: a novel multifunctional nanoparticle platform for targeted delivery of concurrent chemoradiation. Nanomedicine (London, England) 2010, 5 (3), 361-368.

143. Tahir, N.; Madni, A.; Correia, A.; Rehman, M.; Balasubramanian, V.; Khan, M. M.; Santos, H. A., Lipid-polymer hybrid nanoparticles for controlled delivery of hydrophilic and lipophilic doxorubicin for breast cancer therapy. International journal of nanomedicine 2019, 14, 4961-4974.

144. Wang, Y.; Liu, C.-H.; Ji, T.; Mehta, M.; Wang, W.; Marino, E.; Chen, J.; Kohane, D. S., Intravenous treatment of choroidal neovascularization by photo-targeted nanoparticles. Nature Communications 2019, 10 (1), 804.

145. Trabulo, S.; Cardoso, A. L.; Mano, M.; De Lima, M. C. P., Cell-Penetrating Peptides-Mechanisms of Cellular Uptake and Generation of Delivery Systems. Pharmaceuticals (Basel) 2010, 3 (4), 961-993.

146. Mühlebach, S., Regulatory challenges of nanomedicines and their follow-on versions: A generic or similar approach? Adv Drug Deliv Rev 2018, 131, 122-131.

147. Sanchez, F.; Sobolev, K., Nanotechnology in concrete – A review. Construction and Building Materials 2010, 24 (11), 2060-2071.

148. Shrivastava, S.; Dash, D., Applying Nanotechnology to Human Health: Revolution in Biomedical Sciences. Journal of Nanotechnology 2009, 2009, 184702.

149. das Neves, J.; Amiji, M. M.; Bahia, M. F.; Sarmento, B., Nanotechnology-based systems for the treatment and prevention of HIV/AIDS. Adv Drug Deliv Rev 2010, 62 (4-5), 458-77.

150. Duncan, R.; Gaspar, R., Nanomedicine(s) under the Microscope. Molecular Pharmaceutics 2011, 8 (6), 2101-2141.

151. Farokhzad, O. C.; Langer, R., Impact of Nanotechnology on Drug Delivery. ACS Nano 2009, 3 (1), 16-20.

152. Commission, E., Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee: Second Regulatory Review on Nanomaterials. . Parliament, C. t. t. E., Ed. 2012.

153. Commission, E., Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on novel foods. COM/2013/0894 final - 2013/0435 (COD). Commission, E., Ed. 2013.

154. Wagner, V.; Dullaart, A.; Bock, A.-K.; Zweck, A., The emerging nanomedicine landscape. Nature biotechnology 2006, 24 (10), 1211-1217.

155. Alonso, M.; Garcia-Fuentes, M., Nano-Oncologicals: New Targeting and Delivery Approaches. 2014.

156. Gaspar, R.; Duncan, R., Polymeric carriers: preclinical safety and the regulatory implications for design and development of polymer therapeutics. Adv Drug Deliv Rev 2009, 61 (13), 1220-31.

157. Lungu, I. I.; Grumezescu, A. M.; Volceanov, A.; Andronescu, E. Nanobiomaterials Used in Cancer Therapy: An Up-To-Date Overview Molecules (Basel, Switzerland) [Online], 2019. PubMed. http://europepmc.org/abstract/MED/31574993

https://doi.org/10.3390/molecules24193547

https://europepmc.org/articles/PMC6804091

https://europepmc.org/articles/PMC6804091?pdf=render (accessed 2019/09//).

158. Rajasekhar, C.; Kanchi, S., Green Nanomaterials for Clean Environment. In Handbook of Ecomaterials, Martínez, L. M. T.; Kharissova, O. V.; Kharisov, B. I., Eds. Springer International Publishing: Cham, 2018; pp 1-18.

159. Ravichandran, V.; Vasanthi, S.; Shalini, S.; Shah, S. A. A.; Tripathy, M.; Paliwal, N., Green synthesis, characterization, antibacterial, antioxidant and photocatalytic activity of Parkia speciosa leaves extract mediated silver nanoparticles. Results in Physics 2019, 15, 102565.

160. Saldanha, P. L.; Lesnyak, V.; Manna, L., Large scale syntheses of colloidal nanomaterials. Nano Today 2017, 12, 46-63.

161. Qiu Zhao, Q.; Boxman, A.; Chowdhry, U., Nanotechnology in the Chemical Industry – Opportunities and Challenges. Journal of Nanoparticle Research 2003, 5 (5), 567-572.

162. Xu, L.; Liu, D.; Chen, D.; Liu, H.; Yang, J., Size and shape controlled synthesis of rhodium nanoparticles. Heliyon 2019, 5 (1), e01165.

163. Heiligtag, F. J.; Niederberger, M., The fascinating world of nanoparticle research. Materials Today 2013, 16 (7), 262-271.

164. Lead, J. R.; Wilkinson, K. J., Aquatic Colloids and Nanoparticles: Current Knowledge and Future Trends. Environmental Chemistry 2006, 3 (3), 159-171.

165. Engineering, T. R. S. T. R. A. o., Nanoscience and nanotechnologies. Latimer Trend Ltd: Plymouth, UK, 2004.

166. VARANDA, L. C.; SOUZA, C. G. S.; MORAES, D. A.; NEVES, H. R.; SOUZA JUNIOR, J. B.; SILVA, M. F.; BINI, R. A.; ALBERS, R. F.; SILVA, T. L.; BECK JUNIOR, W., Size and shape-controlled nanomaterials based on modified polyol and thermal decomposition approaches. A brief review. Anais da Academia Brasileira de Ciências 2019, 91.

167. Paliwal, R.; Babu, R. J.; Palakurthi, S., Nanomedicine Scale-up Technologies: Feasibilities and Challenges. AAPS PharmSciTech 2014, 15 (6), 1527-1534.

168. Varma, R. S., Greener approach to nanomaterials and their sustainable applications. Current Opinion in Chemical Engineering 2012, 1 (2), 123-128.

169. Cinelli, M.; Coles, S. R.; Sadik, O.; Karn, B.; Kirwan, K., A framework of criteria for the sustainability assessment of nanoproducts. Journal of Cleaner Production 2016, 126, 277-287.

170. Falsini, S.; Bardi, U.; Abou-Hassan, A.; Ristori, S., Sustainable strategies for large-scale nanotechnology manufacturing in the biomedical field. Green Chemistry 2018, 20 (17), 3897-3907.

171. Ehmann, F.; Sakai-Kato, K.; Duncan, R.; Pérez de la Ossa, D. H.; Pita, R.; Vidal, J.-M.; Kohli, A.; Tothfalusi, L.; Sanh, A.; Tinton, S.; Robert, J.-L.; Silva Lima, B.; Amati, M. P., Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines. Nanomedicine : nanotechnology, biology, and medicine 2013, 8 (5), 849-856.

172. Dorbeck-Jung, B.; Chowdhury, N., Is the European Medical Products Authorisation Regulation Equipped to Cope with the Challenges of Nanomedicines? Law & Policy 2011, 33, 276-303.

173. van der Meulen, B.; Bremmers, H.; Purnhagen, K.; Gupta, N.; Bouwmeester, H.; Geyer, L. L., Governing nano foods: principles-based responsive regulation: EFFoST critical reviews# 3. Academic Press: 2014; Vol. 3.

174. Arts, J. H. E.; Hadi, M.; Keene, A. M.; Kreiling, R.; Lyon, D.; Maier, M.; Michel, K.; Petry, T.; Sauer, U. G.; Warheit, D.; Wiench, K.; Landsiedel, R., A critical appraisal of existing concepts for the grouping of nanomaterials. Regulatory Toxicology and Pharmacology 2014, 70 (2), 492-506.

175. Amenta, V.; Aschberger, K.; Arena, M.; Bouwmeester, H.; Botelho Moniz, F.; Brandhoff, P.; Gottardo, S.; Marvin, H. J. P.; Mech, A.; Quiros Pesudo, L.; Rauscher, H.; Schoonjans, R.; Vettori, M. V.; Weigel, S.; Peters, R. J., Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. Regulatory Toxicology and Pharmacology 2015, 73 (1), 463-476.

176. Commission, E., Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee: Second Regulatory Review on Nanomaterials. Committee, t. C. a. t. E. E. a. S., Ed. 2012; Vol. 1272/2008.

177. OECD, Regulatory Frameworks for Nanotechnology in Foods and Medical Products. 2013.

178. Parliament, E., European Parliament Resolution of 24 April 2009 on Regulatory Aspects of Nanomaterials. Parliament, E., Ed. Brussels and Strassbourg, 2009; Vol. 1272/2008.

179. Falkner, R.; Jaspers, N., Regulating Nanotechnologies: Risk, Uncertainty and the Global Governance Gap. Global Environmental Politics - GLOB ENVIRON POLIT 2012, 12, 30-55.

180. Aschberger, K.; Rauscher, H.; Crutzen, H.; Rasmussen, K.; Christensen, F. M.; Sokull-Klüttgen, B.; Stamm, H., Considerations on information needs for nanomaterials in consumer products. European Commission Joint Research Centre Institute for Health and Consumer Protection, Brussels 2014.

181. Council, E. P., European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Union, O. J. o. t. E., Ed. 2008; Vol. 1272/2008.

182. Tinkle, S.; McNeil, S. E.; Mühlebach, S.; Bawa, R.; Borchard, G.; Barenholz, Y. C.; Tamarkin, L.; Desai, N., Nanomedicines: addressing the scientific and regulatory gap. Ann N Y Acad Sci 2014, 1313, 35-56.

183. Dobrovolskaia, M. A.; McNeil, S. E., Understanding the correlation between in vitro and in vivo immunotoxicity tests for nanomedicines. J Control Release 2013, 172 (2), 456-66.

184. Sainz, V.; Conniot, J.; Matos, A. I.; Peres, C.; Zupancic, E.; Moura, L.; Silva, L. C.; Florindo, H. F.; Gaspar, R. S., Regulatory aspects on nanomedicines. Biochem Biophys Res Commun 2015, 468 (3), 504-10.

185. Gaspar, R., Therapeutic products: Regulating drugs and medical devices. In International Handbook on Regulating Nanotechnologies, G. Hodge A., B. D. M., Maynard A.D., Ed. 2010; pp 291-320.

186. Gaspar, R.; Florindo, H.; Silva, L.; Videira, M.; Corvo, L.; Martins, B.; silva lima, B., Regulatory Aspects of Oncologicals: Nanosystems Main Challenges. In Nano-oncologicals, Alonso M.J., G.-F. M., Ed. Springer International 2014.

187. Kumar, S.; Rani, R.; Dilbaghi, N.; Tankeshwar, K.; Kim, K.-H., Carbon nanotubes: a novel material for multifaceted applications in human healthcare. Chemical Society Reviews 2017, 46 (1), 158-196.

188. Rocha, T. L.; Mestre, N. C.; Sabóia-Morais, S. M. T.; Bebianno, M. J., Environmental behaviour and ecotoxicity of quantum dots at various trophic levels: A review. Environment international 2017, 98, 1-17.

189. Ruiz, M. E.; Gantner, M. E.; Talevi, A., Applications of nanosystems to anticancer drug therapy (Part II. Dendrimers, micelles, lipid-based nanosystems). Recent Pat Anticancer Drug Discov 2014, 9 (1), 99-128.

190. Hristozov, D. R.; Gottardo, S.; Critto, A.; Marcomini, A., Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 2012, 6, 880-98.

191. Oberdörster, G., Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. J Intern Med 2010, 267 (1), 89-105.

192. Satalkar, P.; Elger, B. S.; Hunziker, P.; Shaw, D., Challenges of clinical translation in nanomedicine: A qualitative study. Nanomedicine : nanotechnology, biology, and medicine 2016, 12 (4), 893-900.

193. Caster, J. M.; Patel, A. N.; Zhang, T.; Wang, A., Investigational nanomedicines in 2016: a review of nanotherapeutics currently undergoing clinical trials. Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology 2017, 9 (1).

194. Hare, J. I.; Lammers, T.; Ashford, M. B.; Puri, S.; Storm, G.; Barry, S. T., Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv Drug Deliv Rev 2017, 108, 25-38.

195. Maranhao, R. C.; Vital, C. G.; Tavoni, T. M.; Graziani, S. R., Clinical experience with drug delivery systems as tools to decrease the toxicity of anticancer chemotherapeutic agents. Expert Opin Drug Deliv 2017, 14 (10), 1217-1226.

196. Farjadian, F.; Ghasemi, A.; Gohari, O.; Roointan, A.; Karimi, M.; Hamblin, M. R., Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities. Nanomedicine (Lond) 2019, 14 (1), 93-126.

197. D'Mello, S. R.; Cruz, C. N.; Chen, M. L.; Kapoor, M.; Lee, S. L.; Tyner, K. M., The evolving landscape of drug products containing nanomaterials in the United States. Nat Nanotechnol 2017, 12 (6), 523-529.

198. Etheridge, M. L.; Campbell, S. A.; Erdman, A. G.; Haynes, C. L.; Wolf, S. M.; McCullough, J., The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine : nanotechnology, biology, and medicine 2013, 9 (1), 1-14.

199. Bobo, D.; Robinson, K. J.; Islam, J.; Thurecht, K. J.; Corrie, S. R., Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. Pharm Res 2016, 33 (10), 2373-87.

200. Laux, P.; Riebeling, C.; Booth, A. M.; Brain, J. D.; Brunner, J.; Cerrillo, C.; Creutzenberg, O.; Estrela-Lopis, I.; Gebel, T.; Johanson, G.; Jungnickel, H.; Kock, H.; Tentschert, J.; Tlili, A.; Schaffer, A.; Sips, A.; Yokel, R. A.; Luch, A., Biokinetics of Nanomaterials: the Role of Biopersistence. NanoImpact 2017, 6, 69-80.

201. Kolosnjaj-Tabi, J.; Lartigue, L.; Javed, Y.; Luciani, N.; Pellegrino, T.; Wilhelm, C.; Alloyeau, D.; Gazeau, F., Biotransformations of magnetic nanoparticles in the body. Nanotoday 2016, 11 (3), 280-284.

202. Tang, H.; Zhang, H.; Ye, H.; Zheng, Y., Receptor-Mediated Endocytosis of Nanoparticles: Roles of Shapes, Orientations, and Rotations of Nanoparticles. The journal of physical chemistry. B 2018, 122 (1), 171-180.

203. Fernandez, M. N.; Munoz-Olivas, R.; Luque-Garcia, J. L., SILAC-based quantitative proteomics identifies size-dependent molecular mechanisms involved in silver nanoparticles-induced toxicity. Nanotoxicology 2019, 1-15.

204. Wang, J.; Fan, Y., Lung injury induced by TiO2 nanoparticles depends on their structural features: size, shape, crystal phases, and surface coating. Int J Mol Sci 2014, 15 (12), 22258-78.

205. de Jesus, M. B.; Kapila, Y. L., Cellular Mechanisms in Nanomaterial Internalization, Intracellular Trafficking, and Toxicity. In Nanotoxicology, Durán, N.; Guterres, S. S.; Alves, O. L., Eds. Springer New York: New York, 2014; pp 201–227.

206. Oyabu, T.; Myojo, T.; Lee, B. W.; Okada, T.; Izumi, H.; Yoshiura, Y.; Tomonaga, T.; Li, Y. S.; Kawai, K.; Shimada, M.; Kubo, M.; Yamamoto, K.; Kawaguchi, K.; Sasaki, T.; Morimoto, Y., Biopersistence of NiO and TiO(2) Nanoparticles Following Intratracheal Instillation and Inhalation. Int J Mol Sci 2017, 18 (12).

207. Siegrist, S.; Corek, E.; Detampel, P.; Sandstrom, J.; Wick, P.; Huwyler, J., Preclinical hazard evaluation strategy for nanomedicines. Nanotoxicology 2018, 1-27.

208. Sabella, S.; Carney, R. P.; Brunetti, V.; Malvindi, M. A.; Al-Juffali, N.; Vecchio, G.; Janes, S. M.; Bakr, O. M.; Cingolani, R.; Stellacci, F.; Pompa, P. P., A general mechanism for intracellular toxicity of metal-containing nanoparticles. Nanoscale 2014, 6 (12), 7052-61.

209. Filipova, M.; Elhelu, O. K.; De Paoli, S. H.; Fremuntova, Z.; Mosko, T.; Cmarko, D.; Simak, J.; Holada, K., An effective "three-in-one" screening assay for testing drug and nanoparticle toxicity in human endothelial cells. PLoS One 2018, 13 (10), e0206557.

210. Freese, C.; Anspach, L.; Deller, R. C.; Richards, S. J.; Gibson, M. I.; Kirkpatrick, C. J.; Unger, R. E., Gold nanoparticle interactions with endothelial cells cultured under physiological conditions. Biomater Sci 2017, 5 (4), 707-717.

211. Corbo, C.; Molinaro, R.; Parodi, A.; Toledano Furman, N. E.; Salvatore, F.; Tasciotti, E., The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery. Nanomedicine (Lond) 2016, 11 (1), 81-100.

212. Barbalinardo, M.; Caicci, F.; Cavallini, M.; Gentili, D., Protein Corona Mediated Uptake and Cytotoxicity of Silver Nanoparticles in Mouse Embryonic Fibroblast. Small 2018, 14 (34), e1801219.

213. Duran, N.; Silveira, C. P.; Duran, M.; Martinez, D. S., Silver nanoparticle protein corona and toxicity: a mini-review. J Nanobiotechnology 2015, 13, 55.

214. Laloy, J.; Minet, V.; Alpan, L.; Mullier, F.; Beken, S.; Toussaint, O.; Lucas, S.; Dogne, J. M., Impact of Silver Nanoparticles on Haemolysis, Platelet Function and Coagulation. Nanobiomedicine 2014, 1, 4.

215. Jenkins, S. V.; Qu, H.; Mudalige, T.; Ingle, T. M.; Wang, R.; Wang, F.; Howard, P. C.; Chen, J.; Zhang, Y., Rapid determination of plasmonic nanoparticle agglomeration status in blood. Biomaterials 2015, 51, 226-237.

216. Tomasetti, L.; Breunig, M., Preventing Obstructions of Nanosized Drug Delivery Systems by the Extracellular Matrix. Advanced healthcare materials 2018, 7 (3).

217. Ajdary, M.; Moosavi, M. A.; Rahmati, M.; Falahati, M.; Mahboubi, M.; Mandegary, A.; Jangjoo, S.; Mohammadinejad, R.; Varma, R. S., Health Concerns of Various Nanoparticles: A Review of Their in Vitro and in Vivo Toxicity. Nanomaterials (Basel) 2018, 8 (9).

218. Moradi-Sardareh, H.; Basir, H. R. G.; Hassan, Z. M.; Davoudi, M.; Amidi, F.; Paknejad, M., Toxicity of silver nanoparticles on different tissues of Balb/C mice. Life Sci 2018, 211, 81-90.

219. Li, C. H.; Shyu, M. K.; Jhan, C.; Cheng, Y. W.; Tsai, C. H.; Liu, C. W.; Lee, C. C.; Chen, R. M.; Kang, J. J., Gold Nanoparticles Increase Endothelial Paracellular Permeability by Altering Components of Endothelial Tight Junctions, and Increase Blood-Brain Barrier Permeability in Mice. Toxicological sciences : an official journal of the Society of Toxicology 2015, 148 (1), 192-203.

220. Parveen, S.; Misra, R.; Sahoo, S. K., Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomedicine : nanotechnology, biology, and medicine 2012, 8 (2), 147-66.

221. Blanco, E.; Shen, H.; Ferrari, M., Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature biotechnology 2015, 33 (9), 941-51.

222. Crist, R. M.; Grossman, J. H.; Patri, A. K.; Stern, S. T.; Dobrovolskaia, M. A.; Adiseshaiah, P. P.; Clogston, J. D.; McNeil, S. E., Common pitfalls in nanotechnology: lessons learned from NCI's Nanotechnology Characterization Laboratory. Integrative biology : quantitative biosciences from nano to macro 2013, 5 (1), 66-73.

223. Paswan, S. K.; Saini, T. R., Purification of Drug Loaded PLGA Nanoparticles Prepared by Emulsification Solvent Evaporation Using Stirred Cell Ultrafiltration Technique. Pharm Res 2017, 34 (12), 2779-2786.

