UNIVERSIDADE D
COIMBRA

Érica Fernandes da Silva

Relatórios de Estágio e Monografia intitulada "Blood-brain barrier-on-a-chip for central nervous system disease modeling and drug screening" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Sara Terra, da Doutora Ana Catarina Pinto e da Professora Doutora Alexandrina Mendes, apresentados à Faculdade da Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2022

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Setembro 2022

Eu, Érica Fernandes da Silva, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2017255215 declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Blood-brain barrier-on-a-chip for central nervous system disease modeling and drug screening" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 4 de setembro de 2022.

Érica Fernandes da Silva

(Érica Fernandes da Silva)

Agradecimentos

À minha família, em especial aos meus pais, pelo apoio incondicional, por não me deixarem desistir nos momentos mais difíceis e pelo esforço que fazem todos os dias para que possa realizar os meus sonhos.

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A todos o meu sincero obrigada!

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PARTE I

Relatório de Estágio em Farmácia Comunitária

Farmácia Hebel

Estágio sob a orientação da Dra. Sara Terra

Lista de Abreviaturas

COVID- 19- *Coronavirus Disease 2019*

MICF- Mestrado Integrado em Ciências Farmacêuticas

MNSRM- Medicamentos Não Sujeitos a Receita Médica

SWOT- *Strengths, Weaknesses, Opportunities, Threats*

1. Introdução

A farmácia comunitária é um espaço de prestação de cuidados de saúde de elevada diferenciação técnico-científica, representando muito mais do que um local de dispensa de medicamentos. Assim, dada a sua acessibilidade à população, a farmácia comunitária é uma das portas de entrada no Sistema de Saúde, constituindo muitas das vezes o primeiro contacto da população com os cuidados de saúde. [1] Efetivamente, o farmacêutico comunitário é um profissional qualificado que disponibiliza serviços essenciais à saúde do utente, quer na vertente preventiva quer na vertente terapêutica, promovendo a utilização segura, eficaz e racional dos medicamentos. Além disso, este detém competências para evitar deslocações desnecessárias a outros serviços de saúde perante afeções menores. [2] De facto, o papel das farmácias comunitárias e dos seus farmacêuticos na promoção da saúde pública ganhou ainda mais ênfase com o surgimento da pandemia de COVID-19, inicialmente através de uma adaptação de forma a dar continuidade aos serviços básicos que prestam aos utentes e, mais recentemente, através da realização de testes de diagnóstico para despiste de infeção por SARS-CoV-2.

O Mestrado Integrado em Ciências Farmacêuticas (MICF) ministrado na Faculdade de Farmácia da Universidade de Coimbra contempla um plano de estudos abrangente e multidisciplinar, com a duração de 5 anos letivos, culminando na realização de um estágio curricular, em que a farmácia comunitária constitui uma área obrigatória. Este estágio é essencial para os alunos consolidarem e aplicarem os conhecimentos teóricos adquiridos ao longo do percurso académico e adquirirem novas aptidões imprescindíveis ao exercício da profissão.

O meu estágio em farmácia comunitária decorreu entre os dias 10 de janeiro e 29 de abril de 2022 na farmácia Hebel, sob a orientação da Dra. Sara Terra.

O presente relatório, elaborado no âmbito da unidade curricular "Estágio Curricular", reflete os principais aspetos intrínsecos à realização deste estágio, sendo estes apresentados sob a forma de uma análise SWOT, identificativa dos pontos fortes (*Strenghts*), pontos fracos (*Weaknesses*), oportunidades (*Opportunities*) e ameaças (*Threats*).

2. Farmácia Hebel

A farmácia Hebel encontra-se situada no centro da Vila de Souselas, apresentando um horário de funcionamento das 9h às 20h de segunda-feira a sexta-feira e das 9h às 13h ao sábado. O fornecimento de medicamentos é assegurado por dois fornecedores diários e um fornecedor ocasional.

Esta conta essencialmente com utentes de diversas localidades que se localizam nas proximidades de Souselas, tais como Botão, Marmeleira, São Martinho do Pinheiro, Zouparria do Monte, Sargento-Mor, Torre de Vilela, entre outras. Adicionalmente, esta farmácia é responsável pelo fornecimento de medicamentos e produtos de saúde a um lar de idosos situado na Vila de Souselas.

A farmácia Hebel é relativamente pequena, integrando uma área de atendimento ao público, um gabinete de atendimento individualizado, um armazém e um laboratório. Para além disso, inclui instalações sanitárias e um Gabinete de Direção Técnica.

