UNIVERSIDADE EVANGÉLICA DE GOIÁS – UniEVANGÉLICA
PROGRAMA DE PÓS-GRADUAÇÃO EM MOVIMENTO HUMANO E REABILITAÇÃO
PPGMHR

DESENVOLVIMENTO DE UM HIDROGEL PARA APLICAÇÕES EM MEDICINA REGENERATIVA E TERAPIA DE REPARO TECIDUAL

RICARDO SILVA MOURA

Anápolis, GO
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Tese apresentada ao Programa de Pós-graduação *Stricto Sensu* em Movimento Humano e Reabilitação da Universidade Evangélica de Goiás (UNIEVANGÉLICA), como exigência parcial para obtenção de título de Doutor em Movimento Humano e Reabilitação

Doutorando: Ricardo Silva Moura

Orientador: Prof. Dr. Luís Vicente Franco de Oliveira

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FOLHA DE APROVAÇÃO

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Àquele que é capaz de fazer infinitamente mais do que tudo o que pedimos ou pensamos, segundo o seu poder que atua em nós, a ele seja a glória!

Efésios 3:20

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LISTA DE ABREVIATURAS

O_2: Oxigênio singleto

3D: Tridimensional

AMR: Resistência Antimicrobiana

DNA: Ácido Desoxirribonucleico

CHAPS: Detergente 3 - [(3-colamidopropil) dimetilamónio] -1-propanossulfonato

DAPI: Corante 4`-6-diamidino-2-finilindol

dECM: Matriz extracelular descelularizada

Dnase: Desoxirribonuclease

DP: Desvio padrão

DPOC: Doença Pulmonar Obstrutiva Crônica

EDTA: Ácido Etilenodiamino Tetra-Acético

EGTA: Ácido Etilenglicol Tetraacético

FS: Fotossensibilizador

GAGS: Glicosaminoglizanos

HIV: Vírus da Imunodeficiência Humana

LED: Diodo Emissor De Luz

MEC: Matrix extracelular

OCT: Optimal Cutting Temperature compound

PBS: Tampão Fosfato Salino

PDT: Photodynamic Therapy

PEEP: Positive end-expiratory pressure

pH: Potencial Hidrogeniônico

Rnase: Ribonuclease

ROS: Especies reativas de oxigênio

SDS: Detergente Sullfato Dodecil De Sódio

TFD: Terapia Fotodinâmica

TFDA: terapia fotodinâmica antimicrobiana

UV: Radiação ultravioleta

UVA: Radiação ultravioleta A

RESUMO

Introdução: As doenças respiratórias constituem um dos principais desafios da saúde pública mundial, sendo responsáveis por elevada morbimortalidade e pela crescente demanda por transplantes pulmonares, em virtude de danos irreversíveis ao tecido pulmonar. A escassez de órgãos viáveis e os riscos associados ao uso de imunossupressores impulsionam a busca por alternativas na medicina regenerativa e na bioengenharia de tecidos. Paralelamente, o aumento da resistência antimicrobiana e os efeitos tóxicos de agentes químicos, como agrotóxicos, agravam o quadro clínico global e reforçam a necessidade de novas terapias antimicrobianas e materiais biocompatíveis. **Objetivo:** Desenvolver um hidrogel derivado de matriz extracelular descelularizada. **Metodologia:** O estudo baseou-se em três etapas complementares: (i) revisão sistemática sobre hidrogéis e matriz extracelular; (ii) desenvolvimento experimental realizado nos laboratórios da Universidade Evangélica de Goiás e na Universidade de Barcelona, envolvendo a descelularização de pulmões suínos; e (iii) análise da literatura sobre métodos de esterilização de scaffolds. **Resultados e Discussão:** Os resultados demonstraram que o processo de descelularização foi eficaz, resultando em pulmões livres de material genético (17 ± 8 ng/mg, abaixo do limite de 50 ng/mg) e com preservação das estruturas da matriz extracelular, incluindo colágeno, elastina e glicosaminoglicanos. A solubilização e gelificação da matriz produziram um hidrogel transparente, viscoelástico, com alta retenção de água e propriedades mecânicas ajustáveis. O biomaterial mostrou-se potencialmente útil como suporte para cultura celular tridimensional, regeneração pulmonar e liberação controlada de fármacos. Os achados reforçam a necessidade de novos estudos que explorem o uso da terapia fotodinâmica como método estéril e não destrutivo para a produção de hidrogéis de matriz extracelular, abrindo caminho para aplicações clínicas inovadoras que possam reduzir filas de transplante e melhorar os tratamentos de doenças respiratórias.
Conclusão: O desenvolvimento de um hidrogel derivado de pó de pulmão suíno descelularizado representa um avanço relevante para a engenharia de tecidos e a medicina regenerativa, oferecendo um material biocompatível, biodegradável e funcional.

Palavras-chave: Doenças respiratórias; Pulmão; Descelularização; Hidrogel; Medicina regenerativa.

ABSTRACT

Introduction: Respiratory diseases constitute one of the main challenges to global public health, being responsible for high morbidity and mortality and the growing demand for lung transplants due to irreversible damage to lung tissue. The scarcity of viable organs and the risks associated with the use of immunosuppressants drive the search for alternatives in regenerative medicine and tissue bioengineering. In parallel, the increase in antimicrobial resistance and the toxic effects of chemical agents, such as pesticides, worsen the overall clinical picture and reinforce the need for new antimicrobial therapies and biocompatible materials. **Objective:** To develop a hydrogel derived from decellularized extracellular matrix. **Methodology:** The study was based on three complementary stages: (i) systematic review on hydrogels and extracellular matrix; (ii) experimental development carried out in the laboratories of the Evangelical University of Goiás and the University of Barcelona, involving the decellularization of pig lungs; (iii) analysis of the literature on scaffold sterilization methods. **Results and Discussion:** The results demonstrated that the decellularization process was effective, resulting in lungs free of genetic material (17 ± 8 ng/mg, below the limit of 50 ng/mg) and with preservation of extracellular matrix structures, including collagen, elastin, and glycosaminoglycans. Solubilization and gelation of the matrix produced a transparent, viscoelastic hydrogel with high water retention and adjustable mechanical properties. The biomaterial proved potentially useful as a support for three-dimensional cell culture, lung regeneration, and controlled drug release. The findings reinforce the need for further studies exploring the use of photodynamic therapy as a sterile and non-destructive method for the production of extracellular matrix hydrogels, paving the way for innovative clinical applications that can reduce transplant waiting lists and improve treatments for respiratory diseases. **Conclusion:** The development of a hydrogel derived from decellularized porcine lung powder represents a significant advance for tissue engineering and regenerative medicine, offering a biocompatible, biodegradable, and functional material.

Keywords: Respiratory diseases; Lung; Decellularization; Hydrogel; Regenerative medicine.

SUMÁRIO

1. INTRODUÇÃO

1.1 Problemas De Saúde Pública

Doenças respiratórias continuam sendo um grave problema de saúde pública mundial, contribuindo significativamente para a mortalidade e morbidade global. Internação hospitalar prolongada desenvolve complicações significativas e alterações imunológicas (Freire et al., 2024). Doenças respiratórias crônicas frequentemente necessitam de transplante de pulmão devido a danos irreversíveis. Em 2019, aproximadamente 488,9 milhões de casos incidentes de infecções do trato respiratório inferior (ITRI) em todo o mundo (Bender et. al., 2024). No mesmo ano, as mortes atribuíveis às ITRI foram estimadas em cerca de 2,4 milhões de óbitos globais (PMC, 2023).

Em 2021, estimou-se que a Doença Pulmonar Obstrutiva Crônica (DPOC) afetava aproximadamente 213,39 milhões de pessoas no mundo, com uma taxa padronizada por idade de 2.512,86 casos por 100.000 habitantes. (BioMed Central, 2024). Entre os principais fatores de risco para doenças respiratórias crônicas e agudas estão, o tabagismo, a poluição do ar, o uso de combustíveis sólidos em ambientes domésticos e a exposição ocupacional a agentes irritantes (Wang et al., 2025).

A pandemia do covid-19 (SARS-CoV-2) aumentou as mortes por estresse respiratório e sepsia. A infecção pode ocorrer por células do endotélio vascular, que expressam receptores ACE2, com o vírus sendo transportado através do endotélio (Bender et. al., 2024). Um problema crescente em pacientes hospitalizados, principalmente com síndrome respiratória aguda grave (ÖZ et. al., 2022).

Os agrotóxicos são substâncias químicas destinadas aos setores de produção. Brasil é o país que mais utiliza e consome agrotóxicos no mundo (Panis et al., 2022). Os agrotóxicos são substâncias químicas utilizadas para o controle de pragas e doenças que afetam as plantações e, por muitas vezes, ao serem utilizados de maneira incorreta, causam efeitos prejudiciais à saúde humana e ao meio ambiente (Tavares et al., 2020).

A exposição por inalação a produtos à base de glifosato causa problemas respiratórios, incluindo pneumonite química e inflamação pulmonar (Sidthilaw et al., 2022).

Nas últimas cinco décadas, o consumo de agrotóxicos no Brasil vem crescendo de forma expressiva, impulsionado pelo modelo de desenvolvimento agrícola voltado à produção em larga escala e pela dependência econômica do país em relação ao mercado global de commodities. Esse cenário tem gerado sérios riscos à saúde humana e ampla contaminação ambiental — atingindo a água, o ar e o solo. A exposição constante aos agrotóxicos, sobretudo entre trabalhadores rurais, configura-se como um grave problema de saúde pública, representando ameaças tanto às gerações presentes quanto às futuras (Fiocruz, 2018).

O uso intensivo dessas substâncias tem sido alvo de debates nacionais e internacionais sobre seus efeitos nocivos ao meio ambiente e à saúde humana. Diversas pesquisas relatam casos de intoxicações e o surgimento de doenças associadas à exposição prolongada, incluindo câncer, diabetes, enfermidades cardiovasculares e doenças pulmonares (Constante et al., 2022).

A Resistência Antimicrobiana (AMR-antimicrobial resistance) aos antibióticos é um dos problemas médicos e científicos mais importantes do nosso tempo, é considerada uma das mais graves ameaças à saúde global no século XXI. Em 2019 cerca de 4,95 milhões de mortes estavam associadas a infeções bacterianas resistentes a antimicrobianos, das quais cerca de 1,27 milhões foram diretamente atribuíveis à resistência. (Cao et al., 2021). Devido à AMR, infecções que antes eram tratáveis com antibióticos comuns podem tornar-se persistentes, mais graves ou até fatais, resultando em mais tempo de hospitalização, custos elevados e complicações adicionais (Moradi et al., 2022). Sistemas de saúde em países de média e baixa renda enfrentam maiores desafios para acesso a antibióticos eficazes, diagnóstico oportuno, infraestrutura de higiene, controle de infeção, o que agrava o problema da resistência (Nathan et al., 2020).

Uma ameaça significativa vem dos patógenos do grupo ESKAPE clinicamente importante (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa e Enterobacter spp*.), representados principalmente por agentes de infecção nosocomial, que estão associados ao maior risco de mortalidade e aumento dos custos de saúde (Khan et al., 2019).

Apesar da complexidade do desenvolvimento de novos medicamentos com propriedades antibióticas (Nathan et al., 2020), abordagens alternativas foram desenvolvidas recentemente para controlar a disseminação de microrganismos patogênicos. Uma dessas abordagens é a Terapia Fotodinâmica Antimicrobiana (TFDA), que usa agentes fotossensibilizadores (corantes) e hidrogéis capazes de absorver fótons de luz e elevá-los a um estado excitado (Beyer & Paulin, 2020).

Nos últimos anos, o aumento de bactérias resistentes a medicamentos tornou os antibióticos existentes menos eficazes, enquanto o desenvolvimento de biofilmes diminuiu ainda mais seu impacto terapêutico (Youf et al., 2021). O uso massivo e abusivo de antibióticos causou efeitos colaterais graves, tornando imperativo o desenvolvimento de sistemas antibacterianos alternativos, ultraeficientes e seguro (Youf et al., 2021). A técnica mais recente usada para controlar infecções microbianas é a combinação de TFDA com um hidrogel, que parece inovadora e opera de forma não específica em células microbianas, evitando assim o desenvolvimento de resistência (Zheng et al., 2019).

Diante do crescente problema de resistência a antibióticos microbianos, a TFDA administrada usando hidrogéis atraiu interesse como um tratamento antimicrobiano alternativo. Vários estudos *in vitro e in vivo* envolvendo inativação microbiana com resultados bem-sucedidos para bactérias, fungos, leveduras, vírus e parasitas, e na cicatrização de feridas e queimaduras foram conduzidos (Oliveira et al., 2021; Zheng et al., 2019).

1.2 Estado Da Arte – Alternativa Clínica

As alternativas clínicas para problemas pulmonares variam de acordo com a causa e a gravidade da doença. Exitem muitos medicamentos que são utilizados como tratamento para problemas pulmonares, como: broncodilatadores, corticosteroides, antibióticos, antivirais. Outra alternativa é a utilização de oxigenoterapia, de ventilação mecânica e reabilitação pulmonar. Em último caso, recorre-se à cirurgia, restando apenas a alternativa de transplante de pulmão.

A falência de órgãos em pacientes em fase terminal é um grave e dispendioso problema de saúde que apresenta altas índices. Nos últimos anos, o transplante alogênico tem se apresentado como uma única opção de tratamento definitivo. A doença cardíaca coronária, a insuficiência renal, a insuficiência hepática e a doença pulmonar obstrutiva crônica (DPOC) são as patologias com maior indice de mortalidade em países ocidentais desenvolvidos (Heron, Roger et al., 2012; Saran, 2015).

O número de transplantes de órgãos vem crescendo a cada ano, assim como o número de pacientes em lista de espera que tem um crescimento exponencial. No caso do transplante pulmonar, esse número de pacientes no aguardo de um novo órgão duplicou em apenas 10 anos, além disso, a população nos países desenvolvidos ocidentais está em um processo progressivo de envelhecimento significando que no futuro menos doadores estarão disponíveis e mais pacientes necessitarão de transplante (Christie et al., 2012).

Os pacientes que obtem sucesso na lista e recebem um órgão do doador, são obrigados a lidar com um tratamento imunossupressor ao longo da vida, além do risco de rejeição crônica e morbimortalidade associada (Orens, 2009). Dada a necessidade urgente de um maior número de órgãos viáveis para transplante e diante da escassez de doadores, a engenharia de tecidos parece ser uma solução viável como método alternativo na resolução dos problemas de rejeição, devido a utilização de células autólogas (Lee, 2013).

1.3 Estado Da Arte – Alternativa Tecnológica

Como mencionado acima, todas estas alternativas convencionais não são o suficiente para reduzir as filas de transplantas e tratamentos pulmonares. Surgindo novas alternativas paralelas e tecnológicas baseadas no transplante pulmonar através da bioengenharia de órgãos. Estes transplantes podem ocorrer através de transplantes xenogênicos, passando pelo processo de de descelularização e recelularização.

Atualmente, uma abordagem promissora para a substituição de órgãos funcionais é a descelularização prévia de órgão. Os órgãos alogênicos ou xenogênicos, tais como o coração (Ott et al. 2008), fígado (UYGUN et al., 2010) e pulmões (Petersen et al., 2010; Price et al., 2010) fornecem, após o processo de descelularização, um scaffold natural que pode ser subsequentemente repopulados com células tronco. O intuito visando a cliníca seria a utilização da matriz extracelular do órgão descelularizado para ser recelularizado com células autólogas obtidas do próprio paciente, evitando a necessidade de tratamento com imunossupressores (Ott et al., 2010).

Até o momento, a literatura aponta resultados encorajadores, uma vez que a formação de neotecido funcional tem sido demonstrada em modelos pré-clínicos com animais (Atala et al., 2006; Uygun et al., 2010; Petersen et al., 2010;). Estes resultados nos permitem considerar a hipótese de que a engenharia de órgãos é um caminho viável em relação à complexa engenharia de tecidos tridimensionais (Petersen et al., 2010; Heron, 2012).

Para o sucesso da engenharia de órgãos, vários parâmetros primordiais ainda necessitam de ajustes tais como, a determinação da espécie dos candidatos a partir do órgão a ser retirado (diferente de doadores humanos), quais os melhores protocolos para a descelularização, a melhor opção no processo de esterilização, a otimização da recelularização, o tipo celular mais adequado para a recelularização, a endotelização da matriz vascular e o desenvolvimento de biorreatores adequados ao processo de recelularização. A medicina regenerativa in vitro, ex vivo e in vivo também surge como uma alternativa promissora da bioengenharia de órgãos para solucionar este problema da falta de tratamentos para doenças pulmonares.

1.4 Estado Da Arte – Princípio Da Terapia Fotodinâmica

Raab publicou, há mais de cem anos, o primeiro estudo sobre efeitos fotodinâmicos de compostos químicos (eosina e alaranjado de acridina) contra microrganismos observando a toxicidade do hidrocloreto de acridina contra *Paramecium caudatum*. Mais tarde, Von Tappeiner observou que esses efeitos tóxicos não se deviam apenas à presença da luz, criando assim o termo **"**reação fotodinâmica**"** (Von Tappeiner, A. Jodlbauer 1903). Policard, vinte anos depois, publicou as primeiras observações clínicas dessa nova técnica recém-criada (Policard, 1924; Moradi et al., 2022).

A TFD é baseada na administração de um corante não tóxico sensível à luz seguida da irradiação em baixas doses de comprimento de onda de luz visível. Na presença de oxigênio encontrado nas células, o fotossensibilizador (FS) ativado pode reagir com moléculas na sua vizinhança por transferência de elétrons ou hidrogênio, levando à produção de radicais livres ou por transferência de energia ao oxigênio, levando à produção de oxigênio singleto($^1O_2$), ambos os mecanismos podem levar à morte celular e à destruição do tecido doente (Zheng et al., 2021) (Figura 1).