224. Pooja, D.; Tunki, L.; Kulhari, H.; Reddy, B. B.; Sistla, R., Optimization of solid lipid nanoparticles prepared by a single emulsification-solvent evaporation method. Data in brief 2016, 6, 15-9.

225. Ahlawat, J.; Henriquez, G.; Narayan, M., Enhancing the Delivery of Chemotherapeutics: Role of Biodegradable Polymeric Nanoparticles. Molecules 2018, 23 (9).

226. George, A.; Shah, P. A.; Shrivastav, P. S., Natural biodegradable polymers based nano-formulations for drug delivery: A review. Int J Pharm 2019, 561, 244-264.

227. Liu, D.; Yang, F.; Xiong, F.; Gu, N., The Smart Drug Delivery System and Its Clinical Potential. Theranostics 2016, 6 (9), 1306-23.

228. Acharya, S.; Sahoo, S. K., PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Adv Drug Deliv Rev 2011, 63 (3), 170-83.

229. Gou, M.; Wei, X.; Men, K.; Wang, B.; Luo, F.; Zhao, X.; Wei, Y.; Qian, Z., PCL/PEG copolymeric nanoparticles: potential nanoplatforms for anticancer agent delivery. Current drug targets 2011, 12 (8), 1131-50.

230. Weiss, A.-V.; Fischer, T.; Iturri, J.; Benitez, R.; Toca-Herrera, J. L.; Schneider, M., Mechanical properties of gelatin nanoparticles in dependency of crosslinking time and storage. Colloids and Surfaces B: Biointerfaces 2019, 175, 713-720.

231. Yi, X.; Lian, X.; Dong, J.; Wan, Z.; Xia, C.; Song, X.; Fu, Y.; Gong, T.; Zhang, Z., Co-delivery of Pirarubicin and Paclitaxel by Human Serum Albumin Nanoparticles to Enhance Antitumor Effect and Reduce Systemic Toxicity in Breast Cancers. Mol Pharm 2015, 12 (11), 4085-98.

232. Kesharwani, S. S.; Nandekar, P. P.; Pragyan, P.; Rathod, V.; Sangamwar, A. T., Characterization of differences in substrate specificity among CYP1A1, CYP1A2 and

CYP1B1: an integrated approach employing molecular docking and molecular dynamics simulations. Journal of molecular recognition : JMR 2016, 29 (8), 370-90.

233. Zhang, E.; Xing, R.; Liu, S.; Li, K.; Qin, Y.; Yu, H.; Li, P., Vascular targeted chitosan-derived nanoparticles as docetaxel carriers for gastric cancer therapy. Int J Biol Macromol 2019, 126, 662-672.

234. Quinones, J. P.; Peniche, H.; Peniche, C., Chitosan Based Self-Assembled Nanoparticles in Drug Delivery. Polymers 2018, 10 (3).

235. Singh, R. P.; Ramarao, P., Accumulated polymer degradation products as effector molecules in cytotoxicity of polymeric nanoparticles. Toxicological sciences : an official journal of the Society of Toxicology 2013, 136 (1), 131-43.

236. Yu, W.; Liu, C.; Ye, J.; Zou, W.; Zhang, N.; Xu, W., Novel cationic SLN containing a synthesized single-tailed lipid as a modifier for gene delivery. Nanotechnology 2009, 20 (21), 215102.

237. Chantaburanan, T.; Teeranachaideekul, V.; Chantasart, D.; Jintapattanakit, A.; Junyaprasert, V. B., Effect of binary solid lipid matrix of wax and triglyceride on lipid crystallinity, drug-lipid interaction and drug release of ibuprofen-loaded solid lipid nanoparticles (SLN) for dermal delivery. J Colloid Interface Sci 2017, 504, 247-256.

238. Winter, E.; Dal Pizzol, C.; Locatelli, C.; Crezkynski-Pasa, T. B., Development and Evaluation of Lipid Nanoparticles for Drug Delivery: Study of Toxicity In, Vitro and In Vivo. Journal of nanoscience and nanotechnology 2016, 16 (2), 1321-30.

239. Knudsen, K. B.; Northeved, H.; Kumar, P. E.; Permin, A.; Gjetting, T.; Andresen, T. L.; Larsen, S.; Wegener, K. M.; Lykkesfeldt, J.; Jantzen, K.; Loft, S.; Moller, P.; Roursgaard, M., In vivo toxicity of cationic micelles and liposomes. Nanomedicine : nanotechnology, biology, and medicine 2015, 11 (2), 467-77.

240. Shah, V.; Taratula, O.; Garbuzenko, O. B.; Patil, M. L.; Savla, R.; Zhang, M.; Minko, T., Genotoxicity of different nanocarriers: possible modifications for the delivery of nucleic acids. Current drug discovery technologies 2013, 10 (1), 8-15.

241. Nassimi, M.; Schleh, C.; Lauenstein, H. D.; Hussein, R.; Hoymann, H. G.; Koch, W.; Pohlmann, G.; Krug, N.; Sewald, K.; Rittinghausen, S.; Braun, A.; Muller-

Goymann, C., A toxicological evaluation of inhaled solid lipid nanoparticles used as a potential drug delivery system for the lung. Eur J Pharm Biopharm 2010, 75 (2), 107-16.

242. Bourquin, J.; Milosevic, A.; Hauser, D.; Lehner, R.; Blank, F.; Petri-Fink, A.; Rothen-Rutishauser, B., Biodistribution, Clearance, and Long-Term Fate of Clinically Relevant Nanomaterials. Adv Mater 2018, 30 (19), e1704307.

243. Zhang, P.; Sun, F.; Liu, S.; Jiang, S., Anti-PEG antibodies in the clinic: Current issues and beyond PEGylation. J Control Release 2016, 244 (Pt B), 184-193.

244. Wolfram, J.; Zhu, M.; Yang, Y.; Shen, J.; Gentile, E.; Paolino, D.; Fresta, M.; Nie, G.; Chen, C.; Shen, H.; Ferrari, M.; Zhao, Y., Safety of Nanoparticles in Medicine. Current drug targets 2015, 16 (14), 1671-81.

245. Mendonça, M. C. P.; Radaic, A.; F., G.-F.; Cruz-Höfling, M. A.; Vinolo, M. A. R.; de Jesus, M. B., The in vivo toxicological profile of cationic solid lipid nanoparticles 2019, In Press.

246. Mendonca, M. C. P.; Ferreira, L. B.; Rizoli, C.; Batista, A. G.; Marostica Junior, M. R.; da Silva, E. D. N.; Cadore, S.; Duran, N.; Cruz-Hofling, M. A. D.; de Jesus, M. B., N-Acetylcysteine reverses silver nanoparticle intoxication in rats. Nanotoxicology 2019, 13 (3), 326-338.

247. Evans, J. C.; Malhotra, M.; Fitzgerald, K. A.; Guo, J.; Cronin, M. F.; Curtin, C. M.; O'Brien, F. J.; Darcy, R.; O'Driscoll, C. M., Formulation and Evaluation of Anisamide-Targeted Amphiphilic Cyclodextrin Nanoparticles To Promote Therapeutic Gene Silencing in a 3D Prostate Cancer Bone Metastases Model. Mol Pharm 2017, 14 (1), 42-52.

248. Ahn, J.; Cho, C. S.; Cho, S. W.; Kang, J. H.; Kim, S. Y.; Min, D. H.; Song, J. M.; Park, T. E.; Jeon, N. L., Investigation on vascular cytotoxicity and extravascular transport of cationic polymer nanoparticles using perfusable 3D microvessel model. Acta Biomater 2018, 76, 154-163.

249. Ozkan, A.; Ghousifam, N.; Hoopes, P. J.; Yankeelov, T. E.; Rylander, M. N., In vitro vascularized liver and tumor tissue microenvironments on a chip for dynamic determination of nanoparticle transport and toxicity. Biotechnol Bioeng 2019, 116 (5), 1201-1219.

250. Yang, K.; Park, H. J.; Han, S.; Lee, J.; Ko, E.; Kim, J.; Lee, J. S.; Yu, J. H.; Song, K. Y.; Cheong, E.; Cho, S. R.; Chung, S.; Cho, S. W., Recapitulation of in vivo-like paracrine signals of human mesenchymal stem cells for functional neuronal differentiation of human neural stem cells in a 3D microfluidic system. Biomaterials 2015, 63, 177-88.

251. Lopes, C. D.; Gomes, C. P.; Neto, E.; Sampaio, P.; Aguiar, P.; Pego, A. P., Microfluidic-based platform to mimic the in vivo peripheral administration of neurotropic nanoparticles. Nanomedicine (Lond) 2016, 11 (24), 3205-3221.

252. Chaicharoenaudomrung, N.; Kunhorm, P.; Noisa, P., Three-dimensional cell culture systems as an in vitro platform for cancer and stem cell modeling. World J Stem Cells 2019, 11 (12), 1065-1083.

253. Shah, M. K.; Khatri, P.; Vora, N.; Patel, N. K.; Jain, S.; Lin, S., Lipid nanocarriers: Preparation, characterization and absorption mechanism and applications to improve oral bioavailability of poorly water-soluble drugs. In Biomedical Applications of Nanoparticles, Elsevier: 2019; pp 117-147.

254. Tan, Y. L.; Ho, H. K., Navigating albumin-based nanoparticles through various drug delivery routes. Drug discovery today 2018, 23 (5), 1108-1114.

255. Zhang, G.; Jiang, X., Temperature Responsive Nanoparticles Based on PEGylated Polyaspartamide Derivatives for Drug Delivery. Polymers 2019, 11 (2), 316.

256. Kariduraganavar, M. Y.; Heggannavar, G. B.; Amado, S.; Mitchell, G. R., Protein Nanocarriers for Targeted Drug Delivery for Cancer Therapy. In Nanocarriers for Drug Delivery, Elsevier: 2019; pp 173-204.

Capitulo III – Enhancement of the mechanical and drug-releasing properties of poloxamer 407 hydrogels with casein

Louise Lacalendola Tundisi, Rong Yang, Luiz Phellipe Pozzuto Borelli, Thais Alves, Manisha Mehta, Marco Vinícius Chaud, Priscila Gava Mazzola, Daniel S Kohane.

Pharmaceutical Research, p. 1-8

DOI: 10.1007/s11095-021-03017-9

ABSTRACT

Purpose. Topical therapy of local disease (e.g. skin) is advantageous over oral therapy since there is less systemic drug distribution (so fewer side-effects), no first-pass effect, etc. However, patient compliance with topical therapy can be poor as it may require many applications a day and can last months. Here we propose a topical controlled release formulation with thermoresponsive gelation at body temperature and improved adhesiveness, making it easier to remain in contact with the body.

Methods. The formulation contains two excipients, poloxamer 407 (P407) and casein. Casein can modify the properties of the hydrogel through molecular entanglement. In addition, tissue reaction and drug release profile were evaluated.

Results. Changes in casein concentration affected adhesive strength, viscosity, mechanical properties and drug release, presumably by hydrophobic interactions between casein and P407. Two different concentrations of P407 were tested with two different concentrations of casein. Formulations containing 5% and 10% casein released 80% of model drug in 48h, while formulations without casein released the same fraction in around 24h hours. Formulations with 10% casein had almost twice the adhesive strength of those without casein.

Conclusions. Addition of casein modified the mechanical properties and drug release rate of the hydrogel. There was no inflammation or injury after brief exposure in vivo.

Key words: casein, poloxamer 407, drug delivery, mixed micelles, topical treatment

INTRODUCTION

Topical therapy can be advantageous over oral therapy, since it can achieve very high local drug concentrations locally (in absolute terms and in proportion to systemic distribution), there is no first-pass effect, and it usually does not require laboratory monitoring during treatment. Hydrogels have been extensively studied for drug delivery, particularly for topical applications (1, 2). With topical applications, being able to improve the properties of the hydrogel, such as adhesiveness and viscosity, would make it easier for the formulation to remain in contact with the skin, particularly in places that are relatively difficult to access or with high friction (e.g. between toes, intertriginous areas). Improvement of release kinetics could reduce the number of daily applications, enhancing patient compliance. The properties of hydrogels such as drug release and mechanical properties (and therefore duration of release and frequency of administration) can be tuned by changing the composition of the hydrogel (3).

Poloxamer 407 (P407) is an uncharged synthetic amphiphilic triblock copolymer composed of a hydrophobic polypropylene oxide block flanked by two lateral hydrophilic polyethylene oxide blocks. It can be applied as liquid at room temperature and forms a hydrogel by reverse thermal gelation upon contact with the warmer human body (4). With increasing temperature, poloxamer molecules aggregate into micelles due to dehydration of hydrophobic blocks of the poloxamer. Reverse thermal gelation has been useful in a variety of drug delivery applications (5-7). However, P407 presents some deficiencies such as relatively low gel mechanical strength and weak mucoadhesiveness. The mechanical properties of poloxamers, which are important for retention in situ, can be improved by blending with other polymers (3).

Casein, a naturally occurring anionic, amphiphilic protein. It is a diblock copolymer, with hydrophobic and hydrophilic domains at the C- and N-terminal regions, respectively (8, 9). It can be added to a hydrogel to increase its adhesiveness through molecular entanglement (10-12). Casein and poloxamers are miscible and form micelles in solution, forming mixed micelles (13, 14). Casein has been used to improve the mechanical properties of PVA hydrogels (15). Therefore, we hypothesized that casein may improve mechanical properties of P407 hydrogels.

Here we investigate whether the addition of casein to P407 would create a stronger, more adhesive topical hydrogel for extended controlled release.

MATERIALS AND METHODS

Materials

Poloxamer 407 (P407) (BASF, New Jersey, USA), casein (C3400-Sigma-Aldrich, Minnesota, USA) (approximate casein composition of milk is (g/L): □-s1, 12-15; □-s2, 3-4; ß, 9-11; and k, 2-4), bupivacaine hydrochloride (Sigma-Aldrich, Minnesota, USA), sulforhodamine B (230162 -Sigma-Aldrich, Minnesota, USA). Bupivacaine hydrochloride and sulforhodamine B were chosen as model drugs.

Preparation of hydrogels and characterization

Casein solution (50 mg/mL) was prepared by dissolving casein in deionized water and adjusting the solution pH to 7 using a 5 mol/L NaOH solution. The obtained casein solution was stored in a 4ºC refrigerator. P407 at 25% (w/v) and 18% (w/v) of was added and the solutions and stirred overnight at 4°C.

ATR-FTIR

The FTIR spectra were recorded in an attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectrometer (Nicolet iS50, Thermo Fisher Scientific, USA).

Mechanical tests

Rheological experiments (viscosity and G'G")

Hydrogels samples rheological analysis were carried out using a Discovery HR-2 hybrid rheometer (TA Instruments, Delaware, USA). Measurements were performed at a temperature range from 10 to 45 °C using linear oscillatory shear rheology measurements (100 rads−1, 1% strain, and 1°C/min) and 25mm parallel plate, Peltier plate steel (105100), a sample volume of 350 µL, a gap between the plates of 0.300 mm. The oscillatory measurements were used to determine parameters related to the elastic modulus (G′), the viscous modulus (G″). Gelation temperature was taken as the temperature at which G′ becomes greater than G″ .

Gelation temperature and viscosity were quantified using linear oscillatory shear rheology measurements (100 rads−1, 1% strain, and 1°C/min). Gelation temperature was taken as the temperature at which G′ becomes greater than G″ . The changes of G′ and G″ over temperatures ranging from 10°C to 45°C were recorded to reflect changes in mechanical properties.

Bioadhesion properties

Bioadhesive properties of the formulations were evaluated using a Stable Micro Systems texture analyzer (Model TA-XT Plus) in the TPA mode (16-18). Portions of porcine tissue (epidermis/dermis) were fixed horizontally to the lower end of the analytical probe (P/10) using double-sided adhesive tape and suture, respectively. Hydrogels were placed into a fixed volume compartment and conditioned in a water bath at 37 °C. In TPA mode, the analytical probe descended on the surface of each formulation at a constant rate of 0.5 mm/s, penetrated 10 mm in the formulation and a downward tensile force of 2.45 N was applied at fixated time of 90s. In sequence, biological substrate returned vertically to the formulation surface at a constant rate of 10.0 mm/s. After each cycle, hydrogels and biological substrate were replaced.

Drug Release

The release of sulforhodamine B, bupivacaine hydrochloride from each formulation was performed using a diffusion system. Using Yang, et al. (7) adapted, transwell membrane inserts (0.4-mm pore size and 0.33-cm2 area; Costar) and 24-well culture plates were used as the donor and acceptor chambers, respectively. Two hundred microliters of each formulation was pipetted directly onto pre-warmed filter inserts to obtain a solid hydrogel. Filter inserts (donor compartments) with formed gels were suspended in wells (acceptor compartments) filled with pre-warmed phosphate-buffered saline (PBS) pH 7.4, and the plates were then incubated in an oven (37°C). At each sampling time (0.5, 1, 2, 6, 12, 24, 48h), 1 mL aliquots of the PBS-receiving media were collected and inserts were sequentially moved into a new well with fresh PBS. Sample aliquots were analyzed by spectrophotometer UV to determine sulforhodamine B (560 nm) and bupivacaine hydrochloride (263nm) concentrations. Experiments were performed in quadruplicate.

Tissue Reaction

Animals were cared for in compliance with protocol approved by the Boston Children's Hospital Committee, in conformity with NIH guidelines for care and use of laboratory animals (Protocol number: 18-07-3348). In order to prevent animals from rubbing the hydrogel off their skin, these experiments were performed under isoflurane anesthesia. 6h was the maximum time frame allowed at our institutions. Attempts to keep the hydrogel on the

animals for longer periods without anesthesia using occulsive dressings and other devices were unsuccessful.

Male Sprague–Dawley rats weighting 400–600 g were anesthetized under 2% isoflurane in oxygen, and their dorsal aspect was shaved and disinfected with 70% ethanol and betadine. 3 groups of hydrogels (P25; P25C10; P18C5) were tested by applying them on to separate sites / flanks of the rat. Each flank had a different formulation and each formulation group had an N=4. Normal rat skin (with no formulation applied) was used as a negative control. The rats were kept under anesthesia for 6 hours to ensure that the formulation stayed in contact with the skin during the entire experiment. After 6 h, the animals were euthanized with an injection of Pentobarbital 100 mg/Kg. A 1.5 cm2 area that included the site of the formulation application was excised. Tissues were processed, embedded in paraffin, cut into 10 µm sections, and stained with hematoxylin and eosin. Histologic evaluation of tissues by light microscopy was performed by a pathologist in a masked fashion.

Statistics

Data are expressed as means ± standard deviations. All statistical comparisons were made with Student's t-test, unpaired for independent samples. A p value < 0.05 was considered to indicated statistical significance.

RESULTS

Preparation of hydrogels and characterization

Table 1- P407-Casein hydrogels formulation. The concentrations are all in (w/v). See text for definition of abbreviations.

	P18	P18C5	P18C10	P25	P25C5	P25C10
P407	**18%**	**18%**	**18%**	**25%**	**25%**	**25%**
Casein	**-**	**5%**	**10%**	**-**	**5%**	**10%**

We formulated combinations of P407 and casein in varying proportions (Table 1). Formulations are labeled PxCy, where P = P407, C = casein, and x and y are the % (w/v) of each. FT-IR spectroscopy of casein, P407 and P407-casein (Fig. 1), showed the presence of the

two molecules in the hydrogels (peaks for casein: 1644.49 cm-1 [carbonyl groups], 1517.33 cm-1 [N-H bending and C-N stretching vibration]; for P407: 1105.36 cm-1 [C-O stretch] (7, 11)). There was no evidence of new bond formation by FT-IR.