3. Análise SWOT

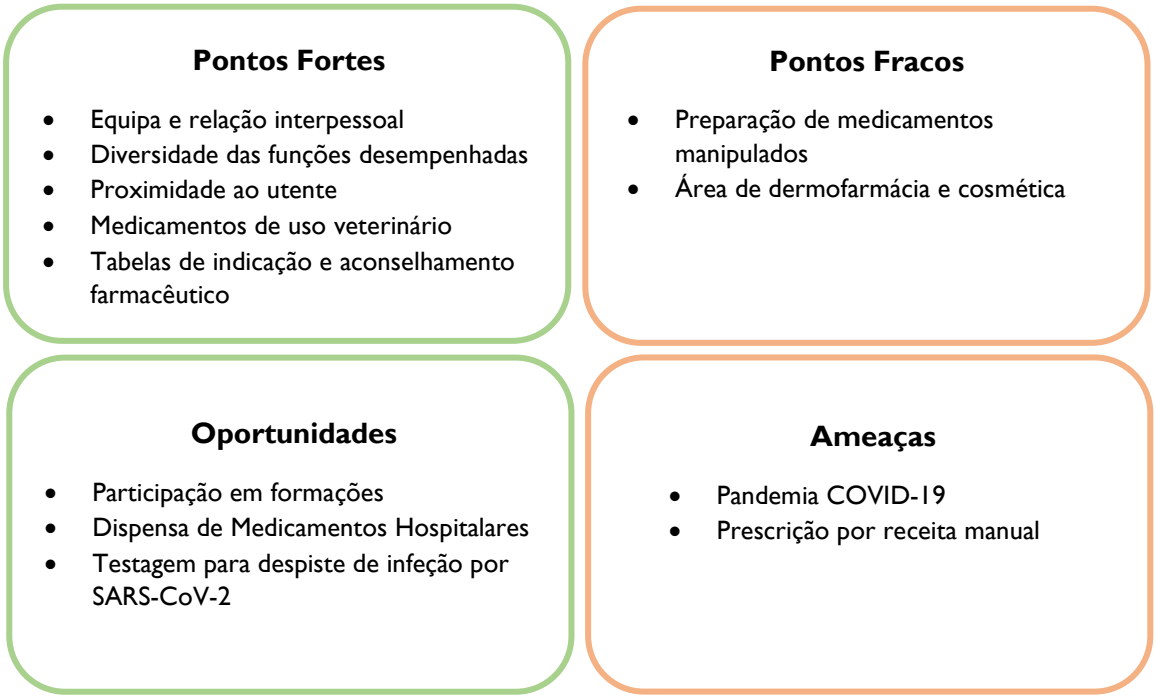

Figura 1- Análise SWOT do estágio realizado na farmácia Hebel.

3.1. Pontos Fortes

3.1.1. Equipa e relação interpessoal

A equipa da farmácia Hebel é constituída por quatro farmacêuticos: a diretora técnica Dra. Sara Terra, o Dr. Pedro Rodrigues, a Dra. Beatriz Craveiro e a Dra. Francisca Janeiro. Para além disso, esta integra também uma técnica de farmácia, Natércia Quinteiro, uma técnica administrativa, Vânia Chouriceiro, e um técnico oficial de contas, Pedro Baganha.

A competência, o espírito de equipa, o bom ambiente vivido na farmácia, aliados à constante disponibilidade da equipa para me auxiliar quando necessário ou para tirar qualquer dúvida, foram fulcrais para que rapidamente me sentisse integrada e para a minha evolução ao longo de todo o estágio, constituindo assim um ponto forte do meu estágio.

3.1.2. Diversidade das funções desempenhadas

Ao longo do período de estágio foi-me permitido desempenhar uma série de funções inerentes ao quotidiano de um farmacêutico comunitário. Enquanto numa fase inicial realizava as diferentes tarefas com o acompanhamento dos diferentes membros da equipa, com o passar do tempo fui adquirindo alguma autonomia na sua elaboração.

Backoffice

No decorrer do estágio tive a oportunidade de desempenhar diversas tarefas de *backoffice*, essencialmente relacionadas com o aprovisionamento, armazenamento e gestão de *stocks*, o que incluiu conferir prazos de validade, regularizar devoluções, rececionar encomendas e armazenar os respetivos produtos no devido local. Estas tarefas condicionam o funcionamento de toda a farmácia, devendo por isso ser realizadas com o mesmo rigor que as demais.

Aquando da receção de encomendas era fundamental conferir se a quantidade encomendada correspondia à quantidade fornecida, de modo a evitar a ocorrência de erros de *stock*. Adicionalmente, eram verificados o prazo de validade dos produtos rececionados e conferidos o preço de custo e o preço de venda ao público. Para além disso, era também nesta fase que segregava os produtos que tinham ficado reservados para determinados utentes, e que iriam ser armazenados em local próprio, dos restantes produtos. Depois de rececionar os medicamentos e outros produtos de saúde e bem-estar, procedia ao seu armazenamento ou exposição, dependendo do tipo de produto, nos devidos locais, seguindo o princípio *first in first out*.

A meu ver, desempenhar este tipo de tarefas foi fundamental para uma maior familiarização com os produtos, permitindo associar os produtos à respetiva embalagem e, no caso dos medicamentos, associar o nome do princípio ativo ao respetivo nome comercial. Estes conhecimentos adquiridos demonstraram-se de grande utilidade na fase de atendimento ao balcão, uma vez que possibilitaram que este se tornasse mais fluente.

Atendimento ao público

Desde o início do estágio, em paralelo com as tarefas realizadas em *backoffice,* comecei a acompanhar os diferentes membros da equipa no atendimento ao público. Nesta fase inicial apenas observava como procediam ao atendimento, prestando atenção à forma como abordavam os utentes, às questões que lhe eram colocadas e informações que eram dadas relativas ao modo de utilização ou toma de um produto ou medicamento. Além disso, tentava acompanhar o modo de funcionamento do programa SIFARMA® ao longo de todo o processo de atendimento. Progressivamente, à medida que fui ganhando mais autonomia e confiança, fui começando a realizar atendimentos com supervisão dos colegas, tentando sempre adaptar o discurso ao tipo de utente e, sempre que pertinente, aconselhar medidas não farmacológicas.

Serviços Farmacêuticos

A farmácia dispõe de vários serviços de promoção da saúde e do bem-estar dos utentes tais como a avaliação de parâmetros antropométricos (peso e altura), medição da pressão arterial, e medição de parâmetros bioquímicos, nomeadamente, medição da glicémia, do colesterol total e triglicerídeos. Também neste âmbito tive oportunidade de, numa fase inicial, acompanhar os colegas no ato de prestação deste tipo de serviços e, posteriormente, começar