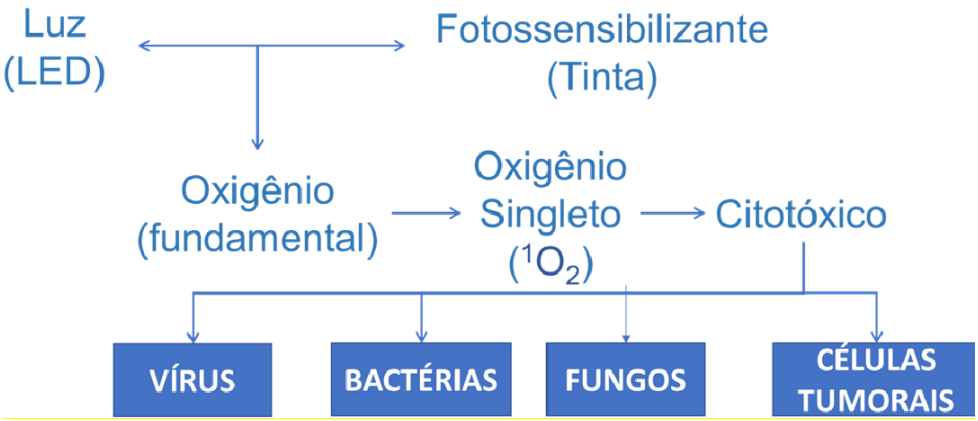

Figura 1. Esquema da reação fotoquímica nos microorganismos.

A membrana celular é a primeira barreira para o $^1O_2$ e esta contém lipídeos insaturados que podem ser danificados, ocorrendo a inviabilidade celular (Kang et al., 2021). Os hidroperóxidos resultantes podem levar à formação de ERO através de reações catalíticas. Uma vez que a reatividade das ERO com moléculas orgânicas não é específica, qualquer macromolécula dentro da célula pode ser um alvo em potencial para a TFD (Zhu et al., 2022). Causando

a morte de microorganismos (tais como bactérias, fungos, leveduras e vírus) por danos oxidativos (Du et al., 2023).

Assim, a multiplicidade de alvos torna mais difícil para as células desenvolverem resistência celular, sendo essa uma das vantagens da fotossensibilização. Esta terapia apresenta um efeito prejudicial mínimo sobre as células humanas, sendo seletivamente tóxico para os microrganismos (Bumah et al., 2020).

A fonte de luz na TFD pode ser oriunda de LED ou laser de baixa potência (Trindade et al., 2015). O LED é considerado uma fonte de luz mais segura, devido à menor produtividade térmica e mínima lesão de tecido, sendo também mais rentáveis, já que o consumo de energia é menor (Trindade et al., 2015). Os comprimentos de luz utilizados na TFD devem estar na faixa de 400 a 800nm, faixa conhecida como “janela terapêutica”, onde há máxima transmitância tecidual da luz. Nessa faixa, os componentes teciduais não absorvem e há penetração da luz no tecido. Os comprimentos de onda abaixo de 400nm têm baixa transmitância tecidual e são prejudiciais aos tecidos (Bumah et al., 2020).

A TFD foi introduzida em 1900, onde um fotossensibilizador não tóxico é ativado com luz irradiada em um comprimento de onda específico no tecido-alvo para gerar espécies reativas de oxigênio (ERO) e eliminar os microrganismos-alvo (Moradi et al., 2022).

A riboflavina (vitamina B2) é um FS natural não tóxico que pode ser usado em conjunto com a irradiação UV para descontaminação de sangue, plasma ou extratos celulares ou para eliminar microrganismos (Goto et al., 2021). Possui baixo custo e é altamente biocompatível, pois possui dois picos de absorção nas regiões ultravioleta A (UVA) (360nm) e azul (440nm) pode ser ativada pelas lâmpadas de LED (Kingsley et al., 2018).

A TFD é um modo de tratamento que tem sido considerado para infecções locais e avaliado por meio de experimentos *in vitro* e *in vivo*. A excitação de um fotossensibilizador por iluminação de luz gera espécies reativas de oxigênio ERO que resulta na eliminação de patógenos (Etemadi et al., 2021). A luz azul (400-470nm) pode eliminar bactérias Gram-positivas e Gram-negativas sem

cromóforo e é considerado menos prejudicial ao ser humano em comparação com a irradiação ultravioleta (UV) (Buchovec, et al., 2023). Os comprimentos de onda entre 402-420nm são aparentemente mais eficazes do que os comprimentos de onda mais longos da luz azul (455-470nm), embora os comprimentos de onda mais longos possam ter algumas vantagens na eliminação de culturas bacterianas densas com altas contagens de unidades formadoras de colônias (Jankowska et al., 2020).

1.5 Motivação

A falta de novos antimicrobianos que avancem ao ritmo da resistência emergente constitui um “colapso silencioso” da capacidade terapêutica moderna. Ainda que estas alternativas sejam poderosas e viáveis, existe um problema grande para poder utilizá-las, a ausência de um processo ouro de esterilização.

Como mecanismo para reduzir este problema quanto a deficiência no processo de esterilização, surge o hidrogel, que é uma classe de rede de polímeros tridimensionais (3D) expandidos com água apresentando propriedades físico-químicas ajustáveis que são exigidas para satisfazer os requisitos específicos sob diferentes condições. Como um tipo de material promissor, eles têm sido extensivamente aplicados no campo biomédico, desde estudos de mecanismos fisiológicos e patológicos até regeneração de tecidos e terapias de doenças (Choi et al., 2019). Os hidrogéis têm sido amplamente investigados como matrizes para aplicações biomédicas devido às suas habilidades de reticulação sob condições suaves, excelente biocompatibilidade e propriedades bioquímicas e biofísicas ajustáveis. Como a estrutura e as propriedades dos hidrogéis se assemelham ao microambiente de muitos tecidos do corpo humano, eles têm sido amplamente utilizados em diversas aplicações biomédicas (Goding et al., 2019).

O hidrogel derivado de pó de órgão descelularizado fornece um microambiente equivalente ao tecido nativo que oferece inúmeros benefícios em relação a outros hidrogéis, que ainda carecem de biofuncionalidade (Piluso et al., 2020). A impressão de células 3D é uma estratégia emergente para criar uma

construção de tecido manipulada, depositando componentes biológicos dentro de micrômetro a milímetro do local desejado (Wang et al., 2019).

Os hidrogéis são caracterizados por suas redes poliméricas tridimensionais altamente inchadas. Esses materiais encontraram ampla aplicação na engenharia biomédica, desde scaffolds de engenharia de tecidos, substituição de órgãos, lentes de contato, adesivos para cicatrização de feridas e até curativos para feridas devido à sua estrutura tridimensional semelhante aos tecidos moles biológicos (Xiang et al., 2019). A semelhança estrutural dos hidrogéis com a dMEC os torna ideais para tais aplicações em tecidos moles (Galdopórpora et al., 2019).

O fator comum que influenciou o amplo uso de materiais de hidrogel é que eles são sistemas de materiais altamente adaptáveis, que podem ser adaptados para atender aos requisitos de aplicações específicas (Choi et al, 2019). Os hidrogéis injetáveis podem ser injetados na ferida para preencher feridas de formato irregular, ou para atuar como agente antibacteriano afim de evitar contaminação no local, devido à sua propriedade esterilizante. Depois disso, esses hidrogéis se solidificam para promover a reconstrução dos tecidos danificados (Tang et. al., 2021). Além disso, o uso excessivo de antibióticos deu origem à resistência bacteriana adquirida aos medicamentos. Portanto, um hidrogel inteligente, que pode matar bactérias de forma eficaz sob controle de estímulos externos e acelerar a cicatrização de feridas sem drogas, é necessário com urgência (Xiang et al., 2019).

Hidrogéis de MEC descelularizada foram projetados com propriedades para atender a uma ou mais funcionalidades (Cao et al., 2021). Por exemplo, é possível projetar um hidrogel para uma determinada aplicação, de modo que possa ser usado como substrato para crescimento celular, preencher buracos em tecidos, reconstruindo o local lesionado ao mesmo tempo em que fornece uma terapêutica antibacteriana e reduz a probabilidade de contaminação ou de rejeição de um tecido transplantado (Le Thi et. al., 2020). Esse potencial de propriedades multifuncionais se deve à capacidade de projetar e manipular sistemas de hidrogéis de MEC descelularizada (Goding et al., 2019). Os hidrogéis têm sido amplamente aplicados para fins biomédicos inclusive como

scaffolds para medicina regenerativa e como transportadores para administração de medicamentos - devido à sua estrutura de rede reticulada e foram modificados de várias maneiras para imitar o ambiente da matriz extracelular nativa (Goto et al., 2021).

Os hidrogéis demonstraram seu bom desempenho como carreador celular em diferentes doenças pulmonares (Li et al., 2021). Apesar de suas boas propriedades, existem algumas preocupações sobre sua esterilidade. Presume-se que durante a preparação da matriz extracelular (MEC) descelularizada existam detergentes e ácidos agressivos que limitam a proliferação bacteriana (Cao et al., 2021). Além disso, a digestão do pó da MEC descelularizada necessária para obter o hidrogel é feita em pH alto e com pepsina e esse processo termina em uma proteólise capaz de matar a maioria das bactérias (Falcones et al., 2021). Porém este é um procedimento não-estéril, pois trata-se de um processo asséptico e seria necessário desenvolver um protocolo estéril que permita ao hidrogel ser totalmente livre de contaminação bacteriana, que permaneça por um longo período de tempo sem apresentar crescimento bacteriano e que não afete a sua capacidade de gelificação nem sua estrutura tridimensional (Li et al., 2021).

O processo de esterilização é fator determinante para que hidrogéis, possam ser submetidos a qualquer procedimento, livres e suprimidos de qualquer risco de contaminação. De fato, o potencial de transmissão de infecções bacterianas e virais, tais como HIV e hepatite C tem sido relatada em aplicações de engenharia de tecidos (Jankowska et al., 2020). Para este fim, é muito importante considerar que os métodos de esterilização agressivos, que asseguram a eliminação completa de agentes patogénicos, podem também deteriorar os componentes estruturais no tecido, especificamente em seu desempenho mecânico (Oliveira et al., 2021).

A esterilidade dos hidrogéis é uma preocupação para algumas aplicações (adesivos/instilação) que precisa ser resolvida (Galdoporpora et al., 2019). Diferentes técnicas de esterilização têm sido usadas com pouco ou nenhum sucesso para hidrogéis de MEC decelularizada, pois têm um efeito negativo nas propriedades do hidrogel (Cao et al., 2021). O carregamento de vários agentes

antibacterianos por hidrogéis é uma estratégia eficiente para aumentar o efeito antimicrobiano. Para evitar o surgimento de bactérias resistentes a drogas, estratégias fototerapêuticas têm sido amplamente utilizadas nas aplicações antibacterianas, como a utilização de hidrogéis carregados com riboflavina + irradiação de diodo emissor de luz (LED) 405nm (Kingsley et al., 2018).

A rigidez dos hidrogéis derivados de MEC é menor que a rigidez do tecido nativo, mas pode ser modificada por processos químicos, como genipina ou fotorreticulação (Li et al., 2021). No entanto, algumas dessas intervenções podem ser potencialmente tóxicas para células ou alterar sua resposta, bem como modificar a estrutura e composição do scaffold. Uma alternativa é utilizar a (TFD) com riboflavina na produção do hidrogel MEC, pois está diretamente relacionada à rigidez do scaffold resultante e este protocolo ainda apresenta vantagens de obter um hidrogel com propriedades estéreis (Du et al., 2023). Em um futuro próximo, hidrogéis antibacterianos multifuncionais e inteligentes, projetados de acordo com a necessidade real, fornecerão amplas perspectivas para terapia de infecção antibacteriana e reconstrução de tecidos simultaneamente (Moura et al., 2024).

Os campos da terapia fotodinâmica antimicrobiana (aPDT) utilizando plataformas de hidrogéis e da engenharia de tecidos pulmonares representam a vanguarda da medicina regenerativa e do controle de infecções. Essas áreas abordam necessidades clínicas críticas, desde o combate a patógenos resistentes a antibióticos como o *Staphylococcus aureus* até a superação da escassez de órgãos para transplante por meio de construções bioengenheiradas (Wang et al., 2025). As revisões sistemáticas fundamentais fornecidas estabelecem a compreensão básica desses domínios até 2024, destacando a promessa dos hidrogéis como veículos de entrega para fotossensibilizadores em aplicações antimicrobianas e as complexidades dos arcabouços de matriz extracelular (MEC) na regeneração de órgãos.

2. HIPÓTESE

O desenvolvimento de um hidrogel baseado em matriz extracelular descelularizada (dECM) pode resultar em uma plataforma biocompatível e funcional para aplicações em medicina regenerativa e terapia de repara tecidual.

3. OBJETIVOS

3.1 Objetivo principal

Desenvoler de um hidrogel baseado em matriz extracelular descelularizada (dECM).

3.2 Objetivos específicos

- Obter um hidrogel de MEC de pulmão de porco descelularizado;
- Verificar e quantificar a presença de DNA nos hidrogéis obtidos.

4. MATERIAIS E MÉTODOS

Este estudo foi realizado em três etapas. Inicialmente foi publicada uma revisão sistemática sobre Hidrogéis, onde pode-se obter a base para a próxima etapa que foi a produção de hidrogel a partir de uma técnica de descelularização de tecidos pulmonares praticada na Universidade Evangélica de Goiás, complementada com um estágio de pesquisa na Unidade de Biofísica e Bioengenharia do Departamento de Biomedicina da Faculdade de Medicina e Ciências da Saúde da Universidade de Barcelona, onde processei a matriz extracelular de pulmão de porco para a obtenção do hidrogel.

Finalmente, na última etapa, foi publicada outra revisão sistemática sobre matriz extracelular (MEC) de scaffolds pulmonares submetidos a diferentes meios de esterilização. Demonstrando que ainda não se tem um padrão ouro para produção de hidrogeis de dMEC que seja estéril e que não danifique as estruturas e os tecidos envolvidos.

4.1 Caracterização do estudo

Trata-se de estudo controlado experimental animal, desenvolvido no Laboratório de Cultura de Células do Programa de Pós-graduação Mestrado e Doutorado em Movimento Humano e Rebilitação – PPGMHR e no Laboratório de Bioengenharia do Departamento de Ciências Fisiológicas da Faculdade de Medicina da Universidade de Barcelona, Espanha.

4.2 Aspectos Éticos e Legais

O projeto do estudo foi aprovado pela Comissão de Ética no Uso de Animais em Pesquisa (CEUA) da Universidade Evangélica de Goiás - UniEVANGÉLICA, sob protocolo de número 006/2020**.** O procedimento foi aprovado pelo Conselho de Ética em Pesquisa Animal da Universidade de Barcelona, em conformidade com os regulamentos regionais, nacionais e europeus.

4.3 Descelularização Pulmonar Suína

Pulmões suínos foram obtidos de um matadouro local e descelularizados seguindo o protocolo de (Falcones et al., 2021) com pequenas adaptações: resumidamente, os pulmões foram perfundidos através da traqueia e da vasculatura com 0,1% Triton X-100, desoxicolato de sódio, DNAse e cloreto de sódio 1M, com perfusão intermediária com água destilada e tampão fosfato-salino do inglês "*phosphate buffered saline* (PBS)" para fins de enxágue. Para verificar a ausência de DNA celular após o processo de descelularização, foi utilizada coloração de fluorescência com 6-diamidino-2-fenilindol (DAPI). Amostras de cada pulmão descelularizado foram imersas em composto de temperatura de corte óptico (OCT, Sakura), congeladas a−80◦C, e posteriormente criosseccionados em 15µm fatias (Cryomicrótomo HM 560, ThermoFisher Scientific, Waltham, MA, EUA). As crioseções foram enxaguadas com PBS para remover a OCT e depois mantidas por 10 min com 1 mg/mL de solução DAPI para coloração. Para avaliar a eficácia da descelularização, o DNA genômico total foi isolado usando o kit PureLink Genomic DNA (ThermoScientific, Waltham, MA, EUA) de scaffolds nativos e descelularizados (n = 4 para cada pulmão descelularizado) seguindo as instruções do fabricante. A quantidade total de DNA foi quantificada usando espectrofotometria e normalizada para o peso seco do tecido da amostra.

4.4 Protocolo da descelularização de pulmão suíno

a. Primeira etapa - Descelularização do pulmão

b. Materiais

- Pulmões suínos
- Tesouras y Grampos
- Bombas (uma por pulmão)
- Bandeja (uma por pulmão)
- Balde com tampa (um por pulmão)
- Recipientes de resíduos

c. Reagentes

Todos os reagentes de perfusão devem estar a 4°C. Como o volume de água MilliQ necessário durante o processo é alto, colete-a nos dias anteriores em recipientes plásticos com tampa preta para não esvaziar o depósito de água MilliQ.

- Água de MilliQ: 10l para cada etapa de limpeza/enxágue
- Triton 0,1%: volume total 10L → 10 ml de Triton 100X em 10L de água de MilliQ
- SDC 2%: volume total 10L → 200g de SDC em 10L de água de MilliQ
- NaCl 1M: volume total 10L → 584.4 g NaCl em 10L de água de MilliQ
- Solução de DNAse: volume total 10L → 300 mg de DNAse + 1.565g de MgSO4 + 2.22 g de CaCl2 em 10L de água de DI.
- PBS 1X: volume total 5 → 0.5L de PBS10X em 4L de água de MilliQ.

d. Primeiro dia

1. Retire o pulmão do freezer -80°C no dia anterior à descelularização e deixe-o descongelar a 4°C.
2. Coloque o pulmão em uma bacia de plástico e remova o pulmão direito conservando o brônquio principal e disseque a vasculatura.
3. Pince a vasculatura e as vias aéreas previamente conectadas ao pulmão direito para criar um sistema fechado para descelularização da perfusão do pulmão esquerdo (Figura 1).
4. Perfundir o pulmão com 1,5L de água MilliQ através da traqueia e 1L através da vasculatura. Repita o procedimento 3 vezes. Entre cada enxágue, a solução é removida passivamente, impulsionada pela elasticidade do tecido e auxiliada com uma massagem suave.
5. Perfundir 1,5L de solução de Triton 0,1% pela traqueia e 1L pela vasculatura.