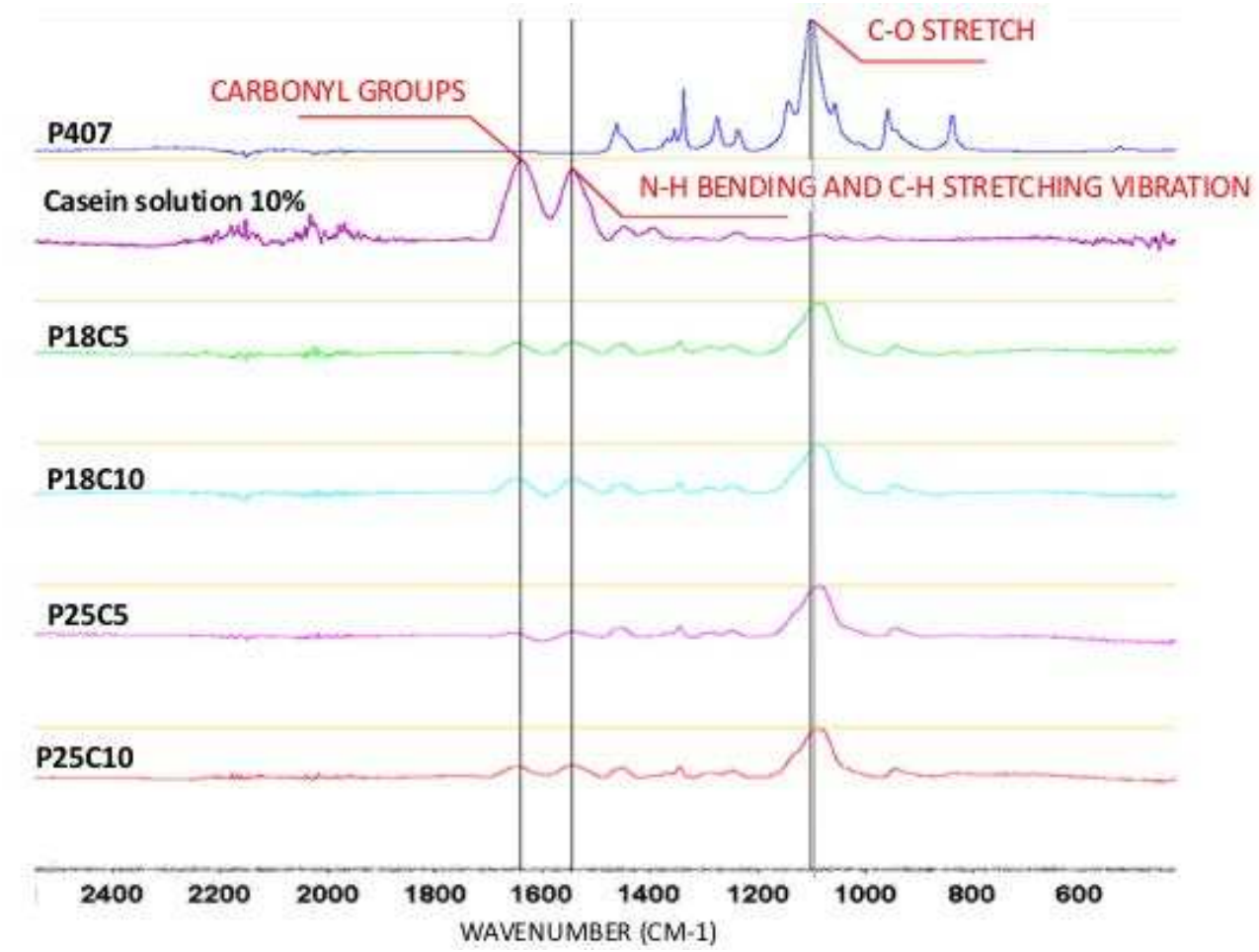


Fig. 1 - ATR-FTIR spectra of P407, Casein 10%, P407 18%-Casein 5% and 10%, P407 25%-Casein 5% and 10%, respectively.

To assess the mechanical effects of the addition of casein to P407, the rheology of the hydrogels was examined by linear oscillatory shear rheology measurements (100 rads−1, 1% strain, and 1°C/min) over a range of temperatures (Fig. 2). Increasing storage modulus (G') relates to mechanical strength, i.e. solid-like properties. The loss modulus (G") relates to liquid-like properties. G' > G" suggests gelation Yang, et al. (7). The addition of casein had little effect on the G' of P25 at 37 oC, 1.07 fold with addition of 10% casein ($p<0.05$) and no increase with 5% casein. However, with P18 the addition of 5% and 10% casein increased the peak G′ 1.43 and 3.73 ($p<0.005$) fold respectively. The gelation temperature of P407, at which G′ became greater than G″, was not affected by the addition of casein at any of the concentrations tested.

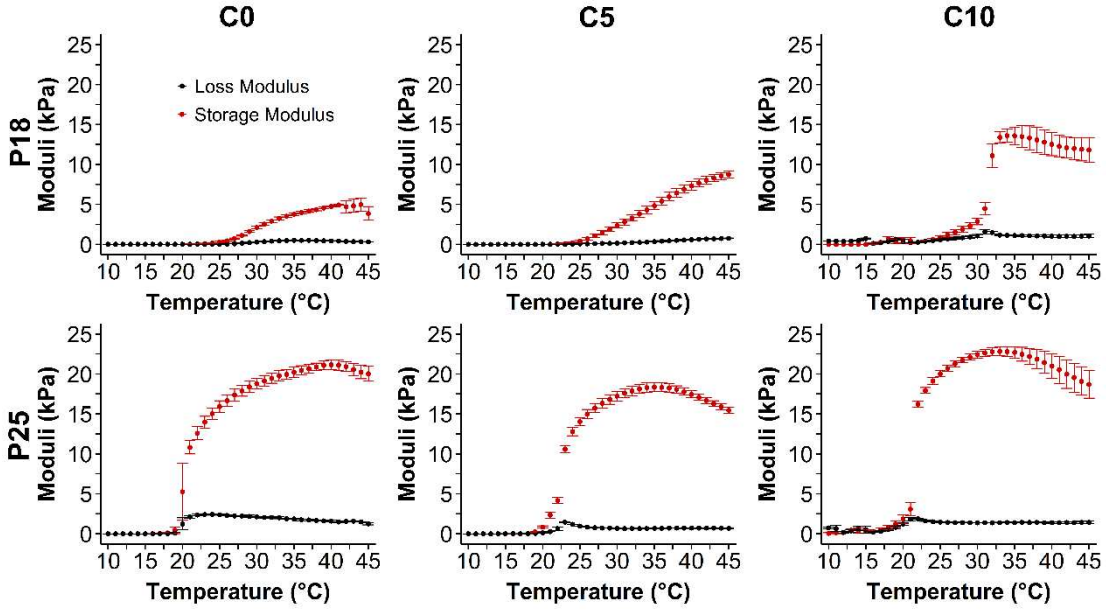


Fig. 2 – Rheology of polymer combinations as a function of temperature. G': storage modulus, G": loss modulus. Data are means ± SD (n=4).

Polymer viscosity was studied at 10 ºC and 37ºC (body temperature). At 10 °C, all formulations had very low viscosity (< 1Pa.s). The enhancement of G' that casein caused in P25 at 37 ºC was also seen with viscosity (Fig. 3). At 37 ºC, i.e. after gelation, the viscosity of P25 was unaffected by the addition of casein (Fig. 3), while that of P18 increased with the

addition of casein. For example, when 10% casein was added to P18, the viscosity of the mixture was 31.83 and 2.23 times higher than that of P18 and P18C5 respectively.

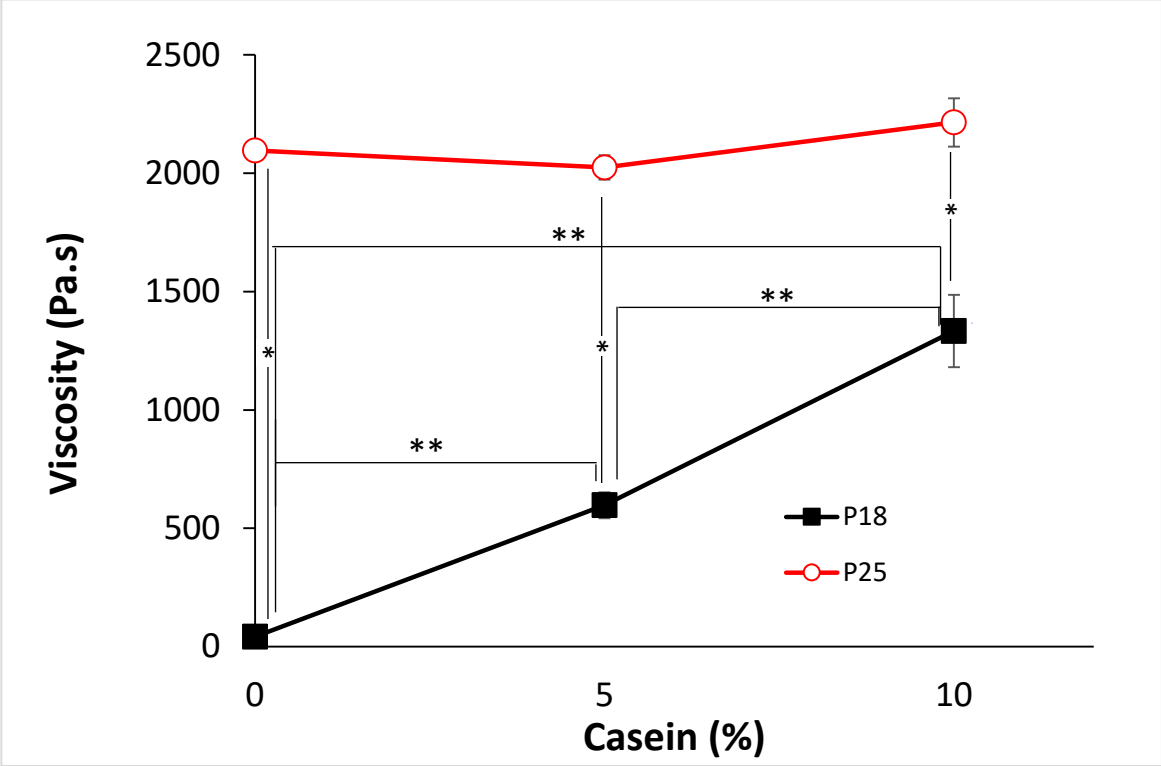


Fig. 3 – Hydrogel viscosity at 37 ºC at different casein concentrations. Data are means ± SD (n=4). *p<0.05 between different P407 concentrations with the same casein concentration. **p< 0.001 between different casein concentrations with the same P407 concentration.

The bioadhesive properties of the P407-casein were assessed by measuring the loading strength required to detach the hydrogel (at 37°C) from porcine skin (Fig. 4). The addition of 5% casein to P18 decreased the adhesive strength of P18 1.36-fold but increased that of P25 by 1.46-fold. 10% casein increased the adhesive strength of P18 1.91-fold and that of P25 1.81-fold.

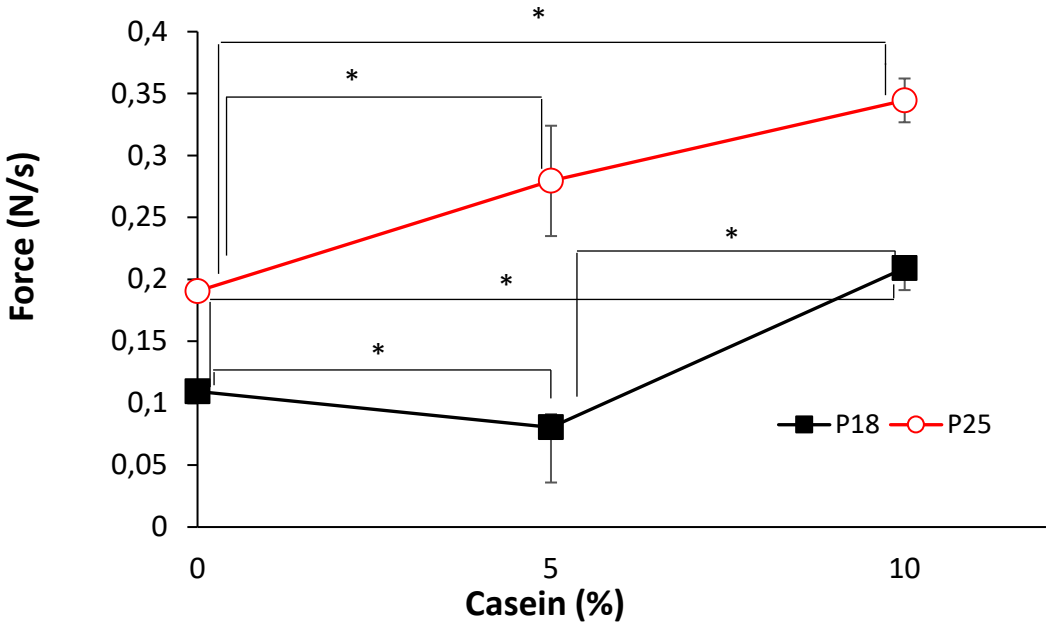


Fig. 4 - Hydrogel bioadhesion to pig skin at 37 ºC. Data are means ± SD (n=3). *p < 0.05.

Drug release

Release kinetics from the hydrogel was assessed with sulforhodamine B (logP = 1.25; ChemAxon Database) and bupivacaine hydrochloride (log P = 3.71 at pH 7; (ChemAxon Database). As described in Methods, formed hydrogels with either sulforhoramine B or bupivacaine hydrochloride, were placed in Transwell ™ inserts then immersed in wells containing pre-warmed PBS pH 7.4, and incubated at 37ºC. At predetermined intervals, aliquots of the PBS (the receiving media) were collected and inserts moved into a new well with fresh PBS.

In the absence of casein, there was little difference in the release rates of sulforhodamine B (Fig.5) or bupivacaine (Fig. 6) from P18 or P25. However, release of both drugs decreased with increasing casein concentration at both P407 concentrations. In the absence of casein, the rate of release of bupivacaine was slower than (P18) or equal to (P25) that of sulforhodamine B release (e.g. at 24h) ($p<0.05$). In the presence of 5% or 10% casein, release of bupivacaine (e.g. at 24 h) was slightly faster than that of sulforhodamine B, by 1.2- to 1.4-fold.

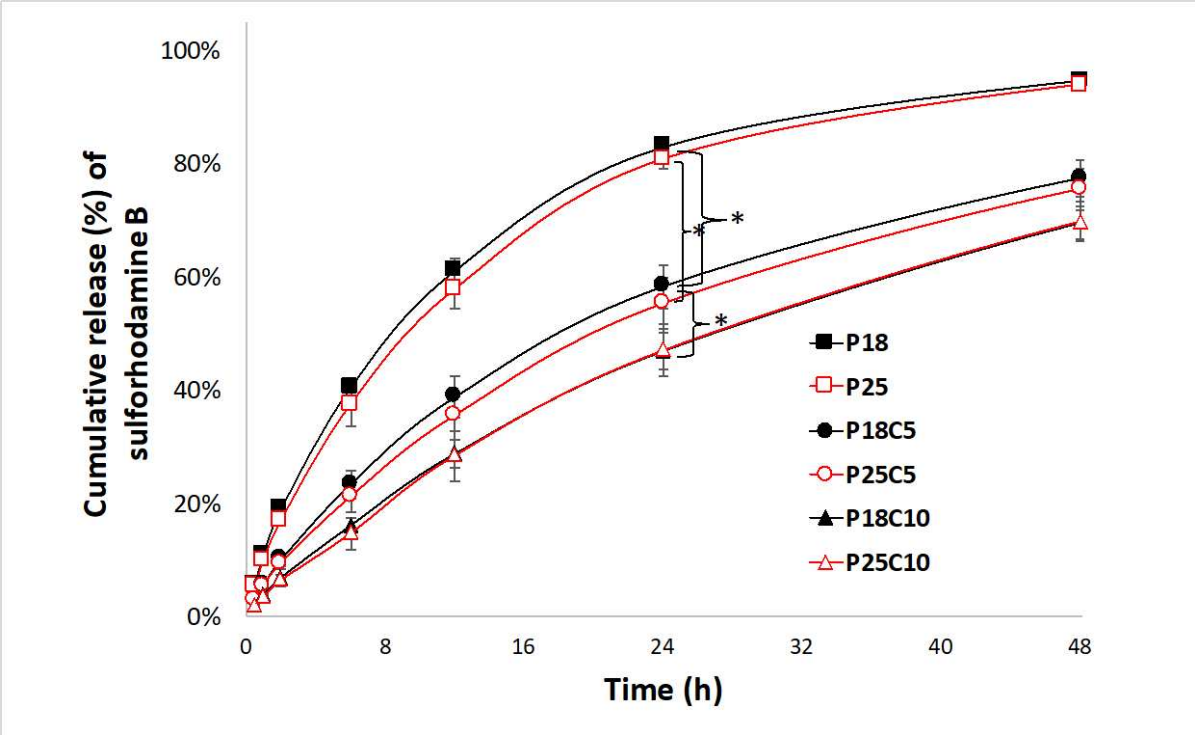


Fig. 5 – Cumulative release (%) of sulforhodamine B from P407/casein hydrogel formulations at 37ºC in PBS buffer pH 7.4. Data are means ± SD (n=4). Some error bars are obscured by the symbols due to their small size. At 24h *$p<0.05$ when compared between different casein concentrations. Please see table 1 for definition of abbreviations.

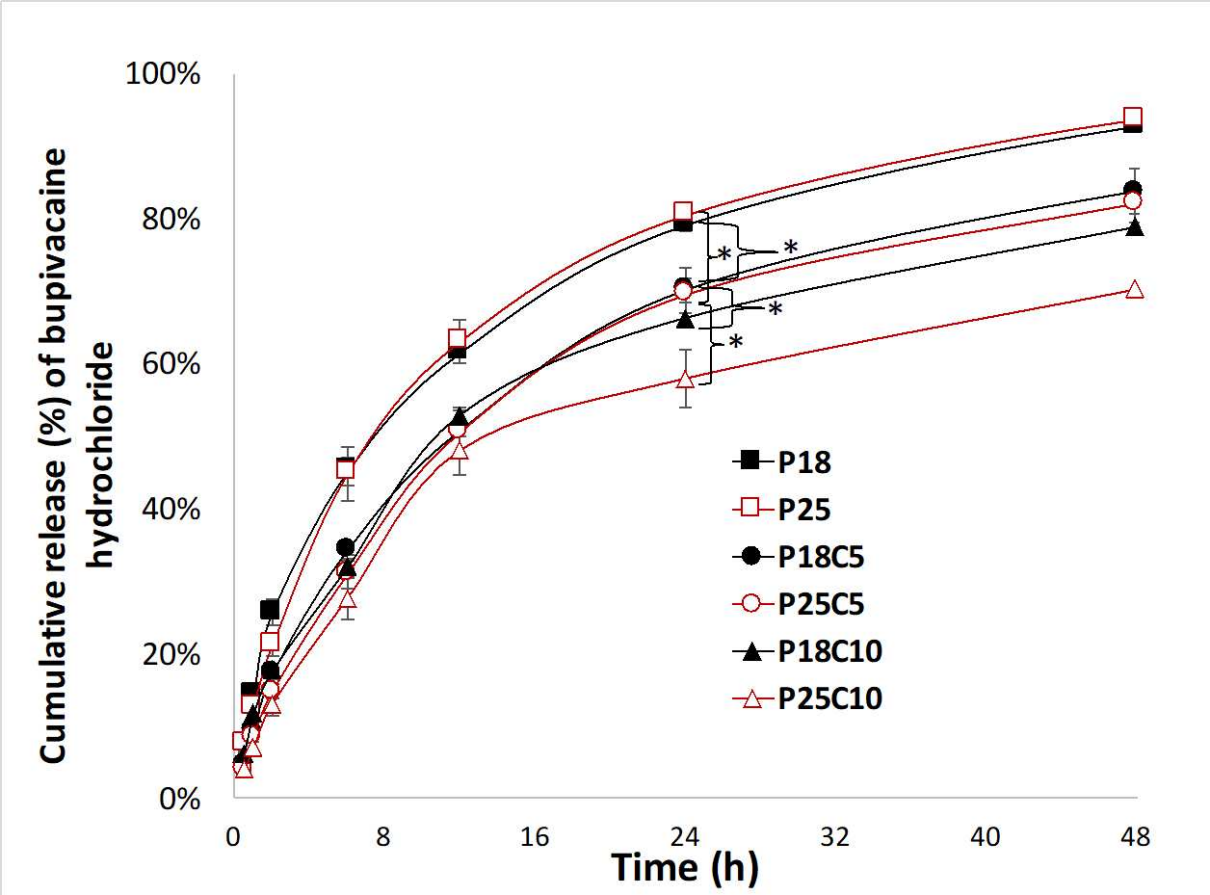


Fig. 6 - Cumulative release (%) of bupivacaine hydrochloride from P407/casein hydrogel formulations at 37ºC in PBS buffer pH 7.4. Data are means ± SD (n=4). Some error bars are obscured by the symbols due to their small size. *p<0.05 when compared between different casein concentrations. Please see table 1 for definition of abbreviations.

Tissue Reaction

To assess tissue reaction in vivo, two areas on the backs of the rats were shaved and 3 hydrogels, including those with the highest and lowest total mass %w/v (P25, P25C10, and P18C5), were applied (Fig. 7a). After 6h, animals were euthanized, and skin samples were collected, and processed into hematoxylin and eosin-stained slides. On histologic evaluation (Fig. 7b), no signs of inflammation or tissue injury were seen in any layers of the skin, subcutaneous tissue, or underlying skeletal muscle layer and fascia.

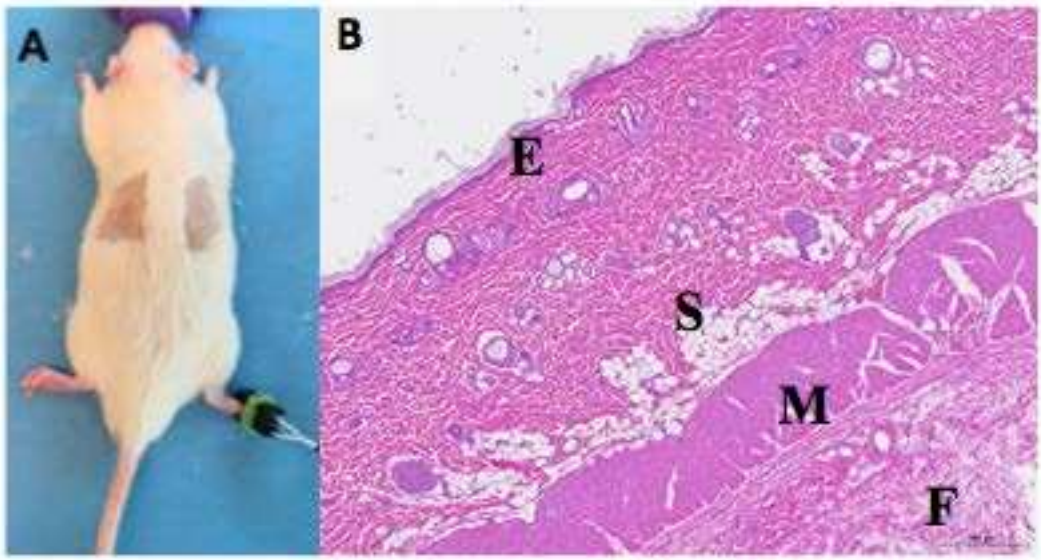


Fig. 7 - Histologic evaluation after surface application of formulations (A) Placement of hydrogels on the skin. (B) Photomicrograph of hematoxylin-eosin-stained section of skin exposed to the P25C10 formulation, showing epidermis (E), subcutaneous tissue (S), underlying skeletal muscle (M) and fascia (F).

DISCUSSION

The addition of casein to P407 generally enhanced the mechanical properties (G' and viscosity) of P407, as well as its adhesive properties, and slowed the release of low-molecular weight compounds. The combination of these macromolecules did not result in new bond formation, suggesting that the interactions that caused those effects occurred by entanglement and the formation of mixed micelles (13). The lack of enhancement of G' with the addition of casein to P25 (which was seen with P18) could be due to that effect having been maximized.

Viscosity can enhance hydrogel retention, helping patients' compliance and the efficiency of the treatment (16, 19). In general, and here, viscosity is driven in part by polymer concentration. In solutions of two polymers, it can also depend on interaction between those polymers, which can have a maximum effect at a given concentration, when all the possible interactions between the two molecules are made (20). (This could explain why the viscosity of P18 was increased by the addition of casein, while that of P25 was not.) In the system, the physical properties of the gel would have also been affected by micellization of P407, the formation of mixed micelles, etc (13).

Viscosity is also an important determinant of the release of drug from hydrogels, as it is harder for the drug to diffuse at higher viscosity (21). In addition to increasing the hydrogel's viscosity, the addition of casein could have altered drug release kinetics by altering the micelle properties. Micellization creates distinct hydrophilic and hydrophobic domains within the hydrogel. The latter would provide the opportunity for relatively hydrophobic drugs to partition into them, potentially extending the duration of release. In that context, it is worth noting that, for example, bupivacaine has an aromatic group and a tertiary amine. Protonation of the tertiary amine confers water solubility and interaction with anionic surfaces, while the aromatic group imparts the ability to partition into hydrophobic domains of drug delivery systems or biological systems (e.g. lipid bilayers in liposomes or cells). The hydrophilicity/-phobicity of the compound is determined by pH-dependent protonation of the amine.

In addition to the creation of micelles, the type of micelles that can be formed can affect drug release. In these formulations, the P407 concentration was always greater than the casein concentration (w/v). If micelle formation is determined by the amount of casein available in each system (22), the number of mixed micelles formed should be similar at a given casein concentration, and might not vary between the two concentrations of P18 and P25. Assuming this is true for our system, one possible explanation for the prolongation of drug release is that increasing the casein concentration might encourage the formation of mixed micelles. As they are much bigger than polymeric micelles, this would make it more difficult for molecules to diffuse through and out of the hydrogel, as the spaces between the micelles became smaller, less free space to diffuse freely (22, 23).

As noted above, bupivacaine is amphiphilic. Based on the potential for interaction with the hydrophobic segments of P407 and with the anionic domains of casein, bupivacaine might have been expected to be released more slowly from the hydrogel than was sulforhodamine B. However, interactions with the hydrophobic domains of P407 and casein were likely impeded by the fact that bupivacaine is mostly in its hydrophilic cationic form at pH 7 (24). Moreover, the long PEO segments of poloxamers can mask the surface charge of α-casein micelles (13). (The isoelectric point of casein is 4.7, so it carries a negative charge at neutral or physiological pH.) This masking could impede electrostatic interactions of the protonated tertiary amine on bupivacaine with anionic casein. The slightly faster release of bupivacaine than of sulforhodamine B may be attributable to the fact that it has a lower molecular weight (25).

Bioadhesion is important to ensure retention at the site of application, improving patient compliance (19). As P407 is a hydrogel with no charge, its interaction with skin is likely

based on hydrogen bonds (26). The addition of casein increased adhesiveness in 3 of the 4 formulations tested. If such charge masking occurred with the mixed micelles formed by P407 and casein in our system as well, then interaction with skin would likely not be due to electrostatic interactions. Increased adhesiveness was therefore likely mediated by the increase of hydrogen bonds, new hydrophobic interactions due to the addition of casein or both (11, 15). Casein can interact with other molecules by several mechanisms, including hydrophobic interactions, van der Waals attraction and hydrogen bonds (12, 27).

Tissue reaction, over the relatively short period of exposure studied here, was benign. This was not surprising given that P407 (3) and casein (12) have good biocompatibility. However, as noted in Methods, we were unable to maintain contact between the hydrogels and skin for longer than six hours. While this might correspond to the duration of a single application in humans, the time frame over which serial applications would be applied to treat most cutaneous diseases is much longer. While the fact that there was no tissue reaction whatsoever within the 6h is somewhat reassuring, it cannot preclude the possibility of tissue reaction with more extended exposure.

CONCLUSION

The addition of casein to P407 increased its adhesiveness, mechanical strength and viscosity, and slowed the release rates of the model drugs. Choosing casein, a food-grade biopolymer, to modify the properties of the gel presented advantages in safety, cost, commercial availability. The properties of the hydrogel could be tuned by changing the proportions of casein and P407. These improvements could have an impact on patient compliance, particularly with topical therapies that may last months and require many applications a day. Bioadhesive hydrogels driven by the addition of readily available proteins have potential as translatable controlled drug release systems.

ACKNOWLEDGMENTS

This study was supported in part by grants FAPESP 2017/10728-2 and 2018/05421-8 (to L.L.T.), FAPESP 2018/07707-6 and CNPQ (to P.G.M.), R35 GM131728 (to D.S.K.)

DATA STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Croisfelt FM, Tundisi LL, Ataide JA, Silveira E, Tambourgi EB, Jozala AF, Souto EMB, Mazzola PG. Modified-release topical hydrogels: a ten-year review. Journal of Materials Science. 2019.

2. Schmolka IR. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. Journal of Biomedical Materials Research. 1972;6(6):571-582.

3. Abou-Shamat MA, Calvo-Castro J, Stair JL, Cook MT. Modifying the Properties of Thermogelling Poloxamer 407 Solutions through Covalent Modification and the Use of Polymer Additives. Macromolecular Chemistry and Physics. 2019;220(16):1900173.

4. Tung IC. Rheological behavior of poloxamer 407 aqueous solutions during sol-gel and dehydration processes. International Journal of Pharmaceutics. 1994;107(2):85-90.

5. Pai-Chie C-C, Frank SG. In vitro release of lidocaine from pluronic F-127 gels. International Journal of Pharmaceutics. 1981;8(2):89-99.

6. P.C. Chen-Chow SGF. Pluronic F-127 Gels as a Vehicle for Topical Administration of Anticancer Agents. Chemical & pharmaceutical bulletin. 1984;32(10):4205-4208.

7. Yang R, Sabharwal V, Okonkwo OS, Shlykova N, Tong R, Lin LY, Wang W, Guo S, Rosowski JJ, Pelton SI, Kohane DS. Treatment of otitis media by transtympanic delivery of antibiotics. Science Translational Medicine. 2016;8(356):356ra120.

8. Hambardzumyan A, Aguié-Béghin V, Daoud M, Douillard R. β-Casein and Symmetrical Triblock Copolymer (PEO−PPO−PEO and PPO−PEO−PPO) Surface Properties at the Air−Water Interface. Langmuir. 2004;20(3):756-763.

9. Portnaya I, Ben-Shoshan E, Cogan U, Khalfin R, Fass D, Ramon O, Danino D. Self-Assembly of Bovine β-Casein below the Isoelectric pH. Journal of agricultural and food chemistry. 2008;56:2192-2198.

10. Shaw DJ. 8 - Colloid stability. In: Shaw DJ, editor. Introduction to Colloid and Surface Chemistry (Fourth Edition). Oxford: Butterworth-Heinemann; 1992. p. 210-243.

11. Xu J, Gao G, Duan L, Sun G. Protein and Hydrophobic Association-Regulated Hydrogels with Adhesive Adjustability in Different Materials. Advanced Materials Interfaces. 2020;7(1):1901541.

12. Elzoghby AO, Abo El-Fotoh WS, Elgindy NA. Casein-based formulations as promising controlled release drug delivery systems. Journal of Controlled Release. 2011;153(3):206-216.

13. Kessler A, Menéndez-Aguirre O, Hinrichs J, Stubenrauch C, Weiss J. αs-Casein—PE6400 mixtures: Surface properties, miscibility and self-assembly. Colloids and Surfaces B: Biointerfaces. 2014;118:49-56.

14. Portnaya I, Khalfin R, Kesselman E, Ramon O, Cogan U, Danino D. Mixed micellization between natural and synthetic block copolymers: β-casein and Lutrol F-127. Physical Chemistry Chemical Physics. 2011;13(8):3153-3160.

15. Xu J, Fan Z, Duan L, Gao G. A tough, stretchable, and extensively sticky hydrogel driven by milk protein. Polymer Chemistry. 2018;9(19):2617-2624.

16. Alves T, Souza J, Rebelo M, Pontes K, Santos C, Lima R, Jozala A, Grotto D, Severino P, Rai M, Chaud M. Formulation and evaluation of thermoresponsive polymeric blend as a vaginal controlled delivery system. Journal of Sol-Gel Science and Technology. 2018;86(3):536-552.

17. Oshiro Junior JA, Carvalho FC, Soares CP, Chorilli M, Chiavacci LA. Development of Cutaneous Bioadhesive Ureasil-Polyether Hybrid Films. International Journal of Polymer Science. 2015;2015:727324.

18. Zurdo Schroeder I, Franke P, Schaefer UF, Lehr CM. Development and characterization of film forming polymeric solutions for skin drug delivery. Eur J Pharm Biopharm. 2007;65(1):111-121.

19. Hurler J, Engesland A, Poorahmary Kermany B, Škalko-Basnet N. Improved texture analysis for hydrogel characterization: Gel cohesiveness, adhesiveness, and hardness. Journal of Applied Polymer Science. 2012;125(1):180-188.

20. Tadros TF. Colloids in Agrochemicals. United Kingdom: Wiley; 2009.

21. Cheong LWS, Heng PWS, Wong LF. Relationship Between Polymer Viscosity and Drug Release from a Matrix System. Pharmaceutical Research. 1992;9(11):1510-1514.

22. Kessler A, Menéndez-Aguirre O, Hinrichs J, Stubenrauch C, Weiss J. αs-Casein–PE6400 mixtures: a fluorescence study. Faraday Discussions. 2013;166(0):399-416.

23. Maolin Z, Min Y, Jian S, Jinshan W, Hongfei H. Radiation preparation and diffusion behavior of thermally reversible hydrogels. Radiation Physics and Chemistry. 1998;52(1):313-317.

24. Stoelting RK, Miller R. Local anesthetics, Basics of anesthesia. New York, NY: Churchill Livingstone Inc.; 1994.

25. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, Assempour N, Iynkkaran I, Liu Y, Maciejewski A, Gale N, Wilson A, Chin L, Cummings R, Le D, Pon A, Knox C, Wilson M. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46(D1):D1074-d1082.

26. Ghobril C, Grinstaff MW. The chemistry and engineering of polymeric hydrogel adhesives for wound closure: a tutorial. Chem Soc Rev. 2015;44(7):1820-1835.

27. Livney YD. Milk proteins as vehicles for bioactives. Current Opinion in Colloid & Interface Science. 2010;15(1):73-83.

Capítulo IV – Terbinafine nanohybrid: proposing a hydrogel carrying nanoparticles for topical release.

Louise Lacalendola Tundisi, Jéssica Heline Lopes da Fonsêca; Janaina Artem Ataide; Marcelo Lancellotti; Marcos Dávila; Daniel S. Kohane, Priscila Gava Mazzola

A ser submetido em: Scientific Reports

ABSTRACT

Poloxamer 407 (P407) – Casein hydrogel were chosen to carry PCL-TBH nanoparticles. In this study, terbinafine hydrochloride (TBH) was encapsulated into polycaprolactone (PCL) nanoparticles, which were further incorporated into poloxamer-casein hydrogel in different addition order, to evaluate the effect of gel formation. Nanoparticles were prepared by nanoprecipitation technique and characterized by evaluating its physicochemical characteristics and morphology. The nanoparticles had a mean diameter of 196.7 ± 0.7 nm, PDI of 0.07, negative ζ potential (-0.713 mV), high encapsulation efficiency (>98%) and did not. show cytotoxic effects in primary human keratinocytes. PCL-NP modulated terbinafine release in artificial sweat. Rheological properties were analyzed by temperature sweep tests at different addition orders of nanoparticles into hydrogel formation. Rheological behavior of nanohybrid hydrogel showed the influence of TBH-PCL nanoparticles addition in the mechanical properties of the hydrogel and a long-term release of the nanoparticles from it.

KEYWORDS: hydrogel, terbinafine, Poloxamer 407, nanoparticle, drug release, rheology, polycaprolactone

1. Introduction

Nanoparticles have shown to be an efficient vehicle to either increase treatment efficiency (increase permeability or bioavailability, decrease side effects) or to protect the active ingredient[1]. When embedding the nanoparticles in a pharmaceutical formulation as hydrogel other characteristics and/or advantages in the treatment and in the product's organoleptic characteristics can be reached. This nanohybrid hydrogels can bring unique physicochemical and mechanical properties, that cannot be reached in single-component systems, also showing multi-functionality [2].

The search for advances in new polymeric materials and the development of drug delivery systems have been stimulated aiming a new approach in the treatments focusing on effectiveness and patient compliance. The use of biodegradable polymers in drug delivery is of great relevance since they decompose into non-toxic natural products [3,4]. Polymeric nanoparticles are generally suitable for topical drug delivery once they tend to accumulate in the skin compared to solid lipid nanoparticles and carriers of lipid nanostructures [5,6], reducing permeation to deeper layers [7,8], avoiding systematic off target delivery and sticking to the local delivery.

Topical therapy show many advantages over oral use, such as no first-pass effect, less drug interactions, high local drug concentration, usually laboratory monitoring during treatment is not required, it is considered a non-invasive route, and reduces systemic toxicity [9-11].

Polycaprolactone (PCL) is a biodegradable polymer that appears to increase drug residence on the skin. It is biocompatible and biodegradable, and its hydrolytic degradation can be designed relative to degradation time, which can be as fast as seconds or even years, offering alternatives for drug delivery [4,12].

P407-Casein hydrogel can provide a modified release and adhesiveness, being an option for local long-term treatment [11]. This nanohybrid hydrogel (hydrogel + PCL-NP) could bring unique properties absent in the individual components.

PCL nanoparticles containing terbinafine were produced by nanoprecipitation technique and further dispersed into poloxamer-based hydrogels. Terbinafine hydrochloride was chosen as a model drug due to its high Log P and because it is used for a local treatment (superficial mycoses) [13]. Here we report the characterization of NPs (mean diameter, polydispersity index (PDI), ζ potential, ATRFT-IR, morphology, encapsulation efficiency, drug release profile and cytotoxicity), as well as the nanohybrid hydrogel rheological properties and particle release.

2. Results and discussion

2.1 Nanoparticle Characterization

The particles were produced using P407 as stabilizers in the aqueous phase aiming their future incorporation into hydrogel formulation. Terbinafine hydrochloride is a very suitable antifungal agent for the treatment of dermatophyte infections. It is a highly lipophilic active that tends to accumulate in skin, nails and adipose tissue [14]. Blank nanoparticles (NP-PCL) and nanoparticles carrying terbinafine (NP-TBH-PCL) were successfully produced and characterized by dynamic light scattering. NPs showed a small difference in size, around 10 nm. We can consider nanoparticle solution as monodisperse as both particles presented a low polydispersity index (PDI) (Table 1). Zeta potential was low as P407, non-charged polymer, was used as a steric hindrance stabilizer for the nanoparticle solution. A one-month stability study was performed with both particles. No significant difference in mean size was observed (Fig.1).

Table 1 – Size distribution and zeta potential of nanoparticles without and with terbinafine by dynamic light-scattering (DLS) procedure employing Zetasizer.

		NP-PCL	NP-TBH-PCL
Size (nm)	**Mean**	186.4 ± 0.4	196.7 ± 0.7
	$D_{(10)}$	127.7 ± 8.1	139.0 ± 2.1
	$D_{(50)}$	195.7 ± 3.1	205.0 ± 2.1
	$D_{(90)}$	306.3 ± 29.5	305.0 ± 11.7
PDI		0.093 ± 0.003	0.069 ± 0.010
Zeta potential (mV)		-11.9 ± 0.7	-0.7 ± 0.1

*Results presented as mean □ SD of three measurements. NP-PCL = nanoparticles without terbinafine; NP-TBH-PCL = nanoparticles with terbinafine; D(10) = 10% of detected particles are equal or smaller than this size; D(50) = 50% of detected particles are equal or smaller than this size; D(90) = 90% of detected particles are equal or smaller than this size; PDI = polydispersity index.

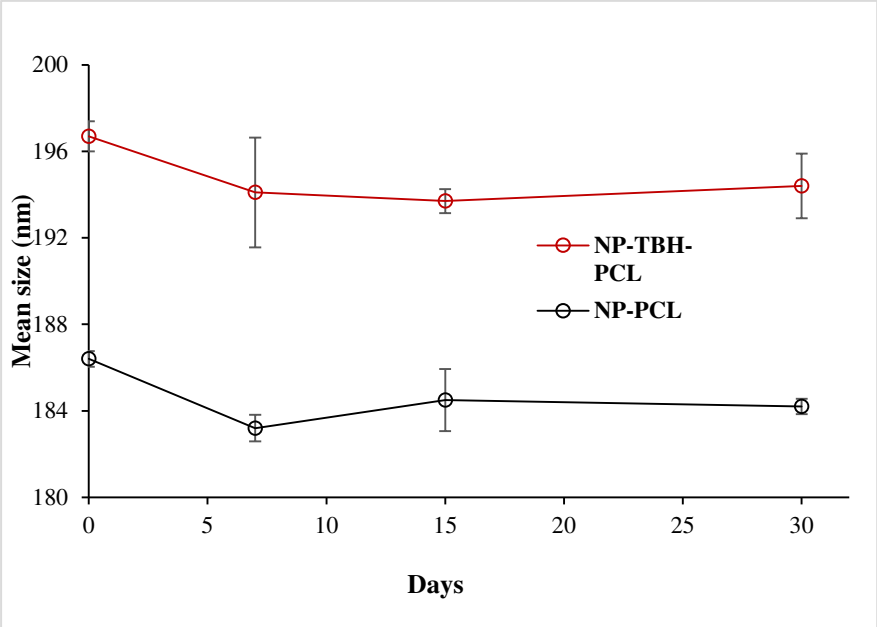


Fig. 1 – Stability study of the nanoparticle mean size (nm) throughout a month. Measures were taken at the 0, 7, 15 and 30 days. Results shown ± SD. Some error bars are obscured by the symbols due to their small size.