6. Encha um balde com solução de Triton 0,1%, mergulhe o pulmão e incube por 24h a 4°C.

e. Segundo dia

7. Enxágue o pulmão 3X com água MilliQ. Ajude a remover a água fazendo uma pressão suave no pulmão.

8. Perfunda 1,5L de solução SCD 2% pela traqueia e 1L pela vasculatura.

9. Encha um balde com solução SCD 2%, mergulhe o pulmão e incube por 24h a 4°C.

f. Terceiro dia

10. Enxágue o pulmão 3X com água MilliQ. Ajude a remover a água fazendo uma pressão suave no pulmão.

11. Perfunda 1,5L de NaCL 1M pela traqueia e 1l pela vasculatura e incube

12. Encha um balde com NaCL 1M, mergulhe o pulmão e incube por 1h a 4°C.

13. Enxágue o pulmão 3X com água MilliQ. Ajude a remover a água fazendo uma pressão suave no pulmão.

14. Perfunda 1,5L de solução de DNAse pela traqueia e 1L pela vasculatura.

15. Encha um balde com solução de DNAse, mergulhe o pulmão e incube por 1h a 4°C.

16. Enxágue o pulmão 2X com PBS1X. Ajude a remover a água fazendo uma pressão suave no pulmão.

17. Enxágue o pulmão 3X com água MilliQ. Ajude a remover a água fazendo uma pressão suave no pulmão.

18. Corte o pulmão em cubos de aproximadamente 1cm e remova todas as vias aéreas cartilaginosas (bronquíolos, alvéolos, etc.). Coloque os pedaços em uma placa de Petri (Figura 2).

19. Armazene a placa de Petri devidamente fechada a -80°C.

Figura 2. Resumo do processo de descelularização. Etapas de enxágua por via aérea e via vascular; processo resumido de 3 dias.

4.5 Preparação de hidrogéis de matriz extracelular pulmonar (L-MEC)

Todos os reagentes foram adquiridos da Sigma-Aldrich, St. Louis, MO, EUA ou ThermoFisher Scientific, Waltham, MA, EUA, a menos que especificado de outra forma. Os hidrogéis L-MEC otimizados para cultura L-MSC foram obtidos descelularizando pulmões suínos, a partir de MEC pulmonar descelularizado (Figura 3).

Figura 3. Resultado da descelularização do pulmão de porco para a obtenção da matriz extracelular descelularizada.

g. Segunda etapa - Liofilização

- Gelo
- Tubos falcons
- Liofilizador

Retire os pedaços de pulmão do freezer -80°C, transfira-os para tubos falcons e coloque-os dentro de uma caixa com gelo, até chegar ao Liofilizador.

Adicionar os tubos ao equipamento Liofilizador, seguindo as instruções contidas no aparelho.

Deixe os pedaços de pulmão no Liofilizador de 3-4 días. Retire-os e transfira-os para os tubos falcons e armazene-os, para posteriormente coloca-los no Molinizador.

Os pedaços de pulmões descelularizados foram fatiados e congelados no refrigerador −80◦C, posteriormente foram liofilizados utilizando o Liofilizador Telstar Lyoquest-55 Plus, Terrassa, Espanha.

h. Terceira etapa – Molinização

- Molinizador
- Falcon de 50 com base
- Tesoura
- Espátula
- Parafilme
- N2 líquido

Coloque os pedaços de pulmão de porco no tubo de moagem do moinho. Não exceda a capacidade máxima recomendada do equipamento.

Feche o tubo de moagem e inicie o processo. O moinho criogênico utiliza batidas rápidas e temperaturas extremamente baixas para triturar a amostra.

Selecione I en el cryomilding se deja 5 minutos por ciclo

Após a moagem, abra o tubo e colete o pó resultante. Utilize uma espátula ou outro instrumento adequado para transferir a amostra para um recipiente apropriado.

Para este experimento os pedaços de pulmão foram pulverizados em partículas micrométricas a −180°C usando um moinho criogênico (6755, SPEX, Metuchen NJA) por 5 min na velocidade máxima.

i. Quarta etapa - Digestão

Material:

- 50ml Falcon com base
- Pipetas de 5ml
- Ímã (fita laranja na caixa de classificação)
- Agitador magnético
- HCl 0,01M
- Pepsina.
- Vf= 5ml para cada 100mg de pó
- Pepsina 1:10 em relação ao pó

Protocolo:

1. Pegue o pó pulmonar do freezer -80 e deixe-o em temperatura ambiente. Não abra o falcon até que esteja em temperatura ambiente. Somente tire a Pepsina da geladeira quando for utilizá-la.
2. Adicione a um falcão de 50 ml com base:
    - 10mg pepsina (após o uso vedar com Parafilm e conservar a -20ºC)
    - 100mg pó (após o uso selar com Parafilm e conservar a -80 ºC)
    - 5ml de HCl 0,1M e pipetar
3. Adicione o ímã ao falcão e coloque no agitador magnético a 400rpm por 16h (Figura 4, Figura 5).

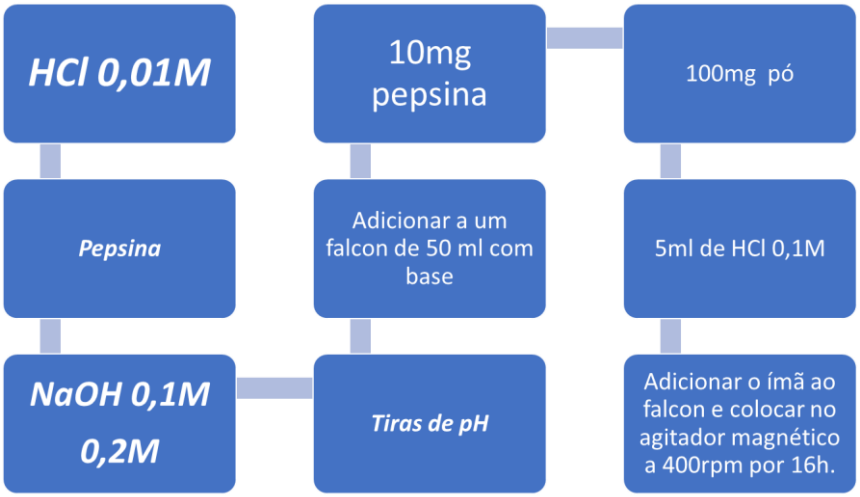

Figura 4. Resumo do processo de Digestão do pó da MEC do pulmão de porco para a solubilização do tecido e produção do hidrogel.

Figura 5. Materiais utilizados durante o processo de digestão.

j. Quinta etapa - Neutralização

Trabalhe na capela de cultura de células do 3º andar com reagentes estéreis.

O procedimento deve ser rápido, pois as amostras gelificam após a neutralização.

Material:

- Gelo
- Eppendorfs
- NaOH 0,1M e 0,2M
- PBS 10X
- Tiras de pH (na gaveta da sala de cultura de células com as câmaras de neubauer)
- Centrifugar

Protocolo:

1. Ligue a centrífuga e defina o programa 7 para atingir uma temperatura de 4ºC.
2. Coloque no gelo:
   - A digestão
   - NaOH 0,1 M
   - NaOH 0,2 M
   - PBS 10X
   - Os eppendorfs que você vai usar (5-6)

3. Adicione à digestão:

   a) 555ul de PBS 10X, leve para o vórtex por alguns segundos, para fazer a homogeneização e depois mantenha no gelo.
   b) 250ul NaOH 0,2M, leve para o vórtex por alguns segundos, para fazer a homogeneização e depois mantenha no gelo.
   c) 85ul de NaOH 0,1M, leve para o vórtex por alguns segundos, para fazer a homogeneização e depois mantenha no gelo.

4. Verifique o pH com 5ul da neutralização em uma tira de pH (pH=7,4). Se o pH estiver alto, adicione HCl 0,01M e leve para o vórtex para homogeneização e verifique o pH novamente, até ele ficar na cor referência.

5. Faça alíquotas de 1ml do hidrogel e centrifugue a neutralização usando o programa 4 para eliminar as bolhas (use-as imediatamente ou guarde-as a -80ºC)

7. Para gelificar o hidrogel:

a) Coloque o ml desejado em um poço ou lâmina de vidro e incube a 37 ºC por 45min (Figura 6, figura 7).

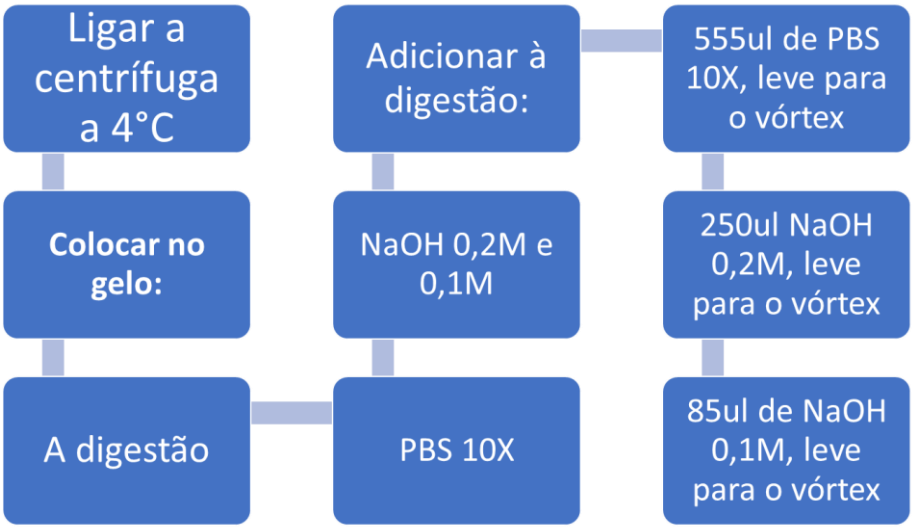

Figura 6. Etapa 1 do processo Neutralização de solução de hydrogel.

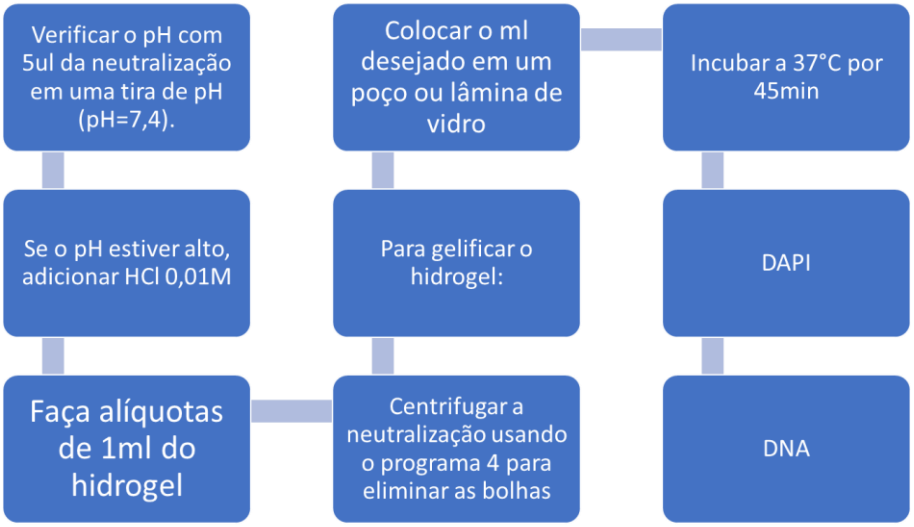

Figura 7. Etapa 2 do processo Neutralização de solução de hydrogel e análises de DNA remanescente.

4.4 Procedimento da produção de L-MEC

Pulmões descelularizados foram fatiados e congelados em −80◦C, liofilizados (Telstar Lyoquest-55 Plus, Terrassa, Espanha) e pulverizados em partículas micrométricas a −180◦C usando um moinho criogênico (6755, SPEX, Metuchen NJA) por 5 min na velocidade máxima. O pó resultante foi digerido na concentração de 20 mg/mL em solução de HCl 0,01 M (pH = 2) com pepsina de mucosa gástrica suína (concentração 1:10) sob agitação magnética em temperatura ambiente por 16 h. A solução resultante (pré-gel) foi ajustada para pH 7,4(±0,4) usando NaOH 0,1 M e PBS 10X e congelado a −80◦C para uso posterior (Figura 8).

Figura 8. Resultado da neutralização da amostra evidenciado o pH dentro do especificado.

5. RESULTADOS

5.1 Estudo 1. Hidrogéis associados à terapia fotodinâmica apresentam efeito antimicrobiano contra Staphylococcus aureus: Uma revisão sistemática

gels

MDPI

Systematic Review

Hydrogels Associated with Photodynamic Therapy Have Antimicrobial Effect against *Staphylococcus aureus*: A Systematic Review

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Abstract: *Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that causes infections ranging from mild superficial cases to more severe, potentially fatal conditions. Many photosensitisers used in photodynamic therapy are more effective against superficial infections due to limitations in treating deeper tissue infections. Recently, attention to this bacterium has increased due to the emergence of multidrug-resistant strains, which complicate antibiotic treatment. As a result, alternative therapies, such as antimicrobial photodynamic therapy (PDT), have emerged as promising options for treating non-systemic infections. PDT combines a photosensitiser (PS) with light and oxygen to generate free radicals that destroy bacterial structures. This systematic review evaluates the effectiveness of PDT delivered via different types of hydrogels in treating wounds, burns, and contamination by *S. aureus*. Following PRISMA 2020 guidelines, a bibliographic search was conducted in PubMed, Web of Science, and Scopus databases, including articles published in English between 2013 and 2024. Seven relevant studies were included, demonstrating evidence of PDT use against *S. aureus* in in vitro and in vivo studies. We concluded that PDT can effectively complement antimicrobial therapy in the healing of wounds and burns. The effectiveness of this technique depends on the PS used, the type of hydrogel, and the lesion location. However, further in vivo studies are needed to confirm the safety and efficacy of PDT delivered via hydrogels.

Keywords: hydrogels; photodynamic therapy; antimicrobial effects; *Staphylococcus aureus*

1. Introduction

The rise of antimicrobial resistance in bacteria, viruses, fungi, and parasites presents a significant global challenge to human health and development [1]. The World Health Organisation recognises it as one of the main concerns for global public health, ranking among the top ten threats to humanity [1]. Inappropriate and excessive use of antimicrobial agents contributes to resistance, making practically all pathogenic microorganisms insensitive to the medications commonly used to control them. The rise in multidrug resistance to key antibiotic classes has led to a surge in hospital-acquired pathogens. These include

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and Enterobacter species, collectively referred to as the ESKAPE group [2]. Despite the complexity of developing new drugs with antibiotic properties [1], alternative approaches have been developed recently to control the spread of pathogenic microorganisms. One such approach is antimicrobial photodynamic therapy (PDT), which uses photosensitising agents (dyes) and hydrogels capable of absorbing light photons and elevating them to an excited state [3].

In recent years, the rise of drug-resistant bacteria has made existing antibiotics less effective, while the development of biofilms has further diminished their therapeutic impact [4]. The massive and abusive use of antibiotics has caused severe side effects, making it imperative to develop alternative, ultra-efficient, and safe antibacterial systems [4]. The most recent technique used to control microbial infections is the combination of PDT with a hydrogel, which appears innovative and operates non-specifically in microbial cells, thus preventing the development of resistance [5].

Photodynamic therapy (PDT) involves administering a non-toxic, light-sensitive photosensitiser (PS), which is then activated by exposure to light, typically using doses that are carefully adjusted to the clinical application and type of PS used. Upon activation by light, and in the presence of oxygen, the PS can undergo a series of reactions leading to the production of reactive oxygen species (ROS). These reactions include the transfer of electrons or hydrogen to nearby molecules, resulting in the formation of free radicals, such as superoxide radicals and hydroxyl radicals, or energy transfer to oxygen, producing singlet oxygen ($^1O_2$). Both pathways result in the generation of ROS that can induce cell death and destruction of diseased tissues [5].

Faced with the growing problem of resistance to microbial antibiotics, PDT delivered using hydrogels has attracted interest as an alternative antimicrobial treatment. Several in vitro and in vivo studies involving microbial inactivation with successful results for bacteria, fungi, yeasts, viruses, and parasites, and in the healing of wounds and burns have been conducted [6]. Furthermore, a recent study has demonstrated that photosensitisation of bacterial cells is independent of the spectrum of antibiotic resistance [5].

The photosensitiser (PS) must be selected so that the available light source provides photons at the appropriate absorption wavelength for effective activation [7]. The penetration of light through tissue can be hindered by dispersion and absorption, which depend on the wavelength of the incident light, the type of tissue, and how the PS is delivered to the area to be decontaminated. Hydrogels are excellent carriers of PSs for photodynamic therapy (PDT) [5]. Hydrogels have demonstrated good performances as cell carriers with different functionalities [8].

Hydrogels are three-dimensional polymer networks expanded with water, offering adjustable physicochemical properties that meet various needs across different conditions. These versatile materials have found extensive use in biomedicine, ranging from investigating physiological and pathological mechanisms to applications in tissue regeneration and disease therapy [9].

Hydrogels have been widely investigated as matrices for biomedical applications because of their crosslinking ability under mild conditions, excellent biocompatibility, and tunable biochemical and biophysical properties. As their structure and properties resemble the microenvironment of many tissues in the human body, they are widely used in various biomedical applications [10].

In summary, multifunctional and intelligent antibacterial hydrogels designed according to the actual needs can simultaneously provide broad prospects for antibacterial infection therapy and tissue reconstruction. This study aimed to conduct a systematic review of studies analysing the effectiveness of PDT delivered via hydrogels for treating infections caused by *S. aureus*.

2. Materials and Methods

2.1. Development

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [11] (Registration ID 586572). Bibliographic

research was conducted using the PubMed, Web of Science, and Scopus databases. Only full articles published in English between 2013 and 2024 from any country of origin (without restrictions) were included. The research was conducted from 15 January 2024 to 15 June 2024 and did not use any automatic bibliographic search tool.

2.2. Data Extraction Process

A two-step process was used to select studies. In the first phase, two reviewers (RSM and JPRA) independently screened the titles and abstracts to identify studies that met the eligibility criteria. Studies that satisfied these criteria were chosen for full-text review. In the second phase, the same reviewers independently assessed the full texts to confirm their inclusion. Any disagreements between the two reviewers were resolved through discussion with a third reviewer (LVFO), if needed.

For each selected database, a bibliographic search was performed using titles and abstracts, with keywords aligned with MeSH terms. The search strategy involved a predefined combination of keywords as follows (Table 1):

Table 1. Database and search strategies.