Particle's morphology was analyzed by TEM (Fig.2), the mean size of the blank nanoparticle was 89.42 nm, and terbinafine nanoparticle was 78.52 nm. NP-TBH-PCL maintained spherical form as NP-PCL. Both TEM results are smaller than the ones obtained from DLS analyzes, as expected, considering that each methodology uses a different technique and state (dry and in solution) to analyze the sample [15]. One can notice a background with smaller spheres probably caused by the formation of P407 micelles.

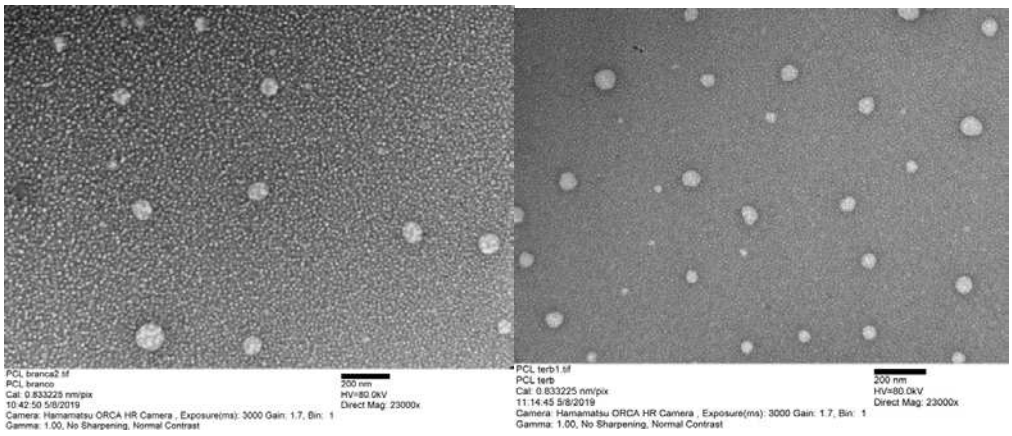


Fig. 2 - TEM image of PCL nanoparticles a) NP-PCL and b) NP-TBH-PCL in P407 2.5% solution.

2.2 Drug Encapsulation Efficiency

Encapsulation efficiency (EE) was performed and quantified using spectrophotometer UV. The EE of terbinafine nanoparticles was 98.81%. Hydrophobic drugs can be encapsulated into PCL particle core due to the interactions with its hydrophobic chains. PEG-PCL nanoparticles showed an encapsulation efficiency of 96.1% of ketoconazole [16]. Terbinafine hydrochloride has LogP of 5.51 (Drug Bank database) higher than ketoconazole that has LogP of 4.30 (PubChem Database). From log P information, we could infer that hydrophobic interactions between PCL and terbinafine would be higher than PCL and ketoconazole, leading to a higher EE% of the nanoparticles.

2.3 ATR-FTIR

The ATR-FTIR from the nanoparticles (Fig. 3) showed a successful terbinafine encapsulation with the PCL nanoparticles.

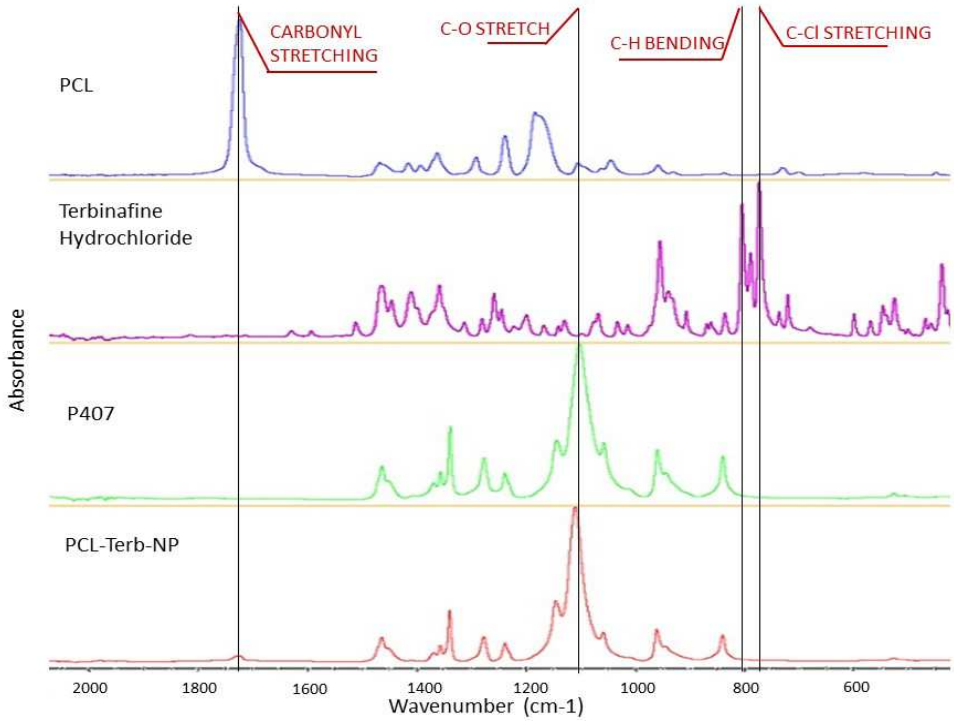


Fig. 3 – ATR-FTIR spectra of PCL, Terbinafine hydrochloride, P407 and the nanoparticle solution with terbinafine respectively.

PCL shows a characteristic peak around 1728.09 cm^{-1} [carbonyl stretching], 2939.97cm^{-1}, 2855.85 cm^{-1} [asymmetric and symmetric CH2 stretching respectively], 1241.29 cm^{-1} [Asymmetric COC stretching] and 1186.97 cm^{-1} [OC=O stretching] [17]. The carbonyl stretching can also be seen in the nanoparticle spectra [1729.09 cm^{-1}] with a low absorbance

considering that the PCL spectra had a pure polymer consequently a higher concentration than in the nanoparticle solution.

P407 show a characteristic peak around 1105.36 cm^{-1} [C-O stretch], and its presence in the nanoparticle spectra overlapped some of the other peaks as the 2946.37cm^{-1} and 2867.44 cm^{-1} from PCL with its own, 2885.37 cm^{-1}.

Terbinafine hydrochloride show peaks 2442.76 cm^{-1} [C=N stretching], 1467.95 cm^{-1} [C-H bending], 807.97 cm^{-1} [CH bending] and 776.53 cm^{-1} [C-Cl stretching]. As the ATR-FTIR analyses the surface of the particle and the medium that it is in [18], terbinafine hydrochloride should appear in NP-TBH-PCL spectra, but when comparing the scale from the terbinafine with the one used in NP-TBH-PCL, it is possible that the terbinafine peak cannot be seen.

2.4 Terbinafine release from nanoparticle formulation

Drug release profile in vitro was evaluated for free-TBH and NP-TBH-PCL for 96 hours (Fig. 4). In the first 6 hours, there are an overlap between free and encapsulated TBH, without significant differences ($p>0.05$) between release percentage, which can be attributed to the diffusion of non-encapsulated TBH [19]. From 8 until 80 hours, there are significant differences ($p<0.05$ in 8 hours and $p<0.001$ in other measured times) between samples, and NP-TBH-PCL presented a lower release of TBH, proving that nanoencapsulation with PCL modified the release of TBH. Free-TBH and NP-TBH-PCL had a significant difference ($p<0.001$) in permeation flux, which was 7.46 ± 0.18 and 6.43 ± 0.06 $\mu g/cm^2/h$, respectively.

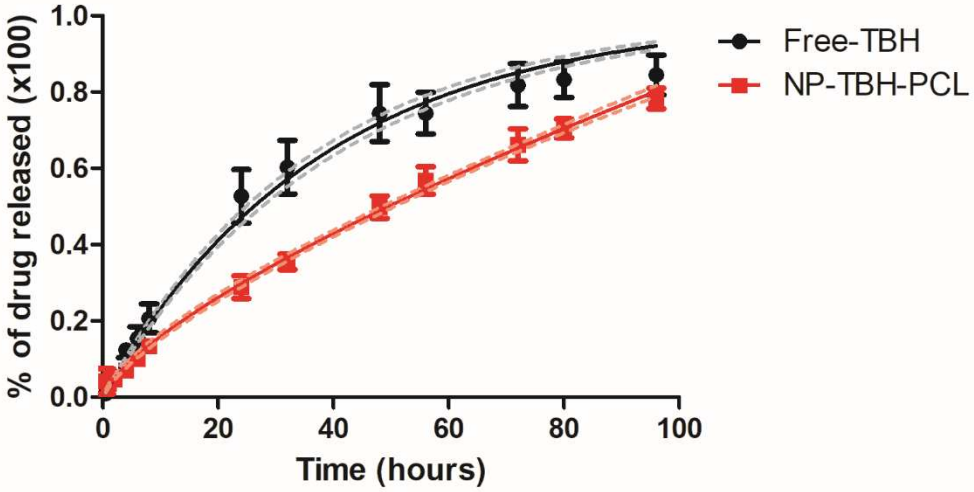


Fig. 4 - *In vitro* drug release profile of free-TBH and NP-TBH-PCL. Data is presented as mean ± standard deviation, n = 6. The continuous curves are the best mathematical fit and their confidence interval (dashed lines).

Mathematical models are a useful tool to fit the release data, helping to better understand data and to predict the release mechanism of a new dosage form [20,21]. After fitting release profiles curves (Table 2), it is possible to conclude that free-TBH presented a release following first order model, while NP-TBH-PCL followed the Korsmeyer-Peppas model. Korsmeyer-Peppas is a model for predicting the release kinetics of polymeric systems [21], and a release type behavior can be indicated according to the release exponent (n). In the case of our nanoformulation, calculated n was 0.715 ± 0.014, indicating an anomalous transport release mechanism, where diffusion and swelling/erosion of polymeric chains influenced TBH release [21-23].

Table 2 – Obtained kinetic parameters of *in vitro* release profile of terbinafine.

	Free-TBH		**NP-TBH-PCL**	
	K_p	**R^2**	**K_p**	**R^2**
Zero Order	0.0114 ± 0.0004	0.7983	0.0091 ± 0,0001	0.9557
First Order	0.0265 ± 0.0007	0.9722	0.0149 ± 0.0002	0.9878
Higuchi model	0.0948 ± 0.0015	0.9459	0.0736 ± 0.0012	0.9518
Korsmeyer-Peppas model	0.0804 ± 0.0082	0.9483	0.0307 ± 0.0019	0.9903

*Results presented as mean ± SD. Free-TBH = terbinafine solution; NP-TBH-PCL = nanoparticles with terbinafine; Kp = release rate constant; R2 = linear correlation coefficient.

2.5 Cell Viability

Cell viability of NP-PCL and NP-TBH-PCL was performed with HaCat cells (karetinocytes) by the MTS colorimetric assay after 24 and 48 hours (Fig. 5).

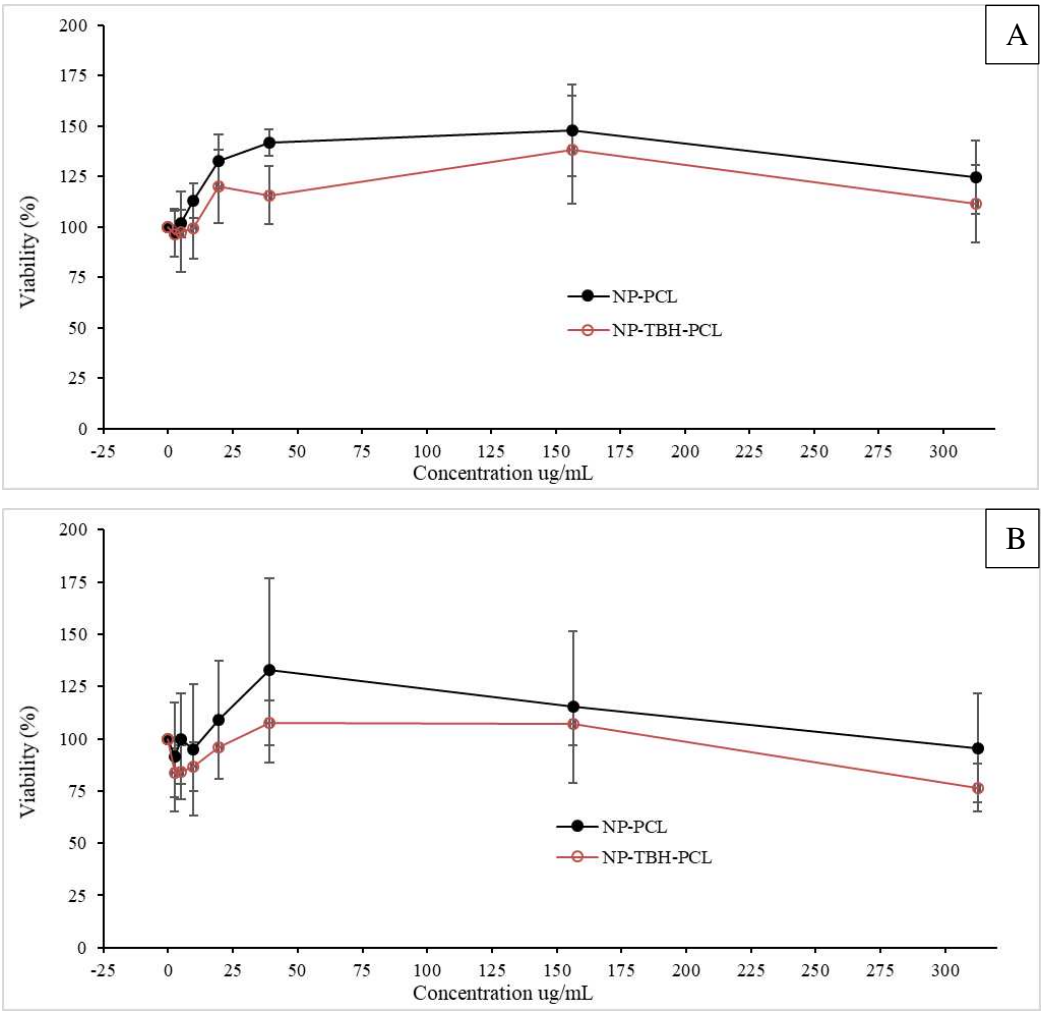


Fig. 5 - HaCat cells viability after 24h (A) and after 48h (B) of NP-PCL and NP-TBH-PCL. Data are means ± SD (n=4).

Even though at 24h NP-PCL viability is higher than NP-TBH-PCL in almost all the concentrations, no significant difference was observed. This difference starts to diminish as the concentrations starts to decrease. Considering the standard deviation, it's possible to say that none of the concentrations showed less than 100% viability, and on the contrary, in most of the concentrations it actually stimulated cell growth. When comparing 24h with 48h there can be found some viabilities less than 100% and lower than 24h. The observation that NP-PCL shows higher viability than NP-TBH-PCL stay constant.

2.6 Nanohybrid hydrogel rheology

Hydrogel's rheological behavior showed the influence of NP-TBH-PCL addition order in hydrogel mechanical properties, mainly in lower concentrations (Fig 6). At 37 °C in both preparation methods, the storage module (G') was greater than the loss module (G''), i.e.,

the capacity of storage energy was improved when compared with the gel without nanoparticles, except to the composition of 0.2 mg in the M1.

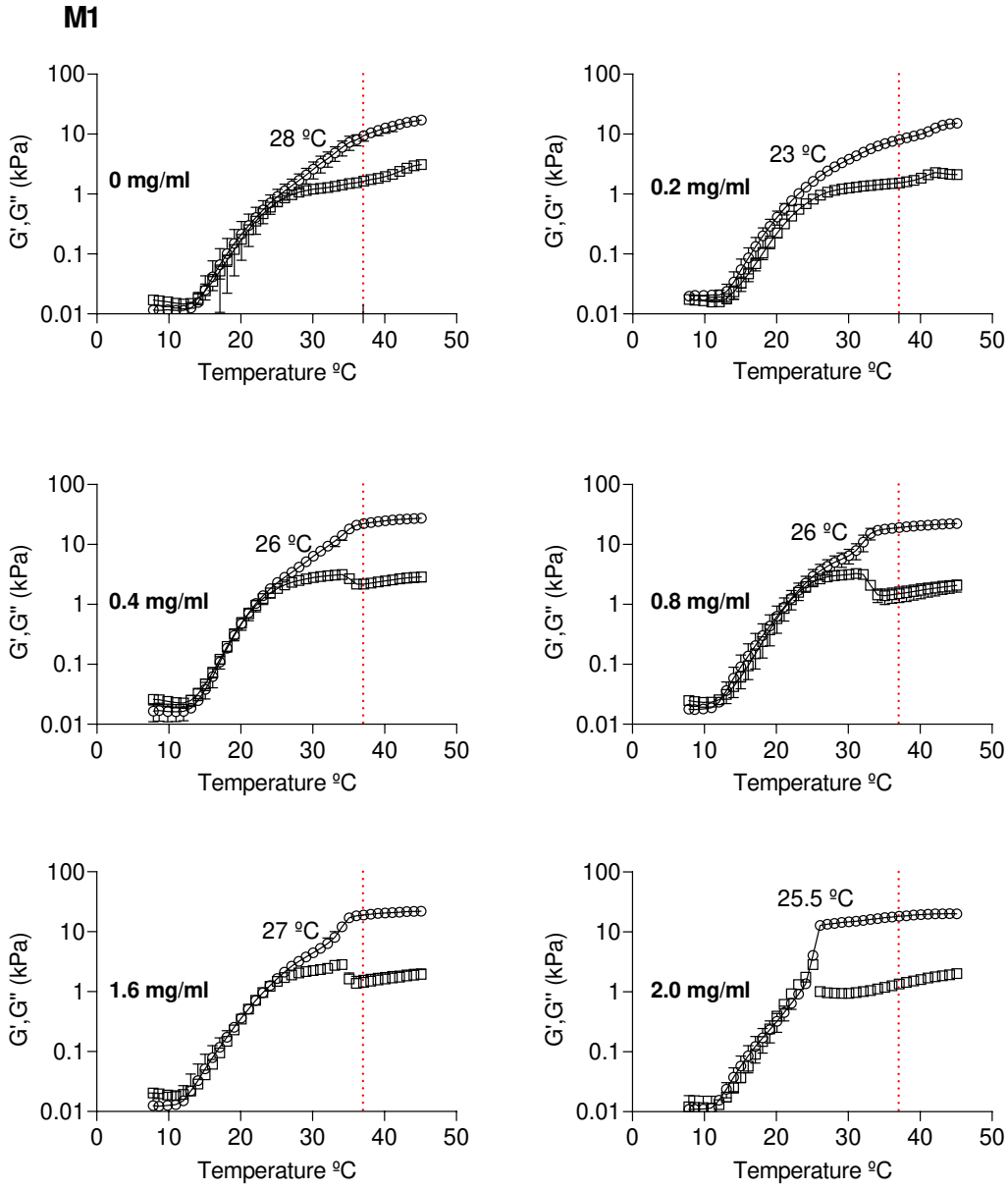


Fig. 6: Rheological behavior of P407 18% Casein 10% hydrogel at different concentrations of the TBH-PCL nanoparticles which were added to the final P407 18% Casein 10% hydrogel (M1). Data are means ± SD (n=4). In the vertical axis, red dotted line refers to 37 ºC.

For the first preparation method (M1), in the composition of 0.2 mg/mL, the decrease in viscoelastic modules could be related to physical interactions occurring between NP-TBH-PCL and the hydrogel of P407 18% casein 10%. However, these physical interactions were able to change the gelation temperature from 28 to 23 ºC ($p<0.05$). Significant changes in

gelation temperature were also observed in the other concentrations. Above 0.4 mg/mL, the gelation temperature shifted from 28 to 26 ºC ($p<0.05$), and strong gels with modules almost temperature-independent were observed. Furthermore, the composition with 0.4 mg/mL showed higher storage module when compared with the others (Fig 6). Then, we can say that to first method 0.4 mg/mL is a suitable concentration to obtain higher mechanical properties gel.

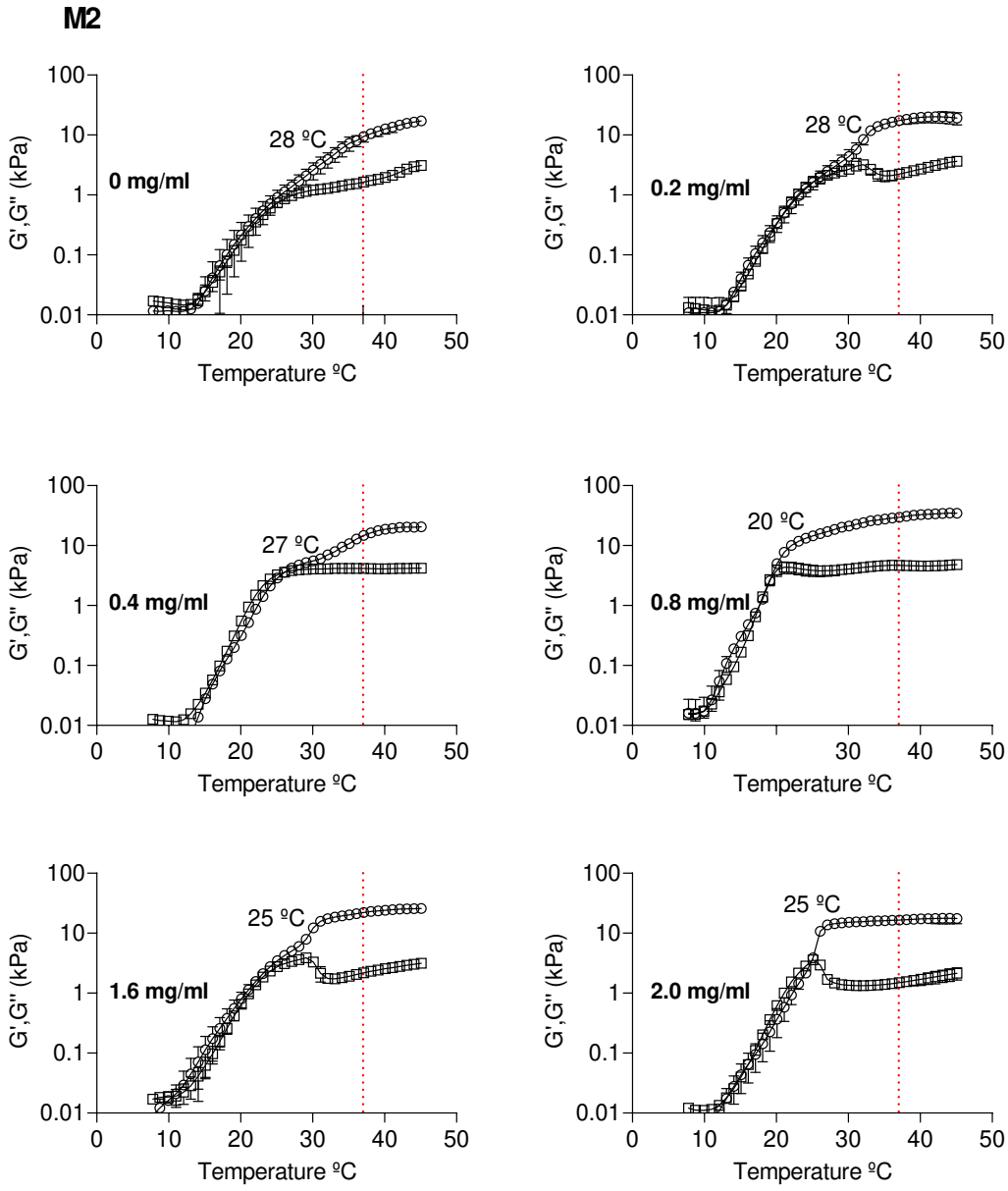


Fig. 7: Rheological behavior of P407 18% Casein 10% hydrogel at different concentrations of the TBH-PCL nanoparticles which were added to the casein solution, and then P407 was added into the solution (M2). Data are means ± SD (n=4). In the vertical axis, red dotted line refers to 37 ºC.

In the second method (M2), the gelation temperature also decreases from 28 to 26 ºC ($p<0.05$) with the incorporation of nanoparticles (Fig 7). To 0.8 mg/mL of nanoparticles, we noted a shift in the gelation temperature from 28 to 20 ºC ($p<0.05$). Moreover, we observe at 37 ºC a significant increase in the storage module from 9.42 to 29.7 kPa ($p<0.05$). This behavior could be related to nanoparticle's addition order. The addition of poloxamer into a previous mixture of casein and NP-TBH-PCL can result in a quickly entangled chain that changes the viscoelastic behavior of the sample. Then, to this method, 0.8 mg/mL is the concentration that showed more significant changes in the rheological behavior.

2.7 Particle release

Nanoparticles release kinetics from the hydrogel was assessed with NP-TBH-PCL and NP-PCL using PCL linked with fluorescein.

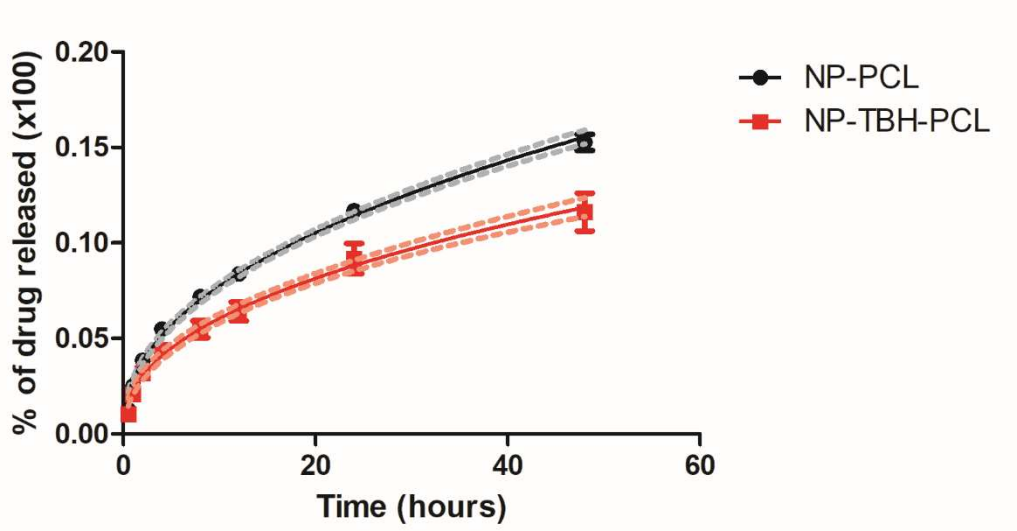


Fig. 8: Cumulative release (%) of PCL-fluorescein-nanoparticle release with/out terbinafine from P407/casein hydrogel at 37ºC in artificial sweat pH 4,7. Data are means ± SD (n=4). Some error bars are obscured by the symbols due to their small size. The continuous curves are the best mathematical fit and their confidence interval (dashed lines).

In the absence of terbinafine, there was a higher release rate than with terbinafine (Fig.8). This difference is not significant $p<0,05$. However, release of both particles were slow if compared with the same hydrogel composition drug release described previously by [11]. This slow release could be justified by PCL nanoparticle being inside the P407 micelles' hydrophobic core, controlling its release. PCL is hydrophobic and its low affinity by the receptor medium together with a storage in the P407 core, may have interfered with the release

rate. This very slow release can be desired in long-term delivery systems an implant or even prothesis with drug release [24].

Both nanoparticles release fitted the Kornsmeyer Peppas model with R^2 of 0.9916 and 0.9743, Kp of 0.028 ± 0.001 and 0.028 ± 0.001 for NP-PCL and NP-TBH-PCL, respectively. Also in both cases, calculated release exponent (*n*) indicate that NP release occurred by diffusion through hydrogel matrix [23], with n values of 0.445 ± 0.009 and 0.429 ± 0.016 for unloaded and TBH-loaded NP.

3. Materials and methods

3.1. Materials

Poloxamer 407 (P407) (BASF- Florham Park, NJ, USA), Casein (Sigma-Aldrich – Atlanta, GA, USA), poly-ε-caprolactone (PCL) 80,000 MW (Sigma-Aldrich – Atlanta, GA, USA), PCL-fluorescein (APM polymer), terbinafine hydrochloride (Frontier Scientific Inc. – Logan, UT, USA). All other reagents were of analytical grade.

3.2 PCL-terbinafine nanoparticle formulation

Terbinafine PCL nanoparticles (NP-TBH-PCL) were produced by nanoprecipitation, where polymer samples were previously dissolved in acetone to reach concentration of 2.5 mg/mL, along to given amounts of terbinafine (0.500 mg, representing 20% w/w). The resulting organic solutions were stirred at 60 °C until polymer dissolution. The formation of nanoparticles was then induced by fast adding 12.5 mL of the organic phase into 50.0 mL of P407 solution 2.5% (w/w) prepared earlier using Milli-Q® water. The stirring speed of the aqueous phase was kept fixed (350 rpm). The resulting solutions were stirred at 37 °C to allow solvent evaporation. Assay was carried out in triplicate. Final aqueous volume after the nanoprecipitation procedure and organic solvent elimination was set to 50.0 mL. Blank nanoparticles (NP-PCL) were prepared similarly excluding the addition of terbinafine.

3.3 Nanopartice characterization

3.2.1 Nanoparticles's size distribution and Zeta potential

Particle size, polydispersity index (PDI) and Zeta potential of NP-TBH-PCL and NP-PCL formulations previously diluted in Milli-Q® water (1:4, v/v) were determined via dynamic light-scattering procedure in Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Particle size and zeta potential were measured in triplicate at room temperature.

3.3.2 Transmission electron microscopy (TEM)

Morphology of particles was observed by transmission electron microscope (TEM) (Tecnai G2 Spirit BioTWIN, FEI, Hillsboro, USA). Carbon film 300 mesh was sunk into the particle suspension (liquid after organic solvent evaporation), dried with filter paper and stained with urinol acetate saturated (UAS) for TEM analyses. Images were acquired using 23 kX magnification.

3.3.3 Terbinafine encapsulation efficiency (EE)

The amount of terbinafine loaded in NP-TBH-PCL was determined by ultra-centrifuging the nanoparticle solution at 25,000 g for 30 min. After that the supernatant was collected and centrifuged using Amicon Ultra-0.5 mL centrifugal filters (10 kDa MWCO) at 14,000 g for 15 min. The filtrate was collected and analyzed for terbinafine by spectrophotometer UV (Multiscan GO, Thermo Scientific, Sweden) at 283 nm. To determinate the terbinafine feeding the whole suspension was lysed adding acetonitrile (1:20) and stirring for 30 min. Cold methanol (1:3) was added and stirred again for 30 min. After that, the solution was ultra-centrifuged 25,000 g for 30 min and the supernatant was collected to analyze. The content of terbinafine encapsulated in the nanoparticles was computed by subtraction of the content that was present in the filtrate from the Terbinafine feeding measured. The percentage encapsulation efficiency (EE%) was calculated using Equation 1:

$$\mathrm{EE}(\%) = \frac{\text{Terbinafine feeding (ug)} - \text{Terbinafine in supernatant (ug)}}{\text{Terbinafine feeding (ug)}} \text{ x } 100 \qquad (1)$$

The quantifications were always performed in triplicate.

3.3.4 ATR-FTIR

The FTIR spectra of NP-TBH-PCL were recorded in an ATR-FTIR (attenuated total reflectance fourier transform infrared) spectrometer (Nicolet iS50, Thermo Fisher Scientific, USA) operating at 2000 to 600 cm−1. Nanoparticles were analyzed in solution as the other compounds (PCL, TBH and P407) were analyzed in its solid state.

3.3.5 Terbinafine release from nanoparticle formulation

In vitro release profile of TBH from nanoparticles was evaluated using vertical diffusion Franz cell system with a diffusion area of 0.64 cm^2. Regenerated cellulose dialysis membranes (14 kDa MWCO) were placed between donor and receptor cells, and subsequently clamped. Receptor cell was filled with pre-warmed artificial sweat pH 4.7 (20 g/L sodium chloride, 18 g/L ammonium chloride, 5 g/L acetic acid, 15 g/L lactic acid adjusting pH to 4.7

with NaOH 3M), and the donor cell with 1 mL of PCL-TBH nanoparticles or free-TBH solution for comparison (sextuplicate for each sample). The system was kept under 37 ºC and at pre-determined intervals 0.3 mL was taken from the receptor compartment for TBH quantification at 283 nm in spectrophotometer UV. Withdrawn volume was replaced with pre-warmed receptor medium right after each sampling. Permeation of TBH across membrane (%) was calculated considering the initial amount of TBH placed in donor compartment, following Equation 2:

$$TBH\ Release\ (\%) = \frac{TBH\ cumulative\ release\ (\mu g)}{TBH\ feeding\ (\mu g)} \times 100 \qquad (2)$$

Steady-state permeation flux (Jss, µg/cm^2/h) was determined following Equation 3:

$$Jss = \left(\frac{dQ}{dt}\right)_{ss} \times \frac{1}{A} \qquad (3)$$

where, (dQ/dt)ss is the amount of TBH (µg) permeated through time (h), and A is the diffusion area (cm^2).

The data from TBH release profile from free-drug solution and nanoformulation were kinetically evaluated using various mathematical models, such as zero order, first order, Higuchi and Korsmeyer-Peppas model equations [21].

3.3.6 Nanoparticle's cytotoxicity

The cell viability was performed with HaCat (keratinocytes) cell line. Cells were plated approximately 5.4 x 10^4 cells/well in 24 well plates, incubated at 37 ºC and 5% CO_2, after 24h and total cells adherence, cells were exposed to 312.5; 156.25; 39.06; 19.53; 9.76; 4.88 and 2.44 µg/mL of NPs (blank and loaded with terbinafine) prior washed in PBS. Cells were exposed for 24 and 48 hours, in incubation at 37 °C and 5% CO_2.

After treatment, culture medium was removed and 500 µl culture medium with 100 µl MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium bromide) were applied to culture, which was incubated for 2h at 37 ºC and 5% CO_2. Then the solution was withdrawal and placed in 96-well plates and the absorbance was read at 570 nm (Readwell Touch, Robonik, India). All tests were performed in quadruplicate.

3.4 P407-casein hydrogel formulation

Casein solution (50 mg/mL) was prepared by dissolving casein in deionized water and adjusting pH to 7 using a 5 M NaOH solution. The obtained casein solution was stored in a 4 °C refrigerator. 18% (w/v) of P407 was added and the solutions and stirred overnight at 4 °C for gel formation.

3.4.1 Rheological analysis

Rheological characterization was performed with P407 18% Casein 10% hydrogel at different concentrations of the NP-TBH-PCL in order to analyze the nanoparticle influence in the rheological behavior. Five different concentrations (0.2, 0.4, 0.8, 1.6 and 2 mg/mL) of NP-TBH-PCL were added to the hydrogel. This addition was done in two different orders. M1: Casein solution, P407, nanoparticles. M2: Casein solution, nanoparticle, P407. Hydrogel's rheological analysis were carried out on a Modular Compact Rheometer (MCR-102, Anton Paar, Graz, Austria) using a plate-plate geometry. The plate diameter was 50 mm and gap of 0.3 mm. Temperature sweep assays were performed at a temperature range from 10 °C to 45 °C at a heating rate of 1 ºC/min, angular frequency of 100 rad/s, and strain of 1% in the linear regime. Experiments were performed in triplicate. Gelation temperature was taken as the temperature at which G' becomes greater than G''.

3.4.2 Particle release from P407-casein hydrogel

Concentrated nanoparticle solution was added to the formulation (NP-PCL and NP-TBH-PCL) and stirred until complete dispersion. Using Yang, et al. [25] adapted method, transwell membrane inserts (0.4 mm pore size and 0.33 cm^2 area; Costar) and 24-well culture plates were used as the donor and acceptor chambers, respectively. Two hundred microliters of each formulation were pipetted directly onto pre-warmed filter inserts to obtain a solid hydrogel. Filter inserts (donor compartments) with formed gels were suspended in wells (acceptor compartments) filled with pre-warmed artificial sweat pH 4.7, and the plates were then incubated in an oven (37 °C). At each sampling time (0.5, 1, 2, 6, 12, 24, 48h), 1 mL aliquots of the artificial sweat-receiving media were collected, and inserts were sequentially moved into a new well with fresh media. Sample aliquots were analyzed by fluorescence spectroscopy (Sinergy HT, Bio-Tek Instruments, Winooski, VT) λ_{ex} 490 nm and $\lambda_{em.}$ 514nm). The same concentrated nanoparticle solution was measured, and its fluorescence was set as the 100%. Experiments were performed in quadruplicate. The data from NP release profile from hydrogel formulation was also kinetically evaluated using mathematical models [21].

3.5 Statistics

Data are expressed as means ± standard deviations. All statistical comparisons were made with Student's t-test, unpaired for independent samples. A p value < 0.05 was considered to indicated statistical significance. For mathematical models fitting Prism 5 software (GraphPad Software Inc.) for windows was used.

4. Conclusion

The nanoparticles were produced by nanoprecipitation method, with P407 as stabilizer showed a low PDI and a stability, besides high TBH encapsulation efficiency. Drug release profile from 8 until 80 hours proved that nanoencapsulation with PCL modified the release of TBH Free-TBH and NP-TBH-PCL had a significant difference ($p<0.001$) in permeation flux, which was 7.46 ± 0.18 and 6.43 ± 0.06 $\mu g/cm^2/h$. NP's did not interfere in the metabolic activity of keratinocytes.

NP-PCL and NPTBH-PCL had a very slow release from the P407-casein hydrogel, being a better choice for long term release treatments.

The nanoparticle's addition to the hydrogel significantly influenced gelation temperature, the viscoelastic properties and provided stable gels at the two preparation methods. Moreover, the nanoparticle addition order leads to different rheological behavior for the same nanoparticle concentration; thus, a suitable nanoparticle concentration to improve hydrogel mechanical properties can be addition order dependent.

Data availability

The raw/processed data required to reproduce these findings can not be shared at this time due to technical or time limitations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Author's acknowledge FAPESP (2017/10728-2, 2018/05421-8 and 2018/07707-6), CNPq (404229/2016–6, 301436/2017-7), FAEPEX and NIH R35 GM131728 for the financial support.

References

1 Ataide, J. A. et al. In vitro performance of free and encapsulated bromelain. Scientific Reports 11, 10195, doi:10.1038/s41598-021-89376-0 (2021).

2 Cha, G. D., Lee, W. H., Lim, C., Choi, M. K. & Kim, D.-H. Materials engineering, processing, and device application of hydrogel nanocomposites. Nanoscale 12, 10456-10473, doi:10.1039/D0NR01456G (2020).

3 Huda, M. K., Das, P. P., Saikia, P. J. & Baruah, S. D. Synthesis of poly(n-octadecyl methacrylate-co-2-hydroxyethyl methacrylate) copolymer and their utilization as polymeric stabilizer in the preparation of PCL microspheres. Polymer Bulletin 74, 1661-1676, doi:10.1007/s00289-016-1795-z (2017).

4 Zelikin, A. N., Ehrhardt, C. & Healy, A. M. Materials and methods for delivery of biological drugs. Nat Chem 8, 997-1007, doi:10.1038/nchem.2629 (2016).

5 Abdel-Mottaleb, M. M. A., Neumann, D. & Lamprecht, A. Lipid nanocapsules for dermal application: A comparative study of lipid-based versus polymer-based nanocarriers. European Journal of Pharmaceutics and Biopharmaceutics 79, 36-42, doi:https://doi.org/10.1016/j.ejpb.2011.04.009 (2011).

6 Rosado, C., Silva, C. & Reis, C. P. Hydrocortisone-loaded poly(ε-caprolactone) nanoparticles for atopic dermatitis treatment. Pharmaceutical Development and Technology 18, 710-718, doi:10.3109/10837450.2012.712537 (2013).