Database	Search Strategy	Results
PubMed	((((“Anti-Infective Agents” [Mesh] OR Anti Infective Agents OR Antiinfective Agents OR Anti-Infective Agent OR Anti Infective Agent OR Microbicides OR Anti-Microbial Agent OR Anti Microbial Agent OR Antimicrobial Agents OR Anti-Microbial Agents OR Anti Microbial Agents OR Microbicide OR Antimicrobial Agent) AND (“*Staphylococcus aureus*” [Mesh])) AND (“Gram-Positive Bacterial Infections” [Mesh] OR Gram Positive Bacterial Infections OR Gram-Positive Bacterial Infection)) AND (“Photochemotherapy” [Mesh] OR Photochemotherapies OR Photodynamic Therapy OR Photodynamic Therapies)) AND (“Hydrogels” [Mesh] OR Hydrogel OR In Situ Hydrogels OR In Situ Hydrogel OR Patterned Hydrogels OR Patterned Hydrogel)	09
Scopus	(“Hydrogels” OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Patterned Hydrogels” OR “Patterned Hydrogel”) AND (“Photochemotherapy” OR “Photochemotherapies” OR “Photodynamic Therapy” OR “Photodynamic Therapies”) AND (“Gram-Positive Bacterial Infections” OR “Gram Positive Bacterial Infections” OR “Gram-Positive Bacterial Infection”) AND (“*Staphylococcus aureus*”) AND (“Anti-Infective Agents” OR “Anti Infective Agents” OR “Antiinfective Agents” OR “Anti-Infective Agent” OR “Anti Infective Agent” OR “Microbicides” OR “Anti-Microbial Agent” OR “Anti Microbial Agent” OR “Antimicrobial Agents” OR “Anti-Microbial Agents” OR “Anti Microbial Agents” OR “Microbicide” OR “Antimicrobial Agent”)	25
Web of Science	((((ALL = (Anti-Infective Agents OR Anti Infective Agents OR Antiinfective Agents OR Anti-Infective Agent OR Anti Infective Agent OR Microbicides OR Anti-Microbial Agent OR Anti Microbial Agent OR Antimicrobial Agents OR Anti-Microbial Agents OR Anti Microbial Agents OR Microbicide OR Antimicrobial Agent)) AND ALL = (*Staphylococcus aureus*)) AND ALL = (Gram-Positive Bacterial Infections OR Gram Positive Bacterial Infections OR Gram-Positive Bacterial Infection)) AND ALL = (Photochemotherapy OR Photochemotherapies OR Photodynamic Therapy OR Photodynamic Therapies)) AND ALL = (Hydrogels OR Hydrogel OR In Situ Hydrogels OR In Situ Hydrogel OR Patterned Hydrogels OR Patterned Hydrogel)	03

“Hydrogels” [Mesh] OR Hydrogel OR In Situ Hydrogels OR In Situ Hydrogel OR Patterned Hydrogels OR Patterned Hydrogel “Photochemotherapy” [Mesh] OR Photochemotherapies OR Photodynamic Therapy OR Photodynamic Therapies “Gram-Positive Bacterial Infections” [Mesh] OR Gram Positive Bacterial Infections OR Gram-Positive Bacterial Infection “*Staphylococcus aureus*” [Mesh]”Anti-Infective Agents” [Mesh] OR Anti Infective Agents OR Antiinfective Agents OR Anti-Infective Agent OR Anti Infective Agent OR Microbicides OR Anti-Microbial Agent OR Anti Microbial Agent OR Antimicrobial Agents OR Anti-Microbial Agents OR Anti Microbial Agents OR Microbicide OR Antimicrobial Agent.

All the titles were manually searched and reviewed for inclusion. Reference lists of the articles containing titles, author names, languages, and publication dates were generated. This systematic review included only scientific articles reporting experimental studies.

2.3. Election Criteria

2.3.1. Design and Interventions

This review examined controlled experimental laboratory studies that employed PDT in conjunction with different hydrogels to combat antibiotic-resistant microorganisms, particularly *S. aureus*. Both in vitro and in vivo experimental models were considered, including

research conducted on animals and studies that investigated the effects of PDT on cells without the use of animals.

2.3.2. Methodological Design

For this scoping review, we queried PubMed (using MeSH), Web of Science, and Scopus databases to identify relevant articles. We used specific search criteria, including keywords such as *Staphylococcus aureus*, photodynamic therapy, photosensitiser, Gram-positive, hydrogel, and antibiotic resistance. The inclusion and exclusion criteria were applied to ensure the relevance of the articles. We included studies that explored the photodynamic activity associated with antimicrobial effects against *S. aureus*, both in vitro and in vivo, considering its clinical applications and synergy with other antimicrobial agents. We excluded unpublished research, studies published before 2013, studies that did not mention the PS used, those that did not involve *S. aureus*, those that did not use hydrogels in PDT, and those that were not clinically relevant to the research. We followed the PRISMA guidelines, which allowed for a systematic approach to article selection. After selecting the studies, data were extracted to ensure consistency in the inclusion and exclusion criteria.

3. Results

3.1. Study Description

The initial search found 37 articles, which, after being subjected to the evaluation criteria, left only seven articles that addressed the topic of this study (Figure 1). Table 2 summarises each article and its particularities.

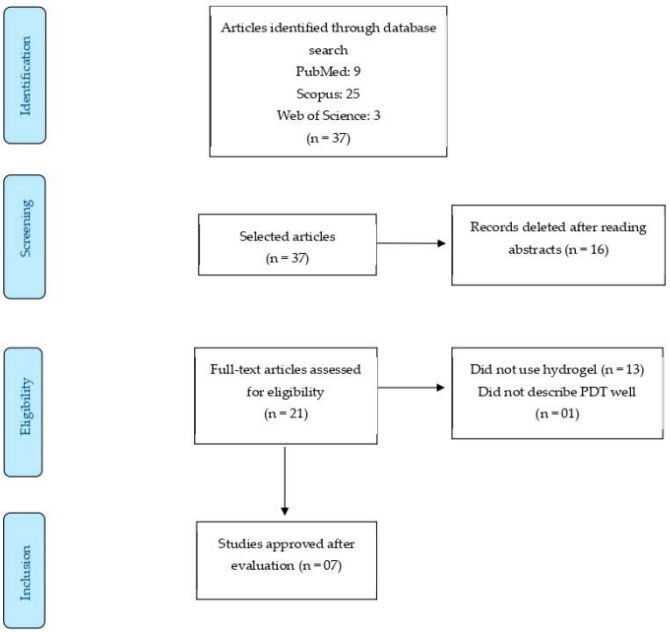

Figure 1. Flow diagram of the current systematic review conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.

Table 2. Information from each study.

Authors	Country	Methods	What Was Analysed?	Conclusion
Chen, C. P., et al., 2015 [12]	Taiwan	Toluidine blue O (TBO) hydrogel = TBO + chitosan + HPMC. Irradiation = 100 J/cm^2 at 635 ± 20 nm.	The photodynamic efficacy of the hydrogel was tested in vitro against *Staphylococcus aureus* biofilms. Confocal laser scanning microscopy was used to assess the penetration of TBO into viable solutions. Adding HMPC improved the physicochemical properties of the chitosan hydrogel, such as hardness, viscosity, and bioadhesion; however, higher HMPC concentrations led to a decreased antimicrobial effect.	The ideal bioadhesive formulation for topical antimicrobial PDT will need to balance the desired drug release rate and the mechanical properties of the formulation, as these factors influence clinical effectiveness and ease of application. The penetration of the TBO biofilm depends on the physicochemical properties of the HTC hydrogel.
Liang, H., et al., 2017 [13]	China	TBO as photosensitiser. TBO hydrogel or light alone (630 nm) showed no antibacterial activity against *S. aureus*.	A new TBO hydrogel was developed for periodontitis treatment, utilising carbomer as the base and NaOH as the neutraliser. TBO hydrogel formulations have been employed as on-demand drug delivery systems for clinical treatments. The antibacterial activity of PDT using TBO hydrogel was tested against *S. aureus*. These TBO hydrogel formulations were optimised using response surface methodology.	A TBO hydrogel was developed for photodynamic therapy against *S. aureus*, yielding better results than PDT with an aqueous TBO solution. The hydrogel released 50% of TBO within 4 h and 68.26% within 24 h. Over six weeks, the TBO hydrogel maintained consistent colour, transparency, pH, and viscosity when stored at 4 °C, 25 °C, and 40 °C. The hydrogel or light alone showed no antimicrobial effect on *S. aureus*; only the combination of light with the TBO hydrogel exhibited antibacterial activity.
Mao, C., et al., 2017 [14]	China	Hydrogel = Ag/Ag@AgCl/ZnO nanostructures. Method = UV light reduction, ZnO added via NaOH precipitation. Irradiation = 300 W xenon lamp (visible light).	A hydrogel composite incorporating carboxymethyl cellulose and Ag/Ag@AgCl/ZnO hybrid nanostructures was developed. This composite demonstrates outstanding photocatalytic activity and broad antibacterial efficacy against Gram-positive bacteria when exposed to visible light.	Leveraging the generation of reactive oxygen species, the system demonstrated significantly improved photocatalytic activity, extensive antibacterial effects against *S. aureus* (a Gram-positive bacterium), and accelerated wound healing. The hydrogel system featured controlled and sustained release of Ag^+ and Zn^{2+}, facilitated by reversible swelling and shrinking in response to pH changes, highlighting its substantial potential for tissue repair and antibacterial applications.

Table 2. *Cont.*

Authors	Country	Methods	What Was Analysed?	Conclusion
Zheng, Y., et al., 2019 [5]	China	Laser = 630 nm diode (5 mW, 4 mW/cm^2, 23 mm periotip). Photosensitiser = toluidine blue.	In vitro antibacterial tests against *S. aureus* utilised response surface methodology to optimise the TBO hydrogel formulation. The stability, pH, and antibacterial activity of the TBO hydrogel remained consistent at 4 °C, 25 °C, and 40 °C over a 6-week period. Additionally, the TBO combined with a carbomer hydrogel demonstrated release rates of 51.28% after 4 h and 69.80% after 24 h.	The optimal TBO hydrogel formulation consisted of 0.5% (*w*/*v*) carbomer 934, a TBO concentration of 0.01 mg/mL, 0.5% (*v*/*v*) ethanol, 0.5% (*v*/*v*) Tween 80, and a NaOH to carbomer mass ratio of 0.4 (*w*/*v*). The hydrogel's properties, including appearance, clarity, viscosity, antibacterial activity, and pH, remained stable at 4 °C, 25 °C, and 40 °C for up to 6 months. It effectively inhibited Propionibacterium acnes, *S. aureus*, and *Escherichia coli*. These results indicate that the new TBO hydrogel is promising for acne treatment, with further studies needed to assess cellular toxicity and conduct animal trials.
Du, P., et al., 2023 [8]	China	Hydrogel = G-quartets + riboflavin (photocatalyst). Function = photodynamic antibacterial therapy with H_2O_2 production. Irradiation = 450 nm, emission recorded from 500–600 nm.	A photoactive supramolecular material based on G-quartets was developed. This material is self-assembled from guanosine (G) and 4-formylphenylboronic acid/1,8-diaminooctane, with riboflavin incorporated as a photocatalyst into the G4 nanowires for post-irradiation photodynamic antibacterial therapy. The G4 materials, which exhibit hydrogel-like properties, act as a scaffold for the riboflavin and guanosine reductant, facilitating the photo-triggered production of therapeutic H_2O_2.	Supramolecular G4 materials loaded with riboflavin, exhibiting gel-like properties, were demonstrated as a proof-of-concept for post-irradiation antibacterial therapy of infected wounds. These G4 hydrogels acted as dressing materials, structurally incorporating riboflavin through covalent bonding and aromatic stacking, while providing guanosine as a reductant to reduce photoexcited riboflavin and facilitate O_2 reduction to generate H_2O_2. The hydrogels, after irradiation, showed strong antibacterial activity, effectively killing Gram-positive bacteria, Gram-negative bacteria, and multi-drug-resistant bacteria both in vitro and in vivo, with biosafety and no significant cytotoxicity. The riboflavin-loaded G4 hydrogels achieved a sterilisation rate greater than 99.999% against *S. aureus*, *E. coli*, and methicillin-resistant *S. aureus*, and demonstrated excellent antibacterial efficacy in infected rat wounds.

Table 2. *Cont.*

Authors	Country	Methods	What Was Analysed?	Conclusion
Elkihel, A., et al., 2023 [15]	France	Hydrogel = Xylan-TCPP with varying PS/Xylan ratios. Irradiation = white LED light for 5 h (25 J/cm^2). Photosensitiser = meso-TCPP.	The antimicrobial activity of the hydrogels was evaluated under visible light irradiation against two strains of Gram-positive bacteria, *S. aureus* and *Bacillus cereus*. The preliminary results demonstrated notable effectiveness against these bacteria, suggesting that these hydrogels hold significant potential for treating bacterial skin infections of these species using photodynamic antimicrobial chemotherapy.	Xylan-based hydrogels containing photosensitisers were developed using TCPP as a crosslinker. Swelling tests revealed that the xyl-TCPP-3 hydrogel, which contained the smallest amount of TCPP, exhibited favourable swelling properties. Preliminary antibacterial tests against two strains of Gram-positive bacteria indicated that this hydrogel showed photodynamic activity only when exposed to light. The covalent attachment of TCPP to the Xylan component appears to reduce the photosensitiser's toxicity in the absence of light. However, the concentration of TCPP required for effective photosensitisation seems to be higher than what is typically reported in the literature.
Zheng, Y., et al., 2023 [16]	China	Hydrogel = Au25Capt18 in carrageenan. Function = dual-mode antibacterial effects (PTT + PDT). Irradiation = NIR light at 808 nm.	Natural polysaccharide carrageenan embedded in atomically precise gold nanoparticles was reported as a novel hydrogel platform for PTT and PDT antibacterial therapy triggered using single infrared light.	Atomically precise gold nanocluster-embedded hydrogels were developed by crosslinking Au25Capt18 with carrageenan, serving as an effective photothermal and photodynamic agent for antibacterial applications with single near-infrared (NIR) laser irradiation. The contribution of photothermal therapy (PTT) to antibacterial efficacy was found to be more substantial than that of photodynamic therapy (PDT) in the Au25Capt18 hydrogels. In vivo studies demonstrated that these hydrogels could effectively eliminate pathogenic bacteria and promote the healing of infected wounds.

3.2. Characteristics and Results of Individual Studies

3.2.1. Xylan–Porphyrin Hydrogels as Light-Triggered Gram-Positive Antibacterial Agents

This study describes the synthesis and characterisation of Xylan-based hydrogels containing a PS (tetra(4-carboxyphenyl) porphyrin; TCPP) for in vitro bacterial photoinactivation. The main results and conclusions are as follows. Hydrogels were prepared by crosslinking Xylan with TCPP using N, N′-carbonyldiimidazole as a coupling agent. Different amounts of TCPP were used to synthesise the hydrogels [15].

Freeze-drying of the hydrogels affected their ability to swell in water, and shrinkage promoted mutual hydrophobic interactions, making it difficult for water to re-enter the hydrogel structure. Hydrogels containing lower amounts of porphyrins (TCPP) exhibited better swelling properties than those containing higher amounts. Covalent bonding between TCPP and Xylan was confirmed through Fourier transform infrared (FTIR) spectroscopy [17]. In vitro bacterial photoinactivation tests showed that the hydrogel functionalised with TCPP exhibited antibacterial activity only under light irradiation and was more effective against Gram-positive bacteria. The concentration of TCPP in the hydrogel affects the antibacterial activity, and further research is needed to optimise the concentration of the PS [15].

This research demonstrates the successful synthesis of TCPP-containing Xylan-based hydrogels that show potential for bacterial photoinactivation applications, particularly against Gram-positive bacteria. However, the optimal concentrations of TCPP and other parameters need to be determined.

3.2.2. Optimisation and Evaluation of a Chitosan/Hydroxypropylmethylcellulose Hydrogel Containing Toluidine Blue for Antimicrobial Photodynamic Inactivation

This study by Brown et al. (1993) describes the formulation and characterisation of hydrogels containing chitosan (HCT) as potential agents for PDI against *S. aureus* and *P. aeruginosa* biofilms. Hydroxypropylmethylcellulose (HPMC) was used as a gelling agent, and the effects of different concentrations of HPMC on the physical and textural properties of hydrogels, including viscosity, hardness, adhesiveness, and compressibility, were evaluated [18]. Chen et al. (2015) showed that increasing the concentration of HPMC in the HCT resulted in greater viscosity, hardness, adhesiveness, and compressibility. The hydrogel with 1% HPMC exhibited textural properties similar to those of a commercial gel. However, the injectability of the hydrogels decreased as the concentration increased [12]. The efficacy of PDI against *S. aureus* and *P. aeruginosa* biofilms was evaluated, and the results show that HCT with low concentrations of HPMC (F-1 and F-2) were effective, similar to those of the mixture of toluidine blue O (TBO) and chitosan. However, increasing the HPMC concentration reduced the effectiveness of PDI, probably due to the restriction of TBO release in the hydrogels with high viscosity. Furthermore, another study investigated the penetration of TBO into biofilms and observed that increasing the concentration of HPMC restricted the penetration of TBO into the deeper layers of the biofilms [19]. In an in vivo study, HCT were tested in a burn model of rat skin infected with *S. aureus*, and the results show a significant reduction in the survival of bacterial cells. However, the effectiveness of this treatment decreased as the HPMC concentration in the hydrogel increased. Overall, this study highlights the importance of HPMC concentration in the formulation of hydrogels for PDI and the need to optimise textural properties and treatment efficacy [12].

3.2.3. Hydrogen Peroxide (H_2O_2)-Supramolecular Material for the Treatment of Post-Irradiation Infected Wounds

This article presents a study on the production of H_2O_2 through a photocatalytic process mediated by riboflavin and its use for antibacterial purposes. The research is based on several steps and discoveries, as described below:

1. Photocatalytic Process and H_2O_2 Production: Riboflavin was used as a photocatalyst and had a strong absorption peak around 460 nm. After irradiation with blue light,

riboflavin was excited and rapidly converted into a triple state with a high oxidation potential, generating H_2O_2. The amount of H_2O_2 was quantified by monitoring the changes in the absorbance at 652 nm [20].