7 Ourique, A. F. et al. Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine. European Journal of Pharmaceutics and Biopharmaceutics 79, 95-101, doi:https://doi.org/10.1016/j.ejpb.2011.03.008 (2011).

8 Alvarez-Román, R., Naik, A., Kalia, Y. N., Guy, R. H. & Fessi, H. Skin penetration and distribution of polymeric nanoparticles. Journal of Controlled Release 99, 53-62, doi:http://dx.doi.org/10.1016/j.jconrel.2004.06.015 (2004).

9 Yang, R. et al. Getting Drugs Across Biological Barriers. Advanced Materials 29, 1606596-n/a, doi:10.1002/adma.201606596 (2017).

10 Havlickova, B. & Friedrich, M. The advantages of topical combination therapy in the treatment of inflammatory dermatomycoses. Mycoses 51, 16-26, doi:10.1111/j.1439-0507.2008.01615.x (2008).

11 Tundisi, L. L. et al. Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein. Pharmaceutical Research, 1-8 (2021).

12 Sinha, V. R., Bansal, K., Kaushik, R., Kumria, R. & Trehan, A. Poly-ϵ-caprolactone microspheres and nanospheres: an overview. International Journal of Pharmaceutics 278, 1-23, doi:http://dx.doi.org/10.1016/j.ijpharm.2004.01.044 (2004).

13 Dias, M. F. R. G. et al. Treatment of superficial mycoses: review - part II. Anais Brasileiros de Dermatologia 88, 937-944 (2013).

14 DrugBank. Terbinafina, <https://www.drugbank.ca/drugs/DB00857> (

15 Bell, N. C., Minelli, C., Tompkins, J., Stevens, M. M. & Shard, A. G. Emerging Techniques for Submicrometer Particle Sizing Applied to Stöber Silica. Langmuir 28, 10860-10872, doi:10.1021/la301351k (2012).

16 Deng, P. et al. Y-shaped methoxy poly (ethylene glycol)-block-poly (epsilon-caprolactone)-based micelles for skin delivery of ketoconazole: in vitro study and in vivo evaluation. Materials Science and Engineering: C 78, 296-304, doi:https://doi.org/10.1016/j.msec.2017.04.089 (2017).

17 Elzein, T., Nasser-Eddine, M., Delaite, C., Bistac, S. & Dumas, P. FTIR study of polycaprolactone chain organization at interfaces. Journal of Colloid and Interface Science 273, 381-387, doi:https://doi.org/10.1016/j.jcis.2004.02.001 (2004).

18 Mudunkotuwa, I. A., Minshid, A. A. & Grassian, V. H. ATR-FTIR spectroscopy as a tool to probe surface adsorption on nanoparticles at the liquid–solid interface in environmentally and biologically relevant media. Analyst 139, 870-881, doi:10.1039/C3AN01684F (2014).

19 Beraldo-de-Araújo, V. L. et al. Excipient-excipient interactions in the development of nanocarriers: an innovative statistical approach for formulation decisions. Scientific Reports 9, 10738, doi:10.1038/s41598-019-47270-w (2019).

20 Siepmann, J. & Peppas, N. A. Higuchi equation: Derivation, applications, use and misuse. International Journal of Pharmaceutics 418, 6-12, doi:https://doi.org/10.1016/j.ijpharm.2011.03.051 (2011).

21 Sriamornsak, P., Limmatvapirat, S., Piriyaprasarth, S., Mansukmanee, P. & Huang, Z. A new self-emulsifying formulation of mefenamic acid with enhanced drug dissolution. Asian Journal of Pharmaceutical Sciences 10, 121-127, doi:http://dx.doi.org/10.1016/j.ajps.2014.10.003 (2015).

22 Giarola, L. R. et al. Pterodon pubescens Benth (sucupira) microencapsulation influence on formulation stability outcome compared to non-encapsulated extract. Journal of

Drug Delivery Science and Technology, 102875, doi:https://doi.org/10.1016/j.jddst.2021.102875 (2021).

23 Siepmann, J. & Peppas, N. A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Advanced Drug Delivery Reviews 48, 139-157, doi:https://doi.org/10.1016/S0169-409X(01)00112-0 (2001).

24 Ahmad Wsoo, M. et al. Development of prolonged drug delivery system using electrospun cellulose acetate/polycaprolactone nanofibers: Future subcutaneous implantation. Polymers for Advanced Technologies 32, 3664-3678, doi:https://doi.org/10.1002/pat.5375 (2021).

25 Yang, R. et al. Treatment of otitis media by transtympanic delivery of antibiotics. Science Translational Medicine 8, 356ra120, doi:10.1126/scitranslmed.aaf4363 (2016).

5. Discussão Geral

A mistura de poloxamer 407 e caseína não resultou na formação de novas ligações químicas. As mudanças nas propriedades mecânicas e reológicas do hidrogel provavelmente se dão a partir das interações causadas pelo emarenhamento e formação de micelas mistas. A modificação gerada nas propriedades mecânicas possivelmente foram as responsáveis pelo perfil de liberação apresentado. O perfil de liberação de um ativo depende do polímero em que ele se encontra, das propriedades físico-químicas do ativo em questão e o meio em que será liberado. O hidrogel se mostrou mais viscoso, assumindo a formações de micelas mistas e houve menos espaço para a difusão de ativo pelo polímero em questão.

Em relação a carga superficial apresentada pela micela de caseína em pH fisiológico, podemos assumir que, provavelmente, elas foram mascaradas pelos segmentos hidrofóbicos PEO do P407, não interferindo na liberação dos ativos estudados (Kessler *et al.*, 2014). As características químicas dos ativos neste caso, se mostraram menos relevantes do que suas características físicas de peso molecular na liberação do hidrogel. Mudanças, misturas e decorações de polímeros podem trazer resultados diferentes para o perfil de liberação do mesmo ativo. Possivelmente, sem o P407, a caseína teria uma interação diferente com os ativos presentes.

Ao escolher a mistura P18C10 para estudo de um nanohibrido, preparamos as nanopartículas de policaprolactona, testando o impacto da ordem de adição de nanopartículas no hidrogel e também da sua concentração. As nanopartículas de PCL mostraram alta eficiência de encapsulação para o cloridrato de terbinafina. Por ser um ativo muito hidrofóbico, corroborou com a interação com a policaprolactona (Deng *et al.*, 2017). Para que um ativo hidrofóbico seja entregue por um hidrogel, sua previa encapsulação do mesmo é comumente utilizada como subterfugio, possibilitando uma maior concentração dele armazenada no sistema de entrega (Rabiee *et al.*, 2020). As partículas se mostraram estáveis pelo período de um mês e sua morfologia permaneceu esférica. A liberação de TBH livre e a NP-TBH-PCL nas primeiras 6 horas se sobrepuseram o que pode ser atribuído a TBH não encapsulada pela nanopartícula de PCL (Beraldo-De-Araújo *et al.*, 2019). TBH livre e NP-TBH-PCL tiveram uma diferença significativa ($p<0,001$) no fluxo de permeação, que foi 7,46 ± 0,18 e 6,43 ± 0,06 µg/cm2/h, mostrando que as nanocapsulas de PCL modificarão a liberação da terbinafina.

A liberação da partícula do hidrogel se mostrou lenta atingindo 15% em 48h. Essa liberação pode ser justificada pela possibilidade das nanopartículas de PCL estarem

armazenadas dentro do núcleo hidrofóbico das micelas de P407. É importante também considerar que por se tratar de uma partícula de PCL (hidrofóbica), sua afinidade pelo meio receptor (mais hidrofílico que o núcleo do P407) é baixa. Uma liberação com esse perfil poderia ser mais adequada quando se busca um tratamento de longa duração como implantes com reservatórios de ativo (Li *et al.*, 2021).

Por se tratar de uma pesquisa que busca de melhorias para os sistemas de entrega tópicos como um todo, além de testar o perfil de liberação da nanopartícula do hidrogel, realizamos a reologia do gel na presença das nananopartículas analisando as mudanças que esta adição causou. Houve mudanças reológicas importantes que impactaram as temperaturas de gelificação. Essa mudança esteve relacionada tanto à ordem de adição quanto à concentração de nanopartículas adicionadas.

O uso de materiais poliméricos no auxílio da entrega tópica de medicamentos se faz por várias formas. Nanopartículas e hidrogéis são formas que trazem ampla possibilidade de funcionalização. Esses polímeros inteligentes ou funcionalizados nos permitem misturar (hidrogéis nanohíbridos) ou mesmo ligar uns aos outros para apresentar uma liberação controlada do ativo.

6. Conclusão

A adição de caseína ao P407 aumentou aderência do hidrogel, mecânica e viscosidade, e diminuiu as taxas de liberação dos fármacos modelo (ao aumentar a viscosidade do hidrogel e sua resistência). A escolha da caseína, um biopolímero de grau alimentício, para modificar as propriedades do gel apresentou vantagens em termos de segurança, custo e disponibilidade comercial. A mistura de P407 e caseína mostrou biocompatibilidade em ratos, demonstrando seu potencial para ser testada e utilizada em humanos.

As propriedades do hidrogel podem ser ajustadas alterando as proporções de caseína e P407. Estas melhorias poderiam ter um impacto na adesão do paciente, particularmente com terapias tópicas que podem durar meses e exigir muitas aplicações por dia. Os hidrogéis bioadesivos impulsionados pela adição de proteínas prontamente disponíveis podem levar a um possível dispositivo de liberação controlada com propriedades mecânicas e adesivas adequadas.

Nanopartículas estabilizadas com P407 e com alta eficiência de encapsulamento, mostraram uma liberação controlada de TBH.

A junção das nanopartículas ao hidrogel apresentou uma liberação muito lenta. A ordem e a concentração adicionada de partículas interferiram na reologia do gel.

As propriedades desejadas para um produto tópico, reologia e liberação do ativo, podem estar contidos na mistura inovadora de componentes já conhecidos e suas formas.

7. Referências

ABDEL-MOTTALEB, M. M. A.; NEUMANN, D.; LAMPRECHT, A. Lipid nanocapsules for dermal application: A comparative study of lipid-based versus polymer-based nanocarriers. European Journal of Pharmaceutics and Biopharmaceutics, v. 79, n. 1, p. 36-42, 9// 2011.

ALVAREZ-ROMÁN, R.; NAIK, A.; KALIA, Y. N.; GUY, R. H.; FESSI, H. Skin penetration and distribution of polymeric nanoparticles. Journal of Controlled Release, v. 99, n. 1, p. 53-62, 2004/09/14/ 2004.

ALVES, T.; SOUZA, J.; REBELO, M.; PONTES, K.; SANTOS, C.; LIMA, R.; JOZALA, A.; GROTTO, D.; SEVERINO, P.; RAI, M.; CHAUD, M. Formulation and evaluation of thermoresponsive polymeric blend as a vaginal controlled delivery system. 2018.

ATAIDE, J. A.; CEFALI, L. C.; FIGUEIREDO, M. C.; BRAGA, L. E. D. O.; RUIZ, A. L. T. G.; FOGLIO, M. A.; OLIVEIRA-NASCIMENTO, L.; MAZZOLA, P. G. In vitro performance of free and encapsulated bromelain. Scientific Reports, v. 11, n. 1, p. 10195, 2021/05/13 2021.

BERALDO-DE-ARAÚJO, V. L.; BERALDO-DE-ARAÚJO, A.; COSTA, J. S. R.; PELEGRINE, A. C. M.; RIBEIRO, L. N. M.; PAULA, E. D.; OLIVEIRA-NASCIMENTO, L. Excipient-excipient interactions in the development of nanocarriers: an innovative statistical approach for formulation decisions. Scientific Reports, v. 9, n. 1, p. 10738, 2019/07/24 2019.

CHA, G. D.; LEE, W. H.; LIM, C.; CHOI, M. K.; KIM, D.-H. Materials engineering, processing, and device application of hydrogel nanocomposites. Nanoscale, v. 12, n. 19, p. 10456-10473, 2020.

DENG, P.; TENG, F.; ZHOU, F.; SONG, Z.; MENG, N.; LIU, N.; FENG, R. Y-shaped methoxy poly (ethylene glycol)-block-poly (epsilon-caprolactone)-based micelles for skin delivery of ketoconazole: in vitro study and in vivo evaluation. Materials Science and Engineering: C, v. 78, n. Supplement C, p. 296-304, 2017/09/01/ 2017.

DIAS, M. F. R. G.; QUARESMA-SANTOS, M. V. P.; BERNARDES-FILHO, F.; AMORIM, A. G. D. F.; SCHECHTMAN, R. C.; AZULAY, D. R. Treatment of superficial mycoses: review - part II. Anais Brasileiros de Dermatologia, v. 88, p. 937-944, 2013.

HAVLICKOVA, B.; FRIEDRICH, M. The advantages of topical combination therapy in the treatment of inflammatory dermatomycoses. Mycoses, v. 51, p. 16-26, 2008.

HEILIGTAG, F. J.; NIEDERBERGER, M. The fascinating world of nanoparticle research. Materials Today, v. 16, n. 7, p. 262-271, 2013/07/01/ 2013.

HUDA, M. K.; DAS, P. P.; SAIKIA, P. J.; BARUAH, S. D. Synthesis of poly(n-octadecyl methacrylate-co-2-hydroxyethyl methacrylate) copolymer and their utilization as polymeric stabilizer in the preparation of PCL microspheres. Polymer Bulletin, v. 74, n. 5, p. 1661-1676, 2017.

HURLER, J.; ENGESLAND, A.; POORAHMARY KERMANY, B.; ŠKALKO-BASNET, N. Improved texture analysis for hydrogel characterization: Gel cohesiveness, adhesiveness, and hardness. Journal of Applied Polymer Science, v. 125, n. 1, p. 180-188, 2012/07/05 2012.

JONES, D. S.; WOOLFSON, A. D.; BROWN, A. F. Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. International Journal of Pharmaceutics, v. 151, n. 2, p. 223-233, 1997/05/26/ 1997.

KESSLER, A.; MENÉNDEZ-AGUIRRE, O.; HINRICHS, J.; STUBENRAUCH, C.; WEISS, J. αs-Casein—PE6400 mixtures: Surface properties, miscibility and self-assembly. Colloids and Surfaces B: Biointerfaces, v. 118, p. 49-56, 2014/06/01/ 2014.

LEAD, J. R.; WILKINSON, K. J. Aquatic Colloids and Nanoparticles: Current Knowledge and Future Trends. Environmental Chemistry, v. 3, n. 3, p. 159-171, 2006.

LI, R.; SONG, Y.; FOULADIAN, P.; ARAFAT, M.; CHUNG, R.; KOHLHAGEN, J.; GARG, S. Three-Dimensional Printing of Curcumin-Loaded Biodegradable and Flexible Scaffold for Intracranial Therapy of Glioblastoma Multiforme. v. 13, n. 4, p. 471, 2021.

OURIQUE, A. F.; MELERO, A.; SILVA, C. D. B. D.; SCHAEFER, U. F.; POHLMANN, A. R.; GUTERRES, S. S.; LEHR, C.-M.; KOSTKA, K.-H.; BECK, R. C. R. Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine. European Journal of Pharmaceutics and Biopharmaceutics, v. 79, n. 1, p. 95-101, 2011/09/01/ 2011.

RABIEE, N.; YARAKI, M. T.; GARAKANI, S. M.; GARAKANI, S. M.; AHMADI, S.; LAJEVARDI, A.; BAGHERZADEH, M.; RABIEE, M.; TAYEBI, L.; TAHRIRI, M.; HAMBLIN, M. R. Recent advances in porphyrin-based nanocomposites for effective targeted imaging and therapy. Biomaterials, v. 232, p. 119707, 2020/02/01/ 2020.

RAVICHANDRAN, V.; VASANTHI, S.; SHALINI, S.; SHAH, S. A. A.; TRIPATHY, M.; PALIWAL, N. Green synthesis, characterization, antibacterial, antioxidant and photocatalytic activity of Parkia speciosa leaves extract mediated silver nanoparticles. Results in Physics, v. 15, p. 102565, 2019/12/01/ 2019.

ROMANHOLE, R. C.; FAVA, A. L. M.; TUNDISI, L. L.; MACEDO, L. M. D.; SANTOS, É. M. D.; ATAIDE, J. A.; MAZZOLA, P. G. Unplanned absorption of sunscreen ingredients: Impact of formulation and evaluation methods. International Journal of Pharmaceutics, v. 591, p. 120013, 2020/12/15/ 2020.

ROSADO, C.; SILVA, C.; REIS, C. P. Hydrocortisone-loaded poly(ε-caprolactone) nanoparticles for atopic dermatitis treatment. Pharmaceutical Development and Technology, v. 18, n. 3, p. 710-718, 2013/06/01 2013.

SINHA, V. R.; BANSAL, K.; KAUSHIK, R.; KUMRIA, R.; TREHAN, A. Poly-ϵ-caprolactone microspheres and nanospheres: an overview. International Journal of Pharmaceutics, v. 278, n. 1, p. 1-23, 2004/06/18/ 2004.

TUNDISI, L. L.; YANG, R.; BORELLI, L. P. P.; ALVES, T.; MEHTA, M.; CHAUD, M. V.; MAZZOLA, P. G.; KOHANE, D. S. Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein. Pharmaceutical Research, p. 1-8, 2021.

YANG, R.; WEI, T.; GOLDBERG, H.; WANG, W.; CULLION, K.; KOHANE, D. S. Getting Drugs Across Biological Barriers. Advanced Materials, v. 29, n. 37, p. 1606596-n/a, 2017.

ZELIKIN, A. N.; EHRHARDT, C.; HEALY, A. M. Materials and methods for delivery of biological drugs. Nat Chem, v. 8, n. 11, p. 997-1007, 11//print 2016.

8. Anexos

8.1. Publicações

8.1.1. Artigos Publicados

- Victória S. Soeiro, **Louise L. Tundisi**, Letícia C. L. Novaes, Priscila G. Mazzola, Norberto Aranha, Denise Grotto, José M.O. Júnior, Daniel Komatsu, Francisco MP Gama, Marco V. Chaud, Angela F. Jozala Production of bacterial cellulose nanocrystals via enzymatic hydrolysis and evaluation of their coating on alginate particles formed by ionotropic gelation. - Carbohydrate Polymer Technologies and Applications, 2021
- Natália A. D'Angelo, Mariana A. Noronha, Isabelle S. Kurnik, Mayra C. C. Câmara, Jorge Vieira, Luís Abrunhosa, Joana T. Martins, Thais F. R. Alves, **Louise L. Tundisi**, Janaina A. Ataide, Juliana S. R. Costa, Angela F. Jozala, Laura O. Nascimento, Priscila G. Mazzola, Marco V. Chaud, António A. Vicente, Andre M. Lopes. Curcumin encapsulation in nanostructures for cancer therapy: a 10-year overview. International Journal of Pharmaceutics. 2021
- Julia Cedran Coco; **Louise Lacalendola Tundisi**; Érica Mendes dos Santos; Ana Laura Masquetti Fava; Thais Francine Alves; Janaína Artem Ataide; Marco Vinícius Chaud; Priscila Gava Mazzola. PVA-co-AAm and PEG-co-AAm Hydrogels as Bromelain Carriers. Journal of Drug Delivery Science And Technology. 2021
- **Tundisi, L. L.**, Mostaço, G. B., Carricondo, P. C., & Petri, D. F. S. (2021). Hydroxypropyl methylcellulose: Physicochemical properties and ocular drug delivery formulations. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences, 159, 105736.
- dos Santos, É. M., de Macedo, L. M., **Tundisi, L. L**., Ataide, J. A., Camargo, G. A., Alves, R. C., ... & Mazzola, P. G. (2021). Coffee by-products in topical formulations: A review. Trends in Food Science & Technology.
- **Tundisi, L. L.,** Yang, R., Borelli, L. P. P., Alves, T., Mehta, M., Chaud, M. V., ... & Kohane, D. S. Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein. Pharmaceutical research. 2021
- Lourenço, C. B., Fava, A. L. M., Dos Santos, É. M., de Macedo, L. M., **Tundisi, L. L.**, Ataide, J. A., & Mazzola, P. G. (2020). Brief descriptions of the principles of prominent methods used to study the penetration of materials into human hair and a review of examples of their use. International Journal of Cosmetic Science.

- Romanhole, R. C., Fava, A. L. M., **Tundisi, L. L.**, de Macedo, L. M., dos Santos, É. M., Ataide, J. A., & Mazzola, P. G. (2020). Unplanned absorption of sunscreen ingredients: Impact of formulation and evaluation methods. International Journal of Pharmaceutics.
- de Macedo, L. M., dos Santos, É. M., Militão, L., **Tundisi, L. L.**, Ataide, J. A., Souto, E. B., & Mazzola, P. G. (2020). Rosemary (Rosmarinus officinalis L., syn Salvia rosmarinus Spenn.) and Its Topical Applications: A Review. Plants, 9(5), 651.
- Croisfelt, F. M.*, **Tundisi, L. L.***, Ataide, J. A., Silveira, E., Tambourgi, E. B., Jozala, A. F., ... & Mazzola, P. G. (2019). Modified-release topical hydrogels: a ten-year review. Journal of Materials Science, 54(16), 10963-10983.
- Coelho, D. F., Huddleston, J. G., **Tundisi, L. L.**, Menezes, P. V., Porto, C., Mazzola, P. G., ... & Tambourgi, E. B. (2019). LLSR-An R package for data analysis of aqueous two-phase systems. Journal of chemical and engineering data.
- de Freitas Coêlho, D., **Tundisi, L. L.**, Cerqueira, K. S., da Silva Rodrigues, J. R., Mazzola, P. G., Tambourgi, E. B., & de Souza, R. R. Microalgae: Cultivation Aspects and Bioactive Compounds.
- Croisfelt, F. M., Ataide, J. A., **Tundisi, L. L.**, Cefali, L. C., Rebelo, M. D. A., Davila Sanchez, J. L., ... & Mazzola, P. G. (2018). Characterization of PNIPAAm-co-AAm hydrogels for modified release of bromelain. EUROPEAN POLYMER JOURNAL, 105, 48.

8.1.2. Artigos submetidos

Louise Lacalendola Tundisi; Janaina Artem Ataide; Juliana S. R. Costa; Diego F. Coêlho; Raquel Bester Liszbinski; Andre Moreni Lopes; Laura Oliveira Nascimento; Marcelo Bispo de Jesus; Angela Faustino Jozala; Carsten Ehrhardt; Priscila Gava Mazzola. Nanotechnology as a Tool to Overcome Macromolecules Delivery Issues. *Journal of Nanoparticle Research.*

Louise Lacalendola Tundisi; Rogério Aparecido Bataglioli; Marisa Masumi Beppu; Priscila Gava Mazzola. Bio-polymeric delivery systems for modified-release. *Journal of Controlled Release.*

8.1.3. Anais de congressos

11 th WORLD BIOMATERIAL CONGRESS

Free Session

Biomaterial synthesis and characterisation

WBC2020-150

Poloxamer 407-Casein - Bioadhesive properties and Biocompatibility of hydrogel for topical control drug release

Louise Lacalendola Tundisi*[1,2], Thais Francine Ribeiro Alves[3], Manisha Mehta[2], Luiz Phellipe Pozzuto Borelli[1], Rong Yang[2], Marcos Vinícius Chaud[3], Priscila Gava Mazzola[1], Daniel S. Kohane[2]

[1]Faculty of Pharmaceutical Sciences, UNICAMP - University of Campinas, Campinas, Brazil, [2]Laboratory for Biomaterials and Drug Delivery, Department of Anaesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, United States, [3]LaBNUS – Biomaterials and Nanotechnology Laboratory, University of Sorocaba, Sorocaba, Brazil

Please select your preferred method of presentation: Free Session - Oral or Poster

Introduction: Topical route has several advantageous to treat skin disorder, considering it provides direct access to the site, avoids first-pass hepatic metabolism, it is not invasive and reduces systemic toxicity. Topical dosage forms for skin application need to have optical mechanical properties as: spreadability, acceptable, bioadhesion proper drug delivery and drug. Here we propose a sustained release formulation that would enhance compliance because it would require less frequent administration. Poloxamer 407 (P407) a polymer with reverse thermal gelation properties combined with casein, a protein from bovine milk that could make the hydrogel more adhesive through molecular entanglement. The adhesiveness of the formulation would make it easier for the treatment to remain in contact with the application site.

Experimental methods: A casein solution 5% and 10% (w/v) was prepared by dissolving casein in deionized water and adjusting the solution pH to 7. 25% (w/v) and 18% (w/v) of P407 was added and the solutions were stirred overnight at 4°C. Forming 4 different formulations

ADHESIVE - Evaluation of the mechanical properties and bioadhesion of the hydrogel preparations were performed using a Stable Micro Systemsntexture analyzer (Model TA-XT Plus) in the texture profile analysis (TPA) mode following (Alves et al., 2018), using pig skin.

DRUG RELEASE - The release of Sulforhodamine B, Bupivacaine Hydrochloride (as drug models) from each formulation was measured using a Transwell membrane inserts. Filter inserts with formed gels were suspended in wells filled with pre-warmed phosphate-buffered saline (PBS) pH 7.4, and the plates were then incubated in an oven (37°C). At each time point 1mL aliquots of the PBS-receiving media were sampled and inserts were sequentially moved into a new well. Sample aliquots were analyzed by spectrophotometer UV.

BIOCOMPATIBILITY - Histologic evaluation for biocompatibility after surface application of formulations. 4 groups of hydrogels (P407 25%; P407 25% + casein 10%; P407 25% + casein 10%; P407 18% + casein 5%) were applied on the skin for 6 hours.

Table: Table 1 – Hydrogels bioadhesion using pig skin at 37ºC in different contact times

	Bioadhesion (N)		
Time	45s	90s	180s
P18	1,22 ± 0,096	1,12 ± 0,078	1,047 ± 0,193
P18C5	1,054 ± 0,045	1,112 ± 0,105	0,687 ± 0,168
P18C10	1,738 ± 0,115	1,833 ± 0,093	1,382 ± 0,081
P25	1,95 ± 0,218	1,91 ± 0,156	1,87 ± 0,159
P25C5	1,912 ± 0,125	1,878 ± 0,215	2,169 ± 0,379
P25C10	2,528 ± 0,51	2,833 ± 0,176	2,726 ± 0,352

Results and discussions: ADHESIVE - The addition and increase of casein concentrations in P25 formulations increased its rigidity from 0.544±0.006 to 0.945±0.031. This could be due to a network by physical interactions between the outer regions of polypeptide chains created by casein micelles (Xu et al., 2018).

It is possible to verify that the formulations P18, P18C5 and P18C10 the bio adhesion work favors the electrostatic interaction since the force (N) to highlight the two surfaces are larger at times 45s and 90s. For P25, P25C% and P25C10 the bio adhesion work is independent of contact time. However, for both P18 and P25 formulations, the incorporation of C5 and C10 increased bio adhesion, reinforcing the hypothesis that the adhesion work occurs mainly by the interlacing of polymeric chains with the biological tissue.

DRUG RELEASE - The drug release profile of Sulforhodamine B showed that the release speed increases when the casein content is lower. Bupivacaine Hydrochloride drug release profile shows us that the increasing the amount of casein prolonged the drug release.

BIOCOMPATIBILITY - No signs of reactivity or inflammation were seen in any of the groups of the biocompatibility test.

Conclusions: The mixture of P407 and casein showed biocompatibility in rats, demonstrating its potential to be tested and used in humans. The introduction of casein not only tackified the hydrogel, but also enhanced its the mechanical properties and prolonged the drug release. The hydrogels formulations showed really interesting results leading to a possible controlled release device with suitable mechanical and adhesive properties.

References/Acknowledgements: São Paulo Research Foudation (2017/10728-2)

XU, J.; et al. A tough, stretchable, and extensively sticky hydrogel driven by milk protein. **Polymer Chem.**, 2018.

ALVES, T. et al. Formulation and evaluation of thermoresponsive polymeric blend as a vaginal controlled delivery system. **J. Sol-Gel Sci. &Tech** 2018.

Disclosure of Interest: None Declared

Keywords: Biocompatibility, Biomaterials for drug delivery, Stimuli-responsive biomaterials

8th International Conference on Bioengineering and Nanotechnology

Poloxamer 407-Casein hydrogel for topical drug delivery

Louise Lacalendola Tundisi[1,2], Rong Yang[1], Priscila Gava Mazzola[3], Daniel S. Kohane[1]
[1] Laboratory for Biomaterials and Drug Delivery, Department of Anaesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA;
[2]Graduate Program in Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, SP, Brazil;
[3]Faculty of Pharmaceutical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil

Topical therapy of superficial mycosis is advantageous over oral in that there is no first-pass effect, no drug interactions and it usually does not require laboratory monitoring during treatment. However, patient compliance with topical therapy can be poor as it last months and requires many applications a day. Here we propose a sustained release formulation that would enhance compliance because it would require less frequent administration. The formulation contains two excipients. Poloxamer 407 (P407) a polymer with reverse thermal gelation properties, allowing easy application as a liquid, which gels upon contact with body temperature. It is combined with casein, a protein from bovine milk that could make the hydrogel more adhesive through molecular entanglement. The adhesiveness of the formulation would make it easier for the treatment to remain in contact with the body, particularly in places that are relatively difficult to access. That, and the reduction in the number of daily applications, could enhance patient compliance. We formulated combinations of P407 and casein in varying proportions, with the presence of the two molecules being confirmed by the presence on FTIR of the characteristic peaks (for casein, 1644.49 cm^{-1} [carbonyl groups]; for P407, 1517.33 cm^{-1} [N-H bending and C-N stretching vibration] and 1105.36 cm^{-1} [C-O stretch]. We studied the effect of changes in the proportion of casein, on viscosity, as this would be an important determinant of the ability of the formulation to stay in place after application. The viscosity at 37°C of the formulation increased with increasing proportion of casein:18% (w/v) P407 with 5 and 10% casein were 1.27 and 2.84 times more viscous respectively than P407 alone (597.60 Pa; 1333.8325 Pa). At 25% P407, addition of 5% and 10% casein increased viscosity 10.03 and 12.21-fold respectively (1825.76 Pa; 2223.03 Pa). The effect of formulation on mechanical properties (e.g. adhesive strength), drug release kinetics, and cytotoxicity will also be demonstrated.

Keywords: P407, Casein, Adhesive, Topical Treatment, Drug Delivery, Control release

II INTERNATIONAL CONFERENCE OF NANOSCIENCE AND NANOBIOTECHNOLOGY

ABSTRACT

PCL-TERBINAFINE NANOPARTICLES FOR LOCAL DRUG DELIVERY

Louise Lacalendola Tundisi[1,2*], Daniel S. Kohane[1], Priscila Gava Mazzola[3]

[1] Laboratory for Biomaterials and Drug Delivery, Department of Anaesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA;

[2]Graduate Program in Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, SP, Brazil;

[3]Faculty of Pharmaceutical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil

* *Correspondence: tundisi.l@gmail.com*

Introduction: Topical therapy of superficial mycosis is advantageous over oral in that there is no first-pass effect, no drug interactions and it usually does not require laboratory monitoring during treatment. However, patient compliance with topical therapy can be poor as treatment last months and requires many applications a day. Here we propose a nanoparticulate sustained release formulation that would enhance patients' compliance by reducing the frequency of administration. Here we report the characterization of nanoparticles containing terbinafine in order to use it as a local controlled release drug delivery system. **Methodology:** Terbinafine PCL nanoparticles were produced by nanoprecipitation in aqueous poloxamer 407 solution of polymer and terbinafine dissolved in acetone. The resulting mixtures were stirred at 37 °C to allow solvent evaporation. Blank nanoparticles were prepared similarly. The particle size, polydispersity index (PDI) and zeta potential of the nanoparticles containing terbinafine were determined via dynamic light-scattering procedure in Zetasizer. The morphology of particles was observed by transmission electron microscope (TEM). The FTIR spectra were recorded in an ATR-FTIR (attenuated total reflectance Fourier transform infrared) spectrometer. The amount of terbinafine encapsulated in the nanoparticles was computed by quantification of non-encapsulated drug. **Results and Discussion:** Nanoparticles with and without terbinafine showed a small difference in size, 196.7 nm and 186.4 nm respectively. Nanoparticle solution were monodisperse, as both particles had a low polydispersity index

(PDI), under 0.1. The zeta potential was very different between the particles, which could be due to the presence of terbinafine adsorbed on the particles' surfaces. A one-month stability study was performed with both particles. Particles morphology was analyzed by TEM. The mean size of the blank nanoparticle was 89.42 nm and that of the terbinafine nanoparticle was 78.52 nm. The ATR-FTIR of the nanoparticles showed successful terbinafine encapsulation in the PCL nanoparticles. Encapsulation efficiency (EE) was 98.81% by UV spectrophotometery.

Conclusions: PCL nanoparticles stabilized with P407 showed low PDI, were stable throughout a month, and had high encapsulation efficiency, suggesting potential utility in a topical formulation.

References

Abdel-Mottaleb, M.M.A., Neumann, D., Lamprecht, A. 2011. Lipid nanocapsules for dermal application: A comparative study of lipid-based versus polymer-based nanocarriers. *European Journal of Pharmaceutics and Biopharmaceutics*, **79**(1), 36-42.

Rosado, C., Silva, C., Reis, C.P. 2013. Hydrocortisone-loaded poly(ε-caprolactone) nanoparticles for atopic dermatitis treatment. *Pharmaceutical Development and Technology*, **18**(3), 710-718.

Yang, R., Wei, T., Goldberg, H., Wang, W., Cullion, K., Kohane, D.S. 2017. Getting Drugs Across Biological Barriers. *Advanced Materials*, **29**(37), 1606596-n/a.

Acknowledgments and Support

FAPESP n° 2017/10728-2 and n° 2018/05421-8

8.2. Declaração

Licence to Publish

SPRINGER NATURE

Journal Name:	Pharmaceutical Research	(the 'Journal')
Manuscript Number:	PHAM-D-20-00799R2	
Proposed Title of Article:	Enhancement of the Mechanical and Drug-Releasing Properties of Poloxamer 407 Hydrogels with Casein	
Author(s) [Please list all named Authors]:	Daniel Kohane, Louise Lacalendola Tundisi, Rong Yang, Luiz Phellipe Pozzuto Borelli, Thais Alves, Manisha Mehta, Marco Vinícius Chaud, Priscila Gava Mazzola, Daniel S. Kohane	(the 'Author')
Corresponding Author Name:	Daniel Kohane	

1 Publication

Springer Science+Business Media LLC (the 'Licensee') will consider publishing this article, including any supplementary information and graphic elements therein (e.g. illustrations, charts, moving images) (the 'Article').
Headings are for convenience only.

2 Grant of Rights

In consideration of the Licensee evaluating the Article for publication, the Author grants the Licensee the exclusive (except as set out in clauses 3, 4 and 5a) iv) and sub-licensable right, unlimited in time and territory, to copy-edit, reproduce, publish, distribute, transmit, make available and store the Article, including abstracts thereof, in all forms of media of expression now known or developed in the future, including pre- and reprints, translations, photographic reproductions and extensions.

Furthermore, to enable additional publishing services, such as promotion of the Article, the Author grants the Licensee the right to use the Article (including the use of any graphic elements on a stand-alone basis) in whole or in part in electronic form, such as for display in databases or data networks (e.g. the Internet), or for print or download to stationary or portable devices. This includes interactive and multimedia use as well as posting the Article in full or in part or its abstract on social media, and the right to alter the Article to the extent necessary for such use. The Licensee may also let third parties share the Article in full or in part or its abstract on social media and may in this context sub-license the Article and its abstract to social media users. Author grants to Licensee the right to re-license Article metadata without restriction (including but not limited to author name, title, abstract, citation, references, keywords and any additional information as determined by Licensee).

If the Article is rejected by the Licensee and not published, all rights under this agreement shall revert to the Author.

3 Self Archiving

Author is permitted to self-archive a preprint and the accepted manuscript version of their Article.

a) A preprint is the version of the Article before peer-review has taken place ("Preprint"). Prior to acceptance for publication, Author retains the right to make a Preprint of their Article available on any of the following: their own personal, self-maintained website; a legally compliant Preprint server such as but not limited to arXiv and bioRxiv. Once the Article has been published, the Author should update the acknowledgement and provide a link to the definitive version on the publisher's website: "This is a preprint of an article published in [insert journal title]. The final authenticated version is available online at: https://doi.org/[insert DOI]"

b) The accepted manuscript version, by industry standard called the "Author's Accepted Manuscript" ("AAM") is the version accepted for publication in a journal following peer review but prior to copyediting and typesetting that can be made available under the following conditions:
(i) Author retains the right to make an AAM of the Article available on their own personal, self-maintained website immediately on acceptance, (ii) Author retains the right to make an AAM of the Article available for public release on any of the following 12 months after first publication ("Embargo Period"): their employer's internal website; their institutional and/or funder repositories; AAMs may also be deposited in such repositories immediately on acceptance, provided that they are not made publicly available until after the Embargo Period.

An acknowledgement in the following form should be included, together with a link to the published version on the publisher's website: "This is a post-peer-review, pre-copyedit version of an article published in [insert journal title]. The final authenticated version is available online at: http://dx.doi.org/[insert DOI]".

4 Reuse Rights

Author retains the following non-exclusive rights for the published version provided that, when reproducing the Article or extracts from it, the Author acknowledges and references first publication in the Journal according to current citation standards. In any event the acknowledgement should contain as a minimum, "First published in [Journal name, volume, page number, year] by Springer Nature".

a) to reuse graphic elements created by the Author and contained in the Article, in presentations and other works created by them;

b) the Author and any academic institution where they work at the time may reproduce the Article for the purpose of course teaching (but not for inclusion in course pack material for onward sale by libraries and institutions);

c) to reuse the published version of the Article or any part in a thesis written by the same Author , and to make a copy of that thesis available in a repository of the Author(s)' awarding academic institution, or other repository required by the awarding academic institution. An acknowledgement should be included in the citation: "Reproduced with permission from Springer Nature"; and

d) to reproduce, or to allow a third party to reproduce the Article, in whole or in part, in any other type of work (other than thesis) written by the Author for distribution by a publisher after an embargo period of 12 months.

5 Warranties & Representations

Author warrants and represents that:

a)

i. the Author is the sole copyright owner or has been authorised by any additional copyright owner(s) to grant the rights defined in clause 2,
ii. the Article does not infringe any intellectual property rights (including without limitation copyright, database rights or trade mark rights) or other third party rights and no licence from or payments to a third party are required to publish the Article,
iii. the Article has not been previously published or licensed,
iv. if the Article contains materials from other sources (e.g. illustrations, tables, text quotations), Author has obtained written permissions to the extent necessary from the copyright holder(s), to license to the Licensee the same rights as set out in clause 2 and has cited any such materials correctly;

b) all of the facts contained in the Article are according to the current body of research true and accurate;

c) nothing in the Article is obscene, defamatory, violates any right of privacy or publicity, infringes any other human, personal or other rights of any person or entity or is otherwise unlawful and that informed consent to publish has been obtained for all research participants;

d) nothing in the Article infringes any duty of confidentiality which Author might owe to anyone else or violates any contract, express or implied, of Author. All of the institutions in which work recorded in the Article was created or carried out have authorised and approved such research and publication; and

e) the signatory who has signed this agreement has full right, power and authority to enter into this agreement on behalf of all of the Authors.

6 Cooperation

a) Author shall cooperate fully with the Licensee in relation to any legal action that might arise from the publication of the Article, and the Author shall give the Licensee access at reasonable times to any relevant accounts, documents and records within the power or control of the Author. Author agrees that the distributing entity is intended to have the benefit of and shall have the right to enforce the terms of this agreement.

b) Author authorises the Licensee to take such steps as it considers necessary at its own expense in the Author's name(s) and on their behalf if the Licensee believes that a third party is infringing or is likely to infringe copyright in the Article including but not limited to initiating legal proceedings.

7 Author List

Changes of authorship, including, but not limited to, changes in the corresponding author or the sequence of authors, are not permitted after acceptance of a manuscript.

8 Corrections

Author agrees that the Licensee may retract the Article or publish a correction or other notice in relation to the Article if the Licensee considers in its reasonable opinion that such actions are appropriate from a legal, editorial or research integrity perspective.

9 Governing Law

This agreement shall be governed by, and shall be construed in accordance with, the laws of New York State. The courts of competent jurisdiction in New York, N.Y. shall have the exclusive jurisdiction.