2. Choice of Guanosine: Among the nucleotides derived from guanine, guanosine generates the most substantial amount of H_2O_2 owing to hydrogen bonds and stacking interactions with riboflavin. Because of the differences in their oxidation potentials, guanosine produces more H_2O_2 than adenosine, uridine, or cytidine [21].
3. G4 Supramolecular Materials: Guanosine was used to develop G4 supramolecular materials, which were formed into nanofibres and crosslinked using 4-formylphenylboronic acid and 1,8-diaminooctane. The properties of these materials were characterised using techniques such as electrospray mass spectrometry and FTIR spectroscopy [8]. It is very interesting to highlight the role of guanosine in the formation of G-quartets, which are essential for the structural integrity and function of certain photoactive materials. These G-quartets, in combination with riboflavin, facilitate the production of ROS, including H_2O_2, which increases the antibacterial efficacy of the treatment.
4. Controlled H_2O_2 Production: The amount of H_2O_2 generated can be controlled by varying the riboflavin concentration and irradiation time. This system maintains its robustness even after irradiation.
5. Antibacterial Activity: The H_2O_2 generated was used to test the antibacterial activity. The post-irradiation riboflavin-loaded hydrogel effectively killed Gram-positive, Gram-negative, and multidrug-resistant bacteria with a sterilisation rate of over 99.999%. Incubation with the catalase inhibited the antibacterial activity [22].
6. In Vivo Assays: The study included in vivo assays using an MRSA-infected rat wound model. The post-irradiation hydrogel exhibited a significant therapeutic effect by eliminating wound infections and reducing the levels of typical inflammatory factors. This study demonstrates the effectiveness of controlled riboflavin-mediated H_2O_2 production for antibacterial purposes with promising results both in vitro and in vivo [23].

3.2.4. Photo-Inspired Antibacterial Activity and Acceleration of Wound Healing by Hydrogel Incorporated with Ag/Ag@Siver Chloride (AgCl)/Zinc Oxide (ZnO) Nanostructures

This study presents the synthesis and characterisation of a nanocomposite hydrogel containing Ag/Ag@AgCl/ZnO. The hydrogel was obtained by absorbing water and opening pores in the gel structure. X-ray diffraction (XRD) results revealed changes in the hydrogel structure after doping with Ag/Ag@AgCl/ZnO, indicating the incorporation of these nanomaterials. The diffraction patterns showed peaks corresponding to the crystal planes of metallic Ag, AgCl, and ZnO [24]. The morphology of the pure hydrogel was similar to that of a sponge, with pores of approximately 10 µm in diameter. After doping with the Ag NPs and Ag@AgCl particles, the Ag NPs were uniformly distributed in the samples. The ZnO hydrogel incorporated one-dimensional ZnO nanostructures, such as nanorods and aggregates. The presence of Ag NPs in the ZnO nanostructures was confirmed using microscopy [25]. An analysis of the release of Ag and Zn ions from the hydrogels revealed different release profiles. The antibacterial activities of the hydrogels were evaluated in vitro, and the results showed significant effects against bacteria. Reactive oxygen species (ROS) formation was detected and was associated with enhanced antibacterial activity. In vivo studies demonstrated the therapeutic efficacy of hydrogels for wound healing, particularly in wounds infected with *S. aureus*. The Ag/Ag@AgCl/ZnO and pure ZnO hydrogels reduced bacterial infection and promoted wound healing [14].

This article introduces a novel approach for the synthesis of nanocomposite hydrogels that exhibit promising antibacterial properties. This study also highlights the potential of these hydrogels for wound healing, particularly in the context of infected wounds. The unique properties of these hydrogels, such as the controlled release of metal ions and generation of ROS, contribute significantly to their antibacterial and therapeutic efficacy.

3.2.5. Carrageenan Embedded in Atomically Precise Au Nanocluster for Single Infrared Light-Driven Photothermal and Photodynamic Antibacterial Therapy

The study addresses the synthesis and characterisation of a nanocomposite hydrogel composed of Ag, Ag@AgCl, and ZnO [26]. The main points of the study are as follows. The synthesis of the nanocomposite hydrogel involves the absorption of water by the carboxymethyl cellulose hydrogel, followed by doping with Ag/Ag@AgCl/ZnO. The materials were characterised via XRD to analyse their crystalline structures [27]. The results indicate the presence of metallic Ag, AgCl, and ZnO in the hydrogel structure. Transmission electron microscopy images showed the presence of cubic metallic Ag and Ag@AgCl nanostructures along with one-dimensional ZnO nanostructures [5]. The swelling behaviour of the hydrogel was investigated at different pH values, and it was found to be pH-sensitive [28]. The study also evaluated the release of Ag^{+} and Zn^{2+} ions from the nanocomposite hydrogel and its antibacterial activity in vitro. These results indicate that the nanocomposite hydrogel had antibacterial properties with different effects against *Escherichia coli* and *S. aureus* [29].

This study also evaluated the cytotoxicity of these materials in cell culture and their effectiveness in wound healing in an animal model. The results show that the nanocomposite hydrogel could potentially be used for the treatment of infections and wound healing. Overall, this study focused on synthesising and characterising a nanocomposite hydrogel, highlighting its antibacterial properties and potential for wound healing [5].

3.2.6. Optimisation of Hydrogel Containing Toluidine Blue for PDT in the Treatment of Acne

This study focused on optimising the formulation of a hydrogel containing the PS, TBO, for PDT to treat bacterial infections, particularly acne vulgaris. Four types of carbomers were used, namely, TBO, Tween 80, and other chemicals. PDT was performed on different types of bacteria, including *S. aureus*, *E. coli*, and *Propionibacterium acnes*, using the TBO hydrogel as a PS [26].

The results demonstrate that various factors, including the type of carbomer, carbomer concentration, TBO concentration, ethanol concentration, Tween 80 ratio, and mass ratio of NaOH to carbomer, significantly influenced the effectiveness of the TBO hydrogel in photodynamic therapy (PDT). The optimal hydrogel formulation was determined through optimisation experiments using response surface methodology (RSM) [30]. This formulation comprised 0.5% (w/v) carbomer 934, 0.01 mg/mL TBO, 0.5% (v/v) ethanol, 0.5% (v/v) Tween 80, and a 0.4 (c/c) mass ratio of NaOH to carbomer. This hydrogel exhibited excellent rheological properties, stability during storage, and antibacterial activity against the tested bacteria [5]. When compared with the optimal TBO hydrogel PDT and antibiotic therapy, the PDT showed promising results in reducing colony-forming units/mL. The release of TBO from the hydrogel was also evaluated, showing that the optimised formulation maintained its properties over time, in terms of pH and viscosity. This study shows that PDT with the optimised TBO hydrogel is a promising approach for treating bacterial infections, such as acne vulgaris, and may represent an effective alternative to traditional antibiotics [28]. The optimisation process involves adjusting parameters, such as light dose, photosensitiser concentration, and irradiation time, to maximise the loss of viable microorganisms while ensuring safety and minimal side effects.

3.2.7. Optimisation of Hydrogel Containing Toluidine Blue for PDT Using RSM

This study involved the preparation of hydrogels containing TBO and their application in the inactivation of *S. aureus* and *E. coli* through antimicrobial photodynamics (PDT) [31]. The materials used included carbomers, TBO, NaOH, and other reagents. The hydrogels were prepared by mixing the components and optimising the concentrations of carbomer, TBO, and the quality ratio between NaOH and carbomer using RSM [13].

The results show that the choice of carbomer type did not significantly affect the antimicrobial activity. However, the carbomer concentration influenced the activity, with very high concentrations impairing the diffusion of TBO in bacterial cells. The TBO

concentration also affected the activity, with a 0.1 mg/mL concentration chosen for further experiments. The ratio of NaOH to carbomer also influenced the antimicrobial activity, with a ratio of 0.4 found to be the most effective [13].

A single-factor and RSM experiment identified an ideal hydrogel formulation comprising 3% carbomer, 0.1 mg/mL TBO, and a quality ratio of NaOH and carbomer of 0.4. This formulation exhibited potent antibacterial activity against *S. aureus* and *E. coli*. This study also evaluated the stability of the hydrogel, which remained stable during 6 weeks of storage at different temperatures. Furthermore, the release of TBO from the hydrogel was monitored, and it showed gradual release over 6 h [32].

It is essential to highlight that the application of the TBO hydrogel or light alone did not result in significant antibacterial activity, highlighting the importance of combining both for PDT. In summary, this study demonstrates an ideal formulation of a hydrogel containing TBO with high antibacterial activity and good stability. Thus, PDT is a promising candidate for clinical treatments involving the inactivation of bacteria such as *S. aureus* and *E. coli* [31].

4. Discussion

4.1. Main PSs

4.1.1. Methylene Blue (MB)

MB, a phenothiazine, is commonly utilised as a photosensitiser (PS) for photodynamic therapy (PDT) due to its high efficiency in producing singlet oxygen ($^1O_2$). In addition to its photodynamic properties, MB possesses intrinsic antimicrobial activity that enhances its light absorption capabilities [33]. Effective across a wavelength range of 625–635 nm, MB targets both Gram-positive and Gram-negative bacteria. Its effectiveness as a PS is partly due to its hydrophilic/lipophilic balance and its strong affinity for cellular membranes, which facilitates its penetration through plasma membranes and the generation of intracellular reactive oxygen species (ROS) upon light activation [34]. MB has long been recognised for its use as a histological stain and antiseptic dye, and it has been employed as a topical disinfectant for wounds and infections for many years. Furthermore, it is one of the most commonly used PSs in photodynamics, alone or in combination with other compounds, such as nanoparticles (NPs) or antibiotics [35].

Previous studies have demonstrated the synergistic effects of MB in combination with ethanol and ethylenediaminetetraacetic acid (EDTA). The addition of these components significantly inhibits bacterial growth, suggesting a synergistic action. While ethanol can prolong singlet oxygen production during PDT, potentially increasing its effectiveness, its use may also denature proteins, affecting both light and photosensitiser penetration. EDTA disrupts biofilms and damages bacterial outer membranes, facilitating the transport and absorption of ethanol and MB molecules within the bacteria, which leads to increased singlet oxygen production and enhanced PDT efficacy [7]. Although optimising PDT with ethanol shows promise, its clinical application in treating superficial wounds remains debated. Therefore, it is crucial to ensure that optimising PDT with ethanol is clinically safe, particularly in wound care settings [36].

4.1.2. Rose Bengal (RB)

RB, first identified by Gnehm, is a water-soluble anionic xanthene dye and a halogenated derivative of fluorescein [37]. It consists of three aromatic rings arranged linearly with an oxygen atom in the centre and functions as a type II photosensitiser [37]. When activated by visible light, RB exhibits maximum absorption at 546 nm in water [38]. Due to its anionic nature, RB may be less effective against Gram-negative bacteria, such as *Pseudomonas aeruginosa*, because of the negative charges on the bacterial membrane. Strategies to improve RB's effectiveness include its incorporation into cationic polymers, both of natural and synthetic origin, which act as carriers to enhance its antimicrobial efficacy [39].

A study by Fernandez et al. (2021) demonstrated that the interaction of RB with potassium iodide in a Poly 2-hydroxyethyl methacrylate (PHEMA) matrix creates a protective

environment for the photosensitiser (PS), preventing photodegradation and prolonging its half-life. This strategy reduced the light dose required for PDT while effectively eradicating planktonic cells. PHEMA, known for its transparency, is widely used in medicine for manufacturing contact lenses and urethral stents [40].

Another approach involves combining RB with polycationic chitosan, a natural biopolymer with high antimicrobial activity, biocompatibility, and biodegradability. This combination showed promising bacterial eradication when activated by light, suggesting its potential for clinical applications. Additionally, combining RB with cation exchange resins, like macroporous polystyrene and Amberlite® IRA-900 (Wilmington, DE, USA), resulted in significant inhibition of bacterial viability. This interaction increased efficacy against Gram-negative bacteria, such as *P. aeruginosa*, with cationic transporters overcoming RB's initial ineffectiveness against these species [41]. These strategies highlight RB's versatility and potential for optimising PDT to treat resistant infections.

4.1.3. Porphyrins

Porphyrins are a group of fluorescent crystalline pigments, either naturally occurring or synthetically derived, that are widely used in PDT. They have notable absorption bands around 392 nm (Soret band) and weaker satellite absorptions between 495 and 616 nm (Q bands) [42]. In living tissues, porphyrins play vital roles in processes such as oxygen transport and photosynthesis [43].

The efficacy of porphyrins in PDT can be compromised by their difficulty in penetrating Gram-negative bacterial cell membranes due to the negatively charged cell wall. To overcome this, cationic porphyrins that interact effectively with negatively charged bacterial structures have been developed. For neutral or anionic porphyrins, this barrier can be circumvented with membrane-disrupting agents or by attaching cationic polypeptides to the PS molecules [44].

A prominent cationic porphyrin, 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) (TMPyP), has shown significant antimicrobial activity against *P. aeruginosa*. TMPyP's photocationic properties enable strong electrostatic interactions with negatively charged bacterial surfaces. When nanoassembled with cyclodextrin (CAPTISOL) as a carrier, TMPyP demonstrates enhanced penetration of the bacterial plasma membrane. The TMPyP/CAPTISOL combination exhibits excellent stability and photostability in biologically relevant environments and as freeze-dried solids. Furthermore, this combination is effective at eliminating 99% of bacteria, functioning as a sustained-release photosensitiser with enhanced stability compared to the free compound, making it a promising candidate for intravenous administration and pre-surgical applications [45].

However, despite TMPyP's effectiveness, its absorbance spectrum at 630 nm and nearby wavelengths may limit its tissue penetration depth. For deeper tissue applications, photosensitisers with longer wavelength absorption, such as chlorins and bacteriochlorins, may offer superior performance by penetrating deeper and reducing scattering at shorter wavelengths. These alternatives could potentially address the limitations of TMPyP's absorption properties.

4.1.4. Riboflavin (RF)

Riboflavin, also known as vitamin B2, is a natural, non-toxic photosensitiser (PS) with various applications, including the decontamination of blood, plasma, or cell extracts and the elimination of microorganisms when combined with UV irradiation [46]. Additionally, riboflavin is inexpensive, highly biocompatible, and can be activated by light-emitting diode (LED) lamps in the ultraviolet A (360 nm) and blue (440 nm) regions [47]. This ability to be activated by visible light expands its applications and makes it a non-toxic photoinitiator.

Recently, riboflavin has been used as a photoinitiator in the preparation of hydrogels exposed to visible light and is recognised as a biocompatible photocrosslinking agent. Furthermore, when associated with a light-emitting source, such as an LED, it can act as a

sterilising agent [48]. Its water solubility and biocompatibility make riboflavin widely used in the biomedical field [46].

It is important to note that riboflavin has effective absorption in the blue (440 nm) and UV-A (360 nm) regions, which is suitable for many PDT applications despite the limited light penetration at shorter wavelengths. Although riboflavin may not be the most effective for deeper tissue penetration, its ability to generate reactive oxygen species (ROS), such as H_2O_2, makes it useful for superficial applications. Riboflavin indeed absorbs most effectively in the UV-A and blue regions (360 nm and 440 nm). However, its utility in photodynamic therapy (PDT) primarily leverages its ability to generate reactive oxygen species (ROS) under visible light irradiation, even if the penetration depth is limited. While riboflavin may not have the ideal absorption spectrum for deep tissue penetration, its high biocompatibility and ability to produce H_2O_2 and other ROS make it effective for surface-level or superficial applications. Upon irradiation, riboflavin can indeed generate H_2O_2 as a major reactive oxygen species. The production of H_2O_2 plays a significant role in the antibacterial activity of riboflavin-mediated PDT, especially in applications where deeper penetration is not necessary. Notably, the amount of riboflavin required for hydrogel formation is small, generally less than 5 mg per 1 g of hydrogel precursor. Thus, riboflavin as a photoinitiator for hydrogel manufacture is considered safe and even beneficial. Moreover, an overdose of riboflavin does not cause significant side effects, as excess riboflavin is excreted in the urine a few hours after ingestion [8]. The visible light absorption spectrum of riboflavin allows for the initiation of photopolymerisation through visible light irradiation [49].

4.2. PDT in Clinical Isolates

PDT studies involving clinical isolates of *P. aeruginosa* are relatively recent, dating back to 2019. A previous study [17] used clinical isolates from wounds infected with *P. aeruginosa* and other ESKAPE strains from Leipzig Hospital. The researchers demonstrated the complete eradication of *P. aeruginosa* clinical isolates through the application of two porphyrin-based PSs, TMPyP and THPTS, embedded in a hydrogel matrix. TMPyP was activated at a wavelength of 420 nm and a light dose of 13 mW/cm^2, while THPTS was activated at a wavelength of 420 nm and a light dose of 18 mW/cm^2 [50].

One of the fundamental objectives of this study was to explore the use of these hydrogels as adhesives or wound dressings to relieve pain, promote adequate wound healing, and absorb exudates. Translucent hydrogels have been highlighted as an excellent choice, as they allow the effective application of doses and wavelengths of light without the need to remove the substrate. This characteristic facilitates the practical and effective application of PDT, similar to other studies on chitosan hydrogels [17].

Additionally, RM24, one of the photosensitisers used, showed significantly greater bactericidal activity against strains in the exponential growth phase compared to those in the steady state, even at concentrations as low as 1 μM. The researchers also noted that the presence of organic compounds can impact the effectiveness of RM24, suggesting that the medium influences the activity of photosensitisers [51].

Sodium azide was used as the antioxidant to characterise the ROS produced by the activation of RM24. However, notably, the use of this highly toxic compound in photodynamics is currently not recommended, highlighting the importance of safety considerations in this type of research [52].

4.3. Synergism with Antibiotics and Other Drugs

Many studies have explored the combination of PSs and antibiotics to optimise the efficacy of PDT against *P. aeruginosa*. Some notable examples are as follows:

1. Amoxicillin with Gold NPs (amoxi@AuNPs): The combination of gold nanoparticles (AuNPs) as a photosensitiser (PS) and amoxicillin has demonstrated significant potential in inhibiting the growth of *Pseudomonas aeruginosa*. When activated with white LED light at 490 nm for 3 h, this combination achieved a bacterial load reduction of

over 70%, equivalent to approximately 1.5 cell divisions. This synergistic approach not only enhances the antibacterial efficacy but also suggests a strategy to reduce the reliance on high doses of antibiotics, thereby minimising their adverse effects. Notably, the use of amoxicillin with AuNPs reduced the required light activation time, making photodynamic therapy (PDT) more practical and efficient. The light dose used in these experiments was precisely calculated to ensure optimal activation of the PS, and the effectiveness was assessed in terms of the reduction in colony-forming units (CFU/mL). This combined approach leverages the photodynamic properties of gold nanoparticles while enhancing the antibacterial action of amoxicillin, offering a promising alternative to traditional antibiotic therapies [34].

2. MB with Gentamicin (Gen + MB): One study used a combination of MB and gentamicin for PDT against *P. aeruginosa*. Red LED light resulted in a notable inhibition of 6 log cm^2 in planktonic cultures and 3 log cm^2 in biofilms. The addition of gentamicin reduced the amount of methylene blue required for photoactivation, indicating potential advantages for the treatment of skin and mucosal infections [52].
3. Polymyxin B combined with a cationic porphyrin derivative demonstrated significant antibacterial activity. The cationic porphyrin derivative, a positively charged porphyrin that enhances interaction with negatively charged bacterial membranes, was conjugated with polymyxin B to create a potent antimicrobial agent. This conjugate exhibited effective bacterial eradication, even after washing, with minimal light exposure required to photoinactivate the concentrated bacterial inocula. The cationic porphyrin derivative's ability to selectively target bacteria reduces the risk of resistance associated with antibiotic-only treatments. This approach highlights the potential of synergistic combinations of photosensitisers (PSs) and antibiotics, emphasising the versatility and promise of photodynamic therapy (PDT) as an effective and resistance-reducing strategy against *P. aeruginosa* infections [53].

4.4. PDT Associated with NPs

NPs play a significant role in the advancement of PDT by improving the efficacy of PS. Some approaches involving NPs include the following:

1. Incorporation of PS into Polymeric NPs: PS can be incorporated into polymeric NPs, providing a stable and targeted platform for the efficient delivery of photosensitising agents. This approach helps overcome the limitations of solubility and bioavailability of PS.
2. PS Attached to the Surface of NPs: PS can be attached to the surface of NPs, allowing for specific and targeted interactions with the target cells. This approach aims to improve the selectivity and effectiveness of PDT. PS Close to NPs: Some strategies exploit the physical proximity of PS to NPs, enhancing their therapeutic effects. These strategies may involve physical proximity without direct connection but with beneficial interactions for the effectiveness of PDT [54].
3. NPs as Photosensitisers (PS): Certain nanoparticles (NPs) can act as photosensitisers, generating photodynamic reactions when exposed to light. Their effectiveness depends on their absorbance spectra. For instance, NPs absorbing in the near-infrared (NIR) range, such as carbon nanotubes and gold, can penetrate deeper into tissues and minimise scattering, offering advantages over traditional visible light PDT. NPs with high photothermal conversion efficiency, like CNTs and polypyrrole, enhance therapeutic outcomes by converting light into heat effectively. Thus, selecting NPs with appropriate absorbance spectra is crucial for optimising both photodynamic and photothermal therapies [55].
4. Photothermal Therapy (PTT): In addition to PDT, NPs are used in PTT. In this context, near-infrared (NIR) laser irradiation is used to generate heat through the mediation of photoabsorbing agents, resulting in the denaturation of proteins, membrane rupture, and degradation of the genetic material of target cells.

5. Microemulsions (MEs): MEs have been reported to improve the efficiency of PDT by overcoming the limitations associated with the use of aqueous media to disperse photosensitising agents. They consist of two phases (aqueous and organic), with the organic phase stabilised by surfactants. Eucalyptus oil was used to destabilise the cell wall, allowing for greater PS penetration and synergistic effects [56].
6. Gold-Based NPs (AuNPs): AuNPs, including smaller gold nanoclusters, have received considerable attention owing to their photoactivatable properties, excellent biocompatibility, and ease of surface functionalisation. They can be used for both PDT and PTT, generating singlet oxygen under NIR light excitation and exhibiting photothermal properties when combined with organic dyes, such as indocyanine green [52]. These approaches highlight the diversity of strategies that utilise NPs to improve the efficacy and specificity of PDT in diverse biomedical applications.

4.5. PDT Delivered via Hydrogels

Hydrogels are a class of water-expanded three-dimensional polymer networks with tunable physicochemical properties that satisfy specific requirements under different conditions [9]. As promising materials, they have been extensively applied in the biomedical field, from studies on physiological and pathological mechanisms to tissue regeneration and disease therapy [9].

Hydrogels have been extensively studied as matrices for biomedical applications due to their ability to crosslink under mild conditions, excellent biocompatibility, and adjustable biochemical and biophysical properties [10]. Because the structure and properties of hydrogels closely mimic the microenvironment of many human tissues, they are widely employed in various biomedical applications [10].

Hydrogels have demonstrated good performance as cell carriers in various clinical applications [57]. Loading various antibacterial agents onto hydrogels is an efficient strategy for enhancing antimicrobial effects. To prevent the emergence of drug-resistant bacteria, phototherapeutic strategies, such as the use of hydrogels loaded with radiofrequency + 405 nm LED irradiation, have been widely used for antibacterial applications [8]. PDT using hydrogels and different types of PSs (Figure 2) has been reported to exhibit antibacterial activity against *S. aureus* in several studies [5,12–14].

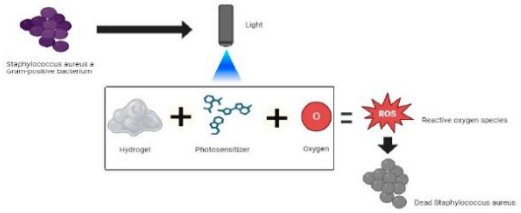

Figure 2. Mechanism of photodynamic therapy combined with hydrogel for combatting *Staphylococcus aureus*. Created with BioRender.com.

4.6. Application of PDT in Biofilm and Its Usefulness In Vivo

Biofilms represent a highly resistant and complex form of bacterial organisation composed of a matrix of exopolysaccharides (EPSs) that protects bacteria against various external stimuli. This structure provides considerable resistance, making treatment, including PDT, more challenging [44]. The following are some important aspects of biofilms.

1. EP Matrix: The EPS matrix forms a three-dimensional structure surrounding the bacterial cells. In some cases, the matrix is composed of polysaccharides, proteins, and metal ions. The presence of metal ions can confer a neutral or polyanionic charge on the matrix, depending on the predominant type of EP [58].

2. Resistance to External Aggression: The biofilm acts as a protective barrier, providing resistance to bacteria against external aggression, such as the host immune response, medications, and other antimicrobial agents. The matrix can trap antimicrobials, preventing them from reaching bacterial cells [58].
3. Greater Resistance Compared to Planktonic Cells: The biofilm formation confers significantly greater resistance, estimated to be between 10 and 1000 times greater than that of planktonic bacterial cells. This feature makes biofilms challenging to eradicate [59].
4. Chemical Signalling and Bacterial Cooperation: Biofilm formation involves chemical signalling between bacteria, allowing the coordination of surface adherence and cell differentiation. This bacterial cooperation results in the creation of a complex and organised microbial community [44].
5. Protection from Environmental Fluctuations: The matrix protects against environmental fluctuations, such as changes in humidity, temperature, and pH. Furthermore, the concentration of nutrients is favoured, and waste can be efficiently eliminated.
6. Challenges for PDT: In PDT, the presence of a biofilm represents a challenge, as the matrix limits the diffusion of PSs into the bacterial plasma membrane, leading to a reduction in the production of singlet oxygen. Specific strategies must be developed to overcome these barriers and make PDT effective against bacteria in biofilms [59].
7. Understanding the complexity of biofilms is crucial for developing effective therapeutic approaches, particularly in clinical situations where persistent biofilm-based infections are common.

5. Conclusions

This systematic review indicates that photodynamic therapy (PDT) delivered through hydrogels can effectively inhibit the growth of *S. aureus* bacteria and biofilms in vitro. Hydrogels, due to their controlled release and localised application capabilities, show potential advantages over traditional methods of delivering photosensitisers. However, it is important to note that PDT is primarily effective for treating local infections, rather than systemic infections, due to limitations in light penetration and photosensitiser activation. While hydrogels offer significant promise, they are not the only method for delivering photodynamic agents. Other delivery systems, such as nanoparticles and microemulsions, also hold potential and should be considered. There is a critical need for comparative studies to evaluate the efficacy of PDT using different delivery methods.

The review highlights a significant gap in the current research: the lack of in vivo studies assessing the effectiveness of PDT in treating *S. aureus* infections, particularly those caused by antibiotic-resistant strains. Future research should focus on conducting in vivo studies using appropriate animal models to confirm the efficacy and safety of PDT. Additionally, there is a need for research to explore how different PDT delivery methods can be adapted for clinical trials. In summary, while PDT with hydrogels shows potential, further research is essential to validate these findings in vivo, explore alternative delivery methods, and develop protocols for translating hydrogel-based PDT into clinical practice. Future studies should also investigate the effectiveness of PDT against multidrug-resistant microorganisms and address the limitations associated with photosensitisers in such contexts.

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5.2 Obteção da dECM

O processo de descelularização do pulmão porcino foi satisfatório, cumprindo com todos os objetivos estabelecidos e entregando um órgão descelularizado e pronto para seguir para as próximas etapas (Figura 9).

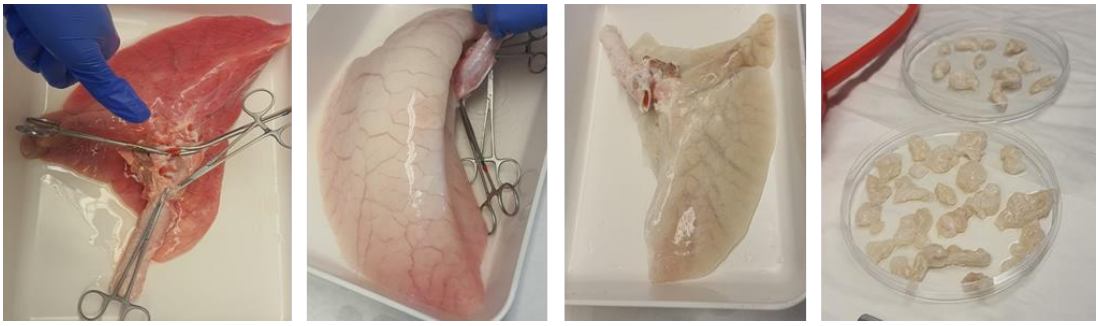

Figura 9. Imagens representativas do processo de descelularização de pulmão de porco.

5.3 Produção do Hidrogel

A produção de um hidrogel derivado da MEC de pulmão de porco descelularizado resultou em um biomaterial com propriedades promissoras para aplicações em engenharia de tecidos e medicina regenerativa (Figura 6). O processo de descelularização foi eficaz na remoção de componentes celulares imunogênicos, preservando a arquitetura estrutural da MEC e componentes bioativos essenciais, como colágeno, elastina, glicosaminoglicanos e fatores de crescimento. Após a liofilização e posterior solubilização enzimática, a matriz foi neutralizada e gelificada a 37°C, formando um hidrogel transparente, viscoelástico e biocompatível. Análises físico-químicas indicaram que o hidrogel apresenta boa capacidade de retenção de água, propridade adequada para difusão de nutrientes e propriedades mecânicas ajustáveis conforme a concentração utilizada na formulação (Figura 10).

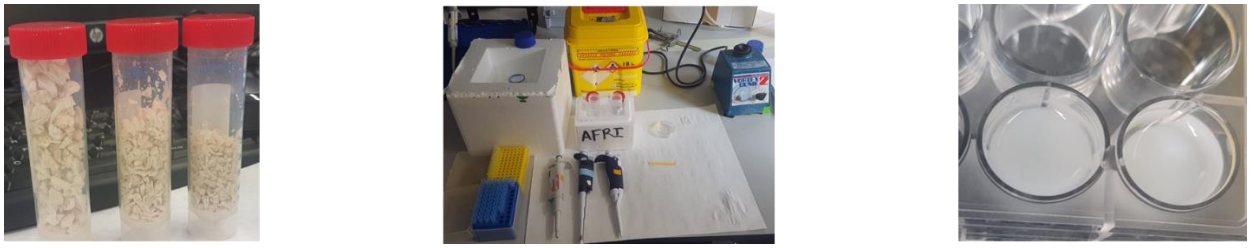

Figura 10. Produção e neutralização do pulmão solubilizado.

Este hidrogel está pronto para futuros ensaios *in vitro* que permitam demonstrar que o hidrogel é citocompatível. Como resultado deste experimento, obtivemos um hidrogel derivado de matriz extracelular de pulmão de porco descelularizado, mantendo as estruturas da matriz extracelular íntegras (Figura 11).

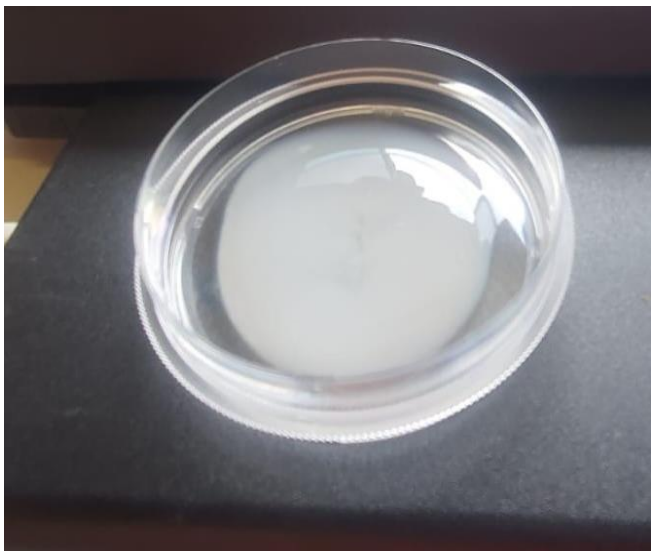

Figura 11. Fotografias macroscópicas das construções desenvolvidas de hidrogéis de HC-L-ECM mostraram que as estruturas eram consistentes, rígidas suficiente para serem manipuladas com pinças e cortadas com bisturi.

Finalmente a quantificação de DNA confirmou que a descelularização foi satisfatória: 17 ± 8ng de DNA por mg de tecido seco. Um valor abaixo do limite atualmente aceito (50 ng/mg) (Figura 12).

Figura 12. Quantificação representativa de análise de DNA presente na amostra de hidrogel.

Em resumo, o hidrogel derivado da MEC de pulmão suíno descelularizado revelou-se um material funcional com alto potencial para uso em modelos tridimensionais de cultura celular, regeneração pulmonar e desenvolvimento de sistemas de liberação controlada de fármacos. Estudos futuro *in vivo* são necessários para validar sua eficácia terapêutica e segurança a longo prazo.

5.4 Estudo 3. Matriz extracelular de scaffolds pulmonares submetidos a diferentes meios de esterilização: Uma revisão sistemática

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SYSTEMATIC REVIEW

Extracellular matrix of lung scaffolds submitted to different means of sterilization: a systematic review [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Chronic respiratory diseases often necessitate lung transplantation due to irreversible damage. Organ engineering offers hope through stem cell-based organ generation. However, the crucial sterilization step in scaffold preparation poses challenges. This study conducted a systematic review of studies that analysed the extracellular matrix (ECM) conditions of decellularised lungs subjected to different sterilisation processes. A search was performed for articles published in the PubMed, Web of Sciences, Scopus, and SciELO databases according to the PRISMA guidelines. Overall, five articles that presented positive results regarding the effectiveness of the sterilisation process were selected, some of which identified functional damage in the ECM. Was possible concluded that regardless of the type of agent used, physical or chemical, all of them demonstrated that sterilisation somehow harms the ECM. An ideal protocol has not been found to be fully effective in the sterilisation of pulmonary scaffolds for use in tissue and/or organ engineering.

Keywords

Extracellular matrix, Scaffolds, Stem cells, Decellularization, Sterilization, Recellularization

Open Peer Review

Approval Status ? ✓

	1	2
version 1 30 May 2024	? view	✓ view

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2. **Yongdae Yoon**, University of Maryland School of Medicine, Maryland, USA

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Moura RS: Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Afonso JPR**: Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Fonseca AL**: Data Curation, Formal Analysis, Methodology, Software; **Cereta AD**: Data Curation, Formal Analysis, Methodology, Visualization; **Mello DACPG**: Formal Analysis, Investigation, Supervision, Visualization; **Oliveira MC**: Investigation, Methodology, Software, Writing – Original Draft Preparation; **Oliveira-Silva I**: Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Oliveira RF**: Formal Analysis, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Oliveira DAAP**: Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Vieira RP**: Data Curation, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Palma RK**: Formal Analysis, Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Insalaco G**: Data Curation, Formal Analysis, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Oliveira LVF**: Conceptualization, Formal Analysis, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing

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Introduction

Tissue engineering has evolved rapidly to allow the development of functional tissue substitutes to improve quality and prolong the life of patients through the regeneration or replacement of tissues and organs compromised by disease.[1] Chronic respiratory illnesses such as chronic obstructive pulmonary disease (COPD), asthma, and lung cancer collectively rank as the third highest contributor to global mortality. Annually, More than four million individuals succumb prematurely to these lung conditions, with projections indicating a further rise in their prevalence in the forthcoming years.[2] Chronic respiratory failure occurs in the advanced stages of these pathologies, and lung transplantation is the only therapeutic indication to allow survival.[3]

The sterilisation process is a crucial factor in obtaining acellular lungs before the recellularisation process, eliminating the risk of transmission of viruses and bacteria from the donor to the recipient to be transplanted.[4] However, not all sterilisation methods used in the healthcare industry apply scaffolds for the regeneration of new structures and/or organs, because of the potential risk of damage to the structure and function of the extracellular matrix (ECM).[4,5] Generally, protocols that involve gamma irradiation, ethylene oxide (ETO), or other chemical and physical agents such as low-frequency LASER are used during the scaffold sterilisation process. In this process, it is extremely important to efficiently eliminate microorganisms, such as fungi, bacteria, and/or viruses, and to preserve the structure and function of the ECM of the scaffolds to be repopulated with stem cells, ensuring the generation of a new functional structure.[5]

In organ engineering, several fundamental criteria still require adjustments, such as determining the species of candidates from the organ to be removed, the best protocols for decellularisation, the best means of sterilisation, the best way to obtain a functional ECM, the optimisation of recellularisation, the most suitable cell type for recellularisation, and the development of suitable bioreactors for the recellularisation process.[6] Diverse investigations have demonstrated progress in whole-organ decellularization methods, facilitating the production of scaffolds for organ engineering.[7–10] These scaffolds, originating from the natural extracellular matrix (ECM), offer biological cues and preserve tissue microarchitecture, including functional vascular networks capable of assimilating into the recipient's circulatory system.[11] Several decellularisation techniques have led to the development of scaffolds for various allogeneic or xenogenic organs, such as the heart, liver, lungs, and kidneys.[12] Although some experimental studies involving the use of scaffolds from decellularised organs have shown encouraging results, the creation of whole functional organs for transplantation still requires further research.[13] The integrity of the ECM structure and function must be preserved during the decellularisation and sterilisation processes.[14] To support cell growth and new structural development, scaffolds must maintain the ECM without the loss of mechanical and elastic properties.[14] In addition, the time required to obtain a native scaffold compatible with this application should be observed. The proper combination of these components can lead to the creation of new in vitro tissue replacements for the implantation of functional tissues.[15] For decades, various materials have been used as scaffolds to build biological tissues. However, currently, synthetic scaffolds are not effective in recreating the complex tridimensional (3D) architecture of the original structures.[16] Therefore, the use of allogeneic decellularised organs would be an excellent solution to this problem.[17] In contrast, tissues and organs are formed by cells associated with the ECM, which are synthesised by unique and tissue-specific resident cells that in turn secrete components and molecules that can ensure their survival.[17]

The ECM, which influences cell migration, proliferation, and differentiation - crucial aspects of the recellularization process - is widely recognized as an ideal scaffold for tissue and organ engineering.[14,17] In accordance with the state of the art, this study aimed to conduct a systematic review of studies that analysed the physiological conditions of the ECM of decellularised lungs subjected to different sterilisation processes.

Methods

Methodology

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines[18,19] and used the Systematics for Experimentation in Laboratory Animals (SYRCLE) risk of bias analysis tool for animal studies. The SYRCLE is a recommended tool for assessing the risk of bias in randomised trials included in the Cochrane Reviews, adjusted for particular aspects of bias that play a role in animal intervention studies. A bibliographic search was performed in the PubMed, Web of Science, SciELO, and Scopus databases. Only complete articles published from 2012 to 2023 in English from any country of origin (without any restrictions) were included. The survey was conducted from 20 July to 20 October 2023 and did not use any automatic bibliographic search tool.

Information sources, research strategy, and data extraction process

A total of 241 scientific articles were identified after a detailed search of the aforementioned databases using keywords chosen according to the Medical Subject Headings (MeSH/NIH). Two researchers worked independently to identify and extract data and verify the quality of the studies using SYRCLE's risk of bias tool for animal studies. Duplicate articles

were removed, and studies were subsequentlyanalysed according to the inclusion and exclusion criteria. In cases where there was disagreement, both investigators reviewed the study designs, employment and exclusion criteria, intervention, and assessment of outcomes to reach a consensus. The third researcher, also involved in this study, was consulted in case of differences between the first two, and together, they reached a consensus.

Keywords

For each selected database, a bibliographic search was performed for the title and abstract using the keywords according to the MeSH. The strategy of a predefined combination of keywords was adopted ('Extracellular matrix' AND 'LASER' OR 'Extracellular matrix' AND 'Recellularization' OR 'Extracellular matrix' AND 'Sterilization' OR 'Extracellular matrix' AND 'Photodynamic therapy') AND ('Extracellular matrix' AND 'lung' AND 'LASER' OR 'Extracellular matrix' AND 'lung' AND 'sterilization' OR 'Extracellular matrix' AND 'lung' AND 'LASER' AND 'Sterilization' OR 'Extracellular matrix' AND 'lung' AND 'Sterilization' AND 'scaffold') AND ('Scaffold' AND 'lung' AND 'sterilization'). All titles were manually searched and analysed for inclusion. Reference lists of articles containing the title, authors' names, language, and publication date were generated. In this systematic review, only scientific articles that reported experimental studies were included.[19]

Election criteria - Design

All manuscripts initially considered relevant by title and abstract were eligible for inclusion in the review. The full text of the manuscript was obtained to verify that the participants met the inclusion criteria. Only studies that presented results from the use of different means of sterilisation of scaffolds and/or ECM of lungs in vitro were included, meeting the criteria of being full text, studies published in scientific journals with a rigorous peer review process, published in English, which described the use and effect of decellularisation methods, and sterilisation of pulmonary scaffolds and ECM in vitro. No restrictions were observed regarding the sample size, sample type, and intervention time for the included studies. Studies that used sterilisation, but did not assess the extracellular matrix; meeting abstracts; studies published in languages other than English; and studies addressing ECM and sterilisation of organs other than the lungs were excluded (Table 2).

Design and interventions

This review analysed controlled experimental laboratory studies that used sterilisation through photodynamic therapy (PDT), physical or chemical techniques, LASER, light emitting diode (LED), and/or gamma irradiation in animal models such as rats, mice, pigs, and cows.

Results

The initial bibliographic survey included 241 studies. Of these, 22 duplicate articles were excluded and 189 articles were rejected because they did not meet the inclusion criteria. After a complete reading of the texts, 13 studies were excluded because they failed to address the subject in question or because the methodology did not include a control group. After applying the exclusion criteria, 13 articles were eliminated, leaving 4 articles that investigated scaffolds and ECM sterilisation processes through chemical and physical means using PDT, LASER, LED, and gamma irradiation, published between the years 2011-2022 were finally analysed in this systematic review (Figure 1). The four articles that were included in this review underwent a risk bias analysis for animals according to the Systematics for Experimentations in Laboratory Animals (SYRCLE) (Table 1). Among the four studies selected for this systematic review, one used rat organs[19] and three used mice[20,21] (Table 2). Despite the small amount of published work in this area, the articles found demonstrated the effectiveness in the sterilisation process of used lung organs or tissues, with some tissue damage occurring during the sterilisation process; however, the ECM structures were preserved. Owing to the important functional and structural role of the ECM in the lungs, early changes are observed in several respiratory diseases. The possibility of analysing the ECM and identifying probable alterations is fundamental to allow a better understanding of future lung diseases; therefore, allowing early potentialisation of the therapeutic approach.[23] Thus, this review gathered articles that evaluated the ECM and mechanical parameters of decellularised lungs subjected to some form of sterilisation through chemical or physical means such as irradiation, LASER, LED, or PDT, demonstrating the effectiveness of the sterilisation process of pulmonary scaffolds for further use in organ bioengineering. It is expected that with the optimisation of these processes, the generation of new organs on a large scale will be possible, solving one of the biggest health problems worldwide, and the availability of organs for transplantation will be possible in the not-too-distant future.[24]

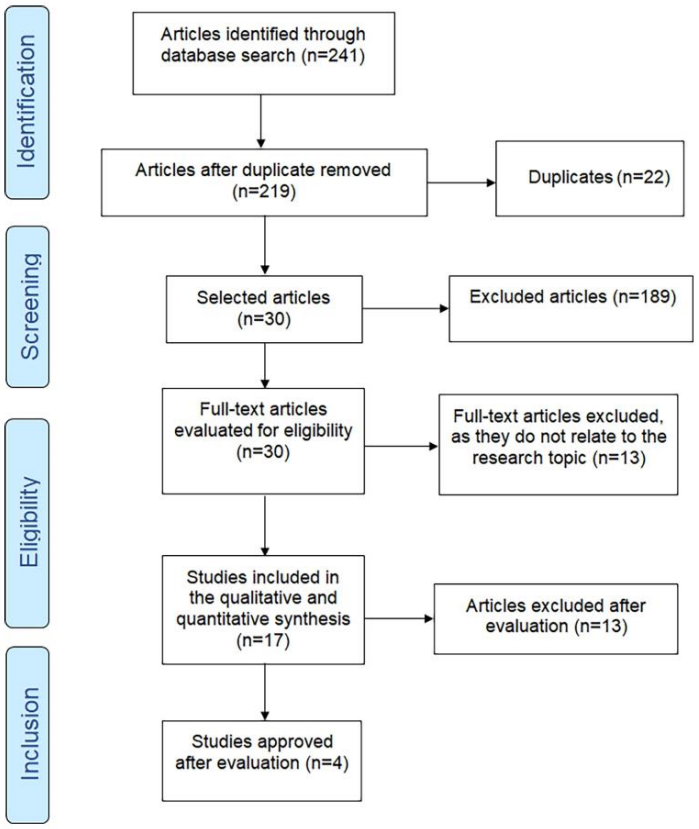

Figure 1. Flowchart of the study.

Table 1. Risk of bias in articles selected according to the Scyrcle tool.

		Bonenfant et al., 2013[20]	Uriarte et al., 2014[21]	Balestrini et al., 2016[22]	Oliveira et al., 2021[23]
Selection bias	Sequence generation	No	Yes	No	No
Selection bias	Basic features	Yes	Yes	Yes	Yes
Selection bias	Allocation concealment	No	Yes	No	Unclear
Performance bias	Random hosting	Yes	Yes	Unclear	Yes
Performance bias	Blindness	No	Unclear	Unclear	Unclear
Detection bias	Random Outcome Evaluation	No	Yes	Unclear	No
Detection bias	Blindness	No	Yes	Unclear	Unclear
Friction bias	Incomplete result data	Yes	Yes	Unclear	Unclear
Reporting bias	Selective result report	Unclear	Yes	Yes	Yes
From others	Other sources of prejudice	Yes	Unclear	Yes	Yes

Table 2. Main findings of selected articles.

Author, year	Decellularizing and sterilizing agents	Animal Model	Results
Bonenfant et al., 2013[20]	Triton-X, deiozinated water, penicillin/ streptomycin, sodium deoxycholate, sodium chloride, peracetic acid. Irradiation by 5 Gy, RadSource 2000 Biological Irradiator.	Mice	These results commonly suggest that used approaches to storage and sterilization of other decellular tissues and other types of biological scaffolding may not be applicable for decellularized lungs. Different cell types may react differently to various storage and sterilization conditions, further complicating the development of an optimal overall strategy for using decellularized lungs.
Uriarte et al., 2014[21]	Gamma irradiation of 31 kGy.	Mice	The application of gamma irradiation with a dose high enough to wait for complete sterilization induced an increase in the mechanical impedance of the decellularized lungs. However, the changes observed were not serious, as the microscopic structure of the pulmonary compartment maintained its integrity and the acellular lung could be ventilated normally. Future research should be approached to verify the effects.
Balestrini et al., 2016[22]	Lung sterilization was completed on NovaSterilis in a Nova 2200 PBS sterilizer, Carbon Dioxide, Peracetic Acid, 2 mL NovaKillTIM Additive Gen2 (Novasterilis).	Mouse	The entire protocol produces a sterile acellular matrix that retains critical structural, adhesive and support proteins such as collagen, elastin, laminin and polysaccharides such as sGAGs. In addition, after 6 months of storage, $ScCO_2$- treated tissues retain their mechanical properties and cell seeding ability. SAL6 sterilization using $ScCO_2$ was evaluated at various processing times and PAA-containing additive levels (Table 1). A minimum amount of 2 h of preconditioning time and 1.5 h of $ScCO_2$ exposure were required for the inactivation of 106 Bacillus atrophaeus spores. To ensure confidence in the sterility of SAL6, all subsequent $ScCO_2$ treatments consisted of 2 h preconditioning time and 2 h $ScCO_2$ exposure. $ScCO_2$ alone, without the addition of PAA, was not sufficient to inactivate lung bioburden.
Oliveira et al., 2021[23]	PBS, SNP, LED 608nm, deionized water, Triton 1%, SDS 1%. Photosensitizer, Red LED 660nm.	Mice	There were no significant differences between the control and GPpIX groups, which represents equal ventilation for both assessments.

Focal points of the included studies

1. Bonenfant et al. (2013):

> Histological evaluation and Masson's trichrome staining demonstrated that Newly decellularised lungs maintained the ECM architecture found in the native lung. Glycosaminoglycans (GAGs) were less evident by Alcian Blue staining in newly decellularised lungs, probably representing the loss of cell-associated GAGs during decellularisation. Lungs that underwent irradiation demonstrated a grossly abnormal appearance, with a scattered heterogeneous pattern of a thickened and fused alveolar septa, associated with large alveolar spaces typical of pulmonary emphysema. The lung architecture of the decellularised organs after three months of storage, with the use of peracetic acid and even at some level of irradiation, better resembled native or newly decellularised lungs after insufflation. In contrast, no significant improvement was observed in the lungs stored for 6 months.[20]

2. Uriarte et al. (2014):

   Scaffolds obtained from the lung decellularisation procedure showed a lack of cell nuclei compared to native lungs, as evaluated by 6-diamidino-2-phenylindole fluorescence solution (DAPI). Qualitative macroscopic evaluation of acellular lungs after sterilisation by gamma irradiation showed changes when compared to non-irradiated control lungs, with reduced organ size and apparent damage to the pleural surface. The relevant components of the ECM (elastin, laminin, and collagens I, III, and IV) remained almost unchanged in the acellular lungs before and after irradiation.[21]

3. Balestrini et al. (2016):

   The authors evaluated sterilisation with a sterility assurance level of 10^{-6} (SAL6) using supercritical carbon dioxide ($ScCO_2$) at various processing times and peracetic acid (PAA)-containing additives. To achieve the desired inactivation of 10^{-6} Bacillus atrophaeus spores, a preconditioning time of at least 2 hours followed by 1.5 hours of exposure to $ScCO_2$ was found to be necessary. To maintain confidence in the sterility of SAL6, all subsequent $ScCO_2$ treatments involved a 2-hour preconditioning period followed by 2 hours of $ScCO_2$ exposure. It was observed that $ScCO_2$ alone, without the inclusion of PAA, did not effectively neutralize lung bioburden.[19]

4. Oliveira et al. (2021):

   The authors evaluated lung mechanics in all lung scaffolds of 12 mice divided into two groups: the control (n=6) administered 1 mL of phosphate buffered saline (PBS) and the experimental group (GPpIX) (n=6) group injected with 1 mL of protoporphyrin IX (PpIX) in the lungs, with both irradiated with 660 nm LED. There were no significant differences between the control and GPpIX groups, which showed equal ventilation. Pulmonary mechanical assessment parameters did not show significant differences between the two PDT intervals. In addition, no changes were observed over the irradiation time, indicating the maintenance of the viscoelastic behaviour of the pulmonary scaffold after 1 h of exposure to LED.[8]

Discussion

This systematic review included studies found in the scientific literature that verified the conditions of the ECM of lung scaffolds subjected to the process of decellularisation and sterilisation through physical and/or chemical resources. This bibliographic survey indicated observed that the components of the ECM responsible for maintaining the 3D lung structure, such as elastin, laminin, and collagens I and IV, remained practically unchanged after sterilisation of decellularised lungs. Analysis of lung scaffolds using scanning electron microscopy suggested that the microscopic lung structure was maintained despite slight alterations after application of the sterilisation method.

Studies such as those by Balestrini et al. (2016) have suggested that traditional sterilisation methods of acellular lungs have allowed a complete reduction of the bioburden. The sterilisation protocol proposed in this study provides a reproducible and efficient method to generate sterile scaffolds for use in tissue engineering. Irradiation, even at a lower dose than that generally used for biological materials, produced significant distortions that were only partially responsive to subsequent lung reinflation.[22] PAA, a denaturing agent used for sterilisation and to eliminate residual detergents and other reagents used during the tissue decellularisation process, has a less deleterious effect on the functional architecture of the resulting structure.[22] The use of $ScCO_2$ in sterilisation, is evidently a promising mechanism for whole-organ sterilization technologies.[22]

Bonenfant et al. (2013) drew attention to the fact that special attention is needed to better understand the various conditions that can compromise acquisition, decellularisation, sterilisation, storage, recellularisation, and implantation of the generated organ or structure. The authors emphasise that because the gold standard for decellularisation and sterilisation techniques, whether by chemical, physical, or biological means, is not established, the use of various protocols for sterilisation of organs and/or structures in vitro has been observed.[20]

Analysis of the literature has shown that some studies have performed sterilisation using chemical methods such as ETO, hydrogen peroxide, PAA, formaldehyde, flutaraldehyde or $ScCO_2$. Other protocols use physical methods such as dry heat (oven), moist heat (steam under pressure – autoclaves), radiation (gamma – cobalt 60, cobalt ultraviolet), electron beam irradiation of 15 kGy, gamma irradiation of 31 kGy, 660 nm red LED, PDT, and certain wavelength LASERs. These resources can be used in conjunction; however, there is still no consensus on the best sterilization method. It is worth noting that this area still requires new experimental studies that show the effects of using traditional methods and new

means that can be used to obtain sterile scaffolds for the recellularisation of organs and/or tissues. Table 2 illustrates the studies and their respective methodologies used in the sterilisation process of lung scaffolds.[20–23]

Sterilisation by chemical means

Bonenfant et al. (2013)

According to this study, the lungs treated with PAA had an overall macroscopic appearance similar to that of native or newly decellularised lungs, although some central regions showed atelectasis. In contrast, lungs sterilised by irradiation (15 and 25 kGy) showed a grossly abnormal appearance with a scattered heterogeneous pattern of thickened and fused alveolar septa, and large emphysematous alveolar spaces. The architecture of lungs decellularised with PAA obtained from storage 3 months after insufflation better resembled native or newly decellularised lungs. In contrast, no significant improvement was observed in the 6-month storage lungs.[20]

Balestrini et al. (2016)

According to the literature, most pulmonary scaffolds are sterilised using high concentrations of PAA, resulting in ECM depletion. Depending on the extent of these injuries, mechanically altered tissues may have little or no storage potential. In this study, Balestrini et al. (2016) demonstrated that a sterilisation technique using $ScCO_2$ can achieve a 10^{-6} sterility assurance level in ECM from decellularised lungs. In this study, we demonstrated that $ScCO_2$ did not cause any major structural or biological degradation in the acellular pulmonary ECM, generating a sterile lung structure that can be stored for a long period. Stored sterile tissue is also suitable to allow cell adhesion and survival.[19] Considering these results, the authors suggest that the proposed sterilisation protocol provides a reproducible and efficient method to generate sterile lung scaffolds for use in tissue engineering. The authors showed that $ScCO_2$ surpassed traditional sterilisation levels with PAA in retaining the key biological and mechanical characteristics. Therefore, the use of $ScCO_2$ in the sterilisation process of lung scaffolds can be a new, powerful, and easy-to-use tool for the generation of sterile lung organs or tissues for subsequent recellularisation. These results indicate that $ScCO_2$ can be used to sterilize acellular lung tissue, as it can preserve the main biological components needed to obtain functional lung scaffolds for regenerative medicine purposes.[22]

Effects of using CO_2 and PAA on the decellularisation of lungs scaffolds

$ScCO_2$ was recently developed as a means of sterilising medical devices, implantable, and allograft tissues using extremely low levels of PAA (0.005–0.05%) to reach the level of probability of the presence of viable microorganisms in a unit load after sterilisation (SAL6) on bacterial endospores.[22] $ScCO_2$ can be sterilised through the enhanced mass transfer of CO_2 during the supercritical phase and by disrupting the bacterial, viral, or fungal outer membrane.[22] Furthermore, because $ScCO_2$ has a diffusion capacity that allows it to penetrate ECM fibres, this process can sterilise at low temperatures and remove unwanted compounds such as blood or potentially residual DNA. In addition, $ScCO_2$ leaves no toxic residue, making it ideal to sterilise the delicate ECM.[22] In studies that used $ScCO_2$ and PAA as sterilisation mechanisms, sterilised tissues showed a significant increase in stiffness in newly decellularised lungs. These data indicate that $ScCO_2$ sterilisation does not compromise the mechanical integrity of acellular lung tissue. The authors demonstrated that $ScCO_2$ surpassed sterilisation levels when compared to traditional PAA in terms of its ability to preserve the main biological and mechanical characteristics. Therefore, the use of $ScCO_2$ in sterilisation can be a powerful tool for whole-organ sterilisation and recellularisation technologies.[22]

Sterilisation by physical means

Bonenfant et al., 2013

In the study by Bonenfant et al. (2013), the effects of sterilisation were evaluated using a protocol commonly applied to the storage of other biological scaffolds through irradiation. Irradiation, even at a dose lower than that usually used for other biological materials (15 and 25 kGy), produced a significant distortion that was only partially responsive to subsequent lung reinsufflation.[20]

Uriarte et al., 2014

Qualitative macroscopic evaluation of acellular lungs after sterilisation by gamma irradiation showed changes when compared to non-irradiated lungs. The main changes observed were a reduction in organ size and apparent damage to the pleural surface. The authors also demonstrated that the relevant components of ECM (elastin, laminin, and IV) collagens were almost unchanged in acellular lungs before and after irradiation. Irradiation of a cellular lungs, with 60A resulting in a significant increase in the mechanical impedance of the lung scaffold, associated with increased pulmonary resistance

and reactance, regardless of whether gamma irradiation was performed when the decellularised lungs were frozen or at room temperature.[21] In principle, other sterilisation methods, such as those based on gamma irradiation, ETO, or other chemical agents, can be used. However, several studies have shown that all sterilisation methods have side effects, since any action to destroy infectious microorganisms potentially compromises the different molecular structures of the scaffold. For example, it has been indicated that both ETO and irradiation can interact with scaffold molecules, potentially degrading their performance.[24]

Oliveira et al., 2021

In a recent study by Oliveira et al. (2021), it was observed that with the use of PDT associated with LED as a means of sterilising pulmonary scaffolds resulted in no functional changes in the tissue. The pulmonary mechanics, resistance, elastance, and viscoelastic behaviour parameters of the pulmonary scaffold were maintained after 1 h of exposure to PDT, and the ECM components remained practically unchanged in acellular lungs. The authors demonstrated a reduction in the fungal infection population after 45 min of PDT, but full sterilisation was not observed. This study provides evidence that the photosensitiser protoporphyrin IX (PpIX) has no antifungal activity when used alone without the application of LED.[8,14] It has also been well described in the literature that the use of isolated LASERs without the addition of a photosensitiser does not reduce fungal populations in the same way. Oliveira et al., 2021 demonstrated that the total reduction in fungal load was dependent on photosensitiser concentration and light parameters using red and methylene blue LASERs on the oral mucosa of a mouse model. It is therefore clear that the use of LASER is effective when used in conjunction with a photosensitizer and when considering the time and concentration of the procedure as a whole.[23]

Sterilisation by gamma irradiation of 31 kGy

Gamma irradiation (31 kGy) induced structural and mechanical changes in the decellularised lungs of the mice. Visual inspection of the acellular lungs revealed a reduction in the volume of the scaffold. This reduction in lung volume may be due to some degree of alveolar atelectasis, consistent with the increase in lung elastance and resistance observed after irradiation. When acellular lungs are irradiated at room temperature, the different lung structures, alveoli, vessel walls, and pleura remain similar to those of non-irradiated lungs.[24] In principle, other sterilisation methods, such as those based on gamma irradiation, ETO, or other chemical agents, can be used. However, all sterilisation methods have side effects, as any action that destroys infectious microorganisms may potentially compromise the different molecular structures of the scaffold. Yoganarasimha et al. (2014) demonstrated that both ETO and irradiation could interact with scaffold molecules, potentially degrading their functionality. From the point of view of effectively reaching all points of the scaffold structure, gamma irradiation may be particularly suitable for sterilising lung scaffolds.[20]

Sterilisation with a photosensitiser

Photosensitisers play a key role in the effectiveness of PDT. PpIX is actively transported into cells via a growth-induced uptake mechanism under nutritionally restrictive conditions.[23] The photosensitiser PpIX did not show antifungal activity when used alone without the application of LED. It is also well described in the literature that the use of isolated LASERs without the addition of a photosensitiser does not reduce fungal populations in the same way. PDT has been successfully used to treat different localised infections, and these results are proof that PDT is also a suitable method to promote microorganism reduction. This systematic review calls for further research to confirm the suitability of PDT as a routine sterilisation technique for lung scaffolds in the organ bio-manufacturing process to replace damaged or deficient organs, and even reduce wait times for organ transplants.[23] In conclusion, our survey of the scientific literature related to lung scaffold sterilisation protocols showed that regardless of the type of agent used (physical or chemical), the sterilisation process affects the ECM in some way. To date, an ideal protocol has not been found to be fully effective in the sterilisation of lung scaffolds for use in tissue and/or organ engineering.

Data availability

No data associated with this article.

Extended data

Reporting guidelines: PRISMA P checklist for 'Extracellular matrix of lung scaffolds submitted to different means of sterilisation: a systematic review'. DOI: https://doi.org/10.6084/m9.figshare.25517137.v1.[25]

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Open Peer Review

Current Peer Review Status: ? ✓

Version 1

Reviewer Report 14 August 2024

https://doi.org/10.5256/f1000research.161894.r310179

✓ **Yongdae Yoon**
University of Maryland School of Medicine, Maryland, USA

The author described various sterilization methods of lung scaffolds and this is a very useful article. However, some articles that should have been covered in the results section was suddenly covered in the conclusion section such as chemical means. I wish the author would have continued the explanation of the part that should have been covered in the results section. They started their explanation based on 4 papers, but it seems that they used more than the papers. Table 2 describes a study using only mice, but on page 4, it mentioned a study using rat organs and there is no more explanation for it, and also it is necessary to check if the source used is correct.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Regenerative medicine and immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 30 July 2024

https://doi.org/10.5256/f1000research.161894.r294964

?

Carolina Herranz Diez
University of Barcelona (UB), Barcelona,, Spain

The topic is of interest as there is a concern about how to sterilize decellularized tissues without compromising their structural properties. The rationale and the objectives of the review are clearly stated. The review strategy is well defined and easy to follow although some choices need a comprehensive justification.
There is no clear reason why studies published before 2012 are excluded. The authors should indicate the reason to avoid works published before 2012 on the relevant topic. It is well known that many of the most pertinent publications use English as the publishing language, but other publications might be relevant to the topic and written in different languages. The authors should take this in consideration.
The review uses the SYRCLE tool to assess the risk of bias in randomized trials included in the Cochrane Reviews, adjusted for particular aspects of bias that play a role in animal intervention studies. The use of the bias guide used in animal experimentation should be justified and the adaptation, if any, to the current sterilization technique and purposes should be described.
Creating a table with the pros and cons of each sterilization technique is advisable to be added to the conclusions section.
Finally, a grammar check is needed as some typo errors have been detected.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Partly

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomaterials, ECM-hydrogels, cell interactions

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

6. DISCUSSÃO

A descelularização de tecidos pulmonares de origem suína tem se consolidado como uma estratégia promissora para o desenvolvimento de biomateriais bioativos destinados à engenharia tecidual e à medicina regenerativa. O processo de remoção celular visou eliminar componentes imunogênicos, preservando a arquitetura tridimensional e os constituintes bioquímicos da matriz extracelular (MEC), como colágeno, elastina, glicosaminoglicanos e proteínas estruturais, fundamentais para a manutenção das propriedades biomecânicas e biofuncionais do tecido (Alcaraz et. al., 2017). A utilização do pó de pulmão descelularizado como matéria-prima para produção de hidrogéis permitiu a obtenção de um material biocompatível e altamente biomimético, capaz de reproduzir aspectos microambientais do tecido nativo. Além disso, a origem suína representa uma alternativa acessível e escalável, reduzindo custos e viabilizando sua aplicação em modelos pré-clínicos (Falcones et. al., 2021).

Os hidrogéis derivados de MEC pulmonar apresentam boa capacidade de gelificação, integridade estrutural e propriedades reológicas ajustáveis, podendo atuar como plataformas para cultivo celular, suporte para reparo tecidual e meios carreadores para liberação controlada de fármacos e fatores de crescimento (Pouliot et. al., 22016). Dessa forma, a combinação de técnicas eficientes de descelularização com a produção de hidrogéis de MEC deste estudo representa um avanço significativo para o desenvolvimento de sistemas biológicos funcionalizados voltados à regeneração de tecidos pulmonares e à bioengenharia de órgãos (Marhuenda, et. al., 2022)

O primeiro artigo aborda uma revisão sistemática de 241 estudos sobre a esterilização de scaffolds pulmonares, dos quais 4 artigos foram selecionados para análise após a exclusão de duplicatas e trabalhos que não atendiam aos critérios de inclusão. Os estudos analisados focaram em processos de esterilização por métodos químicos e físicos, como TFD, LASER, LED e irradiação gama, entre 2011 e 2022. A revisão destacou a eficácia desses

métodos na preservação da MEC dos pulmões, essencial para a bioengenharia de órgãos (Moura, et al. 2024).

Os resultados mostraram que, apesar de algumas alterações teciduais durante a esterilização, a estrutura da MEC, composta por elastina, laminina e colágenos, permaneceu praticamente inalterada. A análise de risco de viés foi aplicada aos estudos selecionados, que incluíram experimentos com ratos. A revisão também discutiu a necessidade de mais pesquisas para otimizar os protocolos de esterilização, uma vez que não há consenso sobre o melhor método, e todos os métodos têm efeitos colaterais que podem comprometer a funcionalidade dos scaffolds (Shakir et al., 2022)

Dentre os métodos avaliados, o uso de dióxido de carbono supercrítico (ScCO2) foi destacado como promissor, pois preservou as características biológicas e mecânicas da MEC, enquanto outros métodos, como a irradiação, mostraram distorções significativas na estrutura pulmonar. A revisão conclui que, embora os métodos de esterilização sejam eficazes, ainda não existe um protocolo ideal que garanta a preservação total da MEC para uso em engenharia de tecidos e transplantes (Balestrini et al., 2016).

O Segundo artigo destaca o azul de metileno (AM) como um FS eficaz, especialmente por sua capacidade de gerar oxigênio singleto (1O2) quando ativado por luz em comprimentos de onda específicos. Essa propriedade é crucial para a TFD, pois o oxigênio singleto é uma espécie reativa que pode danificar as células bacterianas. Além disso, o AM possui atividade antimicrobiana intrínseca, o que aumenta sua eficácia no combate a bactérias Gram-positivas e Gram-negativas (Moura, R.S., et al 2024).

Outro ponto interessante é a combinação do AM com outros compostos, como etanol e ácido etilenodiamino tetra-acético (AETA), que potencializa sua ação antimicrobiana. O etanol pode prolongar a produção de oxigênio singlete, enquanto o AETA ajuda a romper biofilmes, facilitando a penetração do FS nas células bacterianas. Isso é especialmente relevante, pois os biofilmes representam um desafio significativo na erradicação de infecções, tornando as bactérias mais resistentes a tratamentos convencionais (Yanten, 2023).

O texto também menciona outros FSs, como a bengala a riboflavina (RF) e as porfirinas, que têm suas próprias características e desafios. Por exemplo, a RF é menos eficaz contra bactérias Gram-negativas devido à sua carga aniônica, mas estratégias como a incorporação em polímeros catiônicos podem melhorar sua eficácia (Vanerio, 2019). As porfirinas, por sua vez, enfrentam dificuldades em penetrar as membranas bacterianas, mas porfirinas catiônicas têm mostrado promissora atividade antimicrobiana (Meerovich, 2020).

A Riboflavina, um FS natural, é destacada por sua biocompatibilidade e capacidade de gerar espécies reativas de oxigênio sob irradiação de luz visível, embora sua penetração em tecidos mais profundos seja limitada. Isso levanta a questão de como otimizar a TFD para diferentes tipos de infecções, especialmente em tecidos mais profundos (Kingsley et al., 2018).

Além disso, o texto aborda a sinergia entre FSs e antibióticos, como a combinação de amoxicilina com nanopartículas de ouro, que demonstrou aumentar a eficácia antibacteriana e reduzir a dependência de altas doses de antibióticos. Essa abordagem é promissora, pois pode ajudar a combater a resistência bacteriana, um dos maiores desafios na medicina moderna (Du et al., 2023).

Por fim, a utilização de hidrogéis como matrizes para a entrega de FSs é uma estratégia inovadora que pode melhorar a eficácia da TFD, especialmente em infecções resistentes. Os hidrogéis podem facilitar a liberação controlada dos PSs e melhorar a penetração na área afetada (Falcones et al., 2021).

Em resumo, o artigo apresenta uma visão abrangente sobre as diversas abordagens e desafios na aplicação da terapia fotodinâmica no combate a infecções bacterianas. A produção e aplicações de hidrogéis derivados de pó de órgão suíno. A combinação de diferentes FSs, a sinergia com antibióticos e o uso de novas tecnologias, como hidrogéis e nanopartículas, são estratégias promissoras para otimizar a eficácia da TFD e enfrentar a crescente resistência bacteriana (Moura et al., 2024).

Os fotossensibilizadores são elementos-chave para a efetividade da terapia fotodinâmica (TFD). O composto PpIX, por exemplo, é internalizado pelas células por meio de um mecanismo ativo, especialmente quando estas se desenvolvem em ambientes com restrições nutricionais. Por si só, o PpIX não apresenta ação antifúngica na ausência da luz LED, assim como o uso isolado de LASERs também não é eficaz na redução de populações fúngicas sem a presença de um fotossensibilizador — fato já bem documentado na literatura científica. A TFD já foi aplicada com êxito no tratamento de diversas infecções localizadas, o que comprova seu potencial na eliminação de microrganismos (Balestrini et al., 2016).

A segunda revisão sistemática mostrou que a terapia fotodinâmica (TFD) usando hidrogéis pode ser eficaz contra o crescimento de *Staphylococcus aureus* e seus biofilmes em testes laboratoriais. Os hidrogéis se destacam por permitir liberação controlada dos agentes ativos e aplicação direcionada, apresentando vantagens em relação aos métodos convencionais. Contudo, a TFD é mais indicada para infecções localizadas, pois a penetração da luz e a ativação do fotossensibilizador são limitadas em infecções sistêmicas. Além dos hidrogéis, outros veículos como nanopartículas e microemulsões também têm potencial e merecem ser explorados (Moura et al., 2024).

Um ponto crítico levantado é a carência de estudos *in vivo*, especialmente em infecções causadas por cepas resistentes a antibióticos. Pesquisas futuras devem incluir modelos animais para confirmar a segurança e eficácia da TFD, além de avaliar diferentes formas de aplicação em cenários clínicos. Em resumo, embora os resultados iniciais com hidrogéis sejam promissores, mais estudos são necessários para validar esses achados, investigar outras estratégias de aplicação e viabilizar o uso clínico da TFD, principalmente contra microrganismos multirresistentes.

7. CONCLUSÃO

As duas revisões sistemáticas ressaltam a necessidade de novos estudos que avaliem o uso da TFD como uma técnica padrão de esterilização em scaffolds pulmonares, especialmente no contexto da bioprodução de órgãos, com o objetivo de substituir órgãos danificados ou reduzir filas de transplantes. A análise da literatura científica sobre métodos de esterilização de scaffolds revela que, independentemente do agente esterilizante utilizado — físico ou químico —, sempre há algum impacto na matriz extracelular (MEC). Até o momento, ainda não se chegou a um protocolo considerado ideal para garantir a esterilização eficaz desses scaffolds sem comprometer sua integridade para aplicação em engenharia de tecidos ou órgãos.

A produção de um hidrogel derivado de pó de pulmão suíno descelularizado representa um avanço significativo na área de biomateriais e engenharia de tecidos. Esse tipo de hidrogel possui características únicas, como biocompatibilidade, biodegradabilidade e a capacidade de mimetizar a matriz extracelular, conservando uma quantidade mínima de DNA no hidrogel o que o torna altamente promissor para aplicações em medicina regenerativa e terapias de reparo tecidual.

Em suma, a produção de hidrogel não só abre novas possibilidades para tratamentos médicos inovadores, mas também representa um passo importante em direção a soluções mais sustentáveis e eficazes na medicina moderna. A pesquisa e o desenvolvimento contínuos nessa área podem levar a melhorias significativas na qualidade de vida dos pacientes e na eficácia dos tratamentos.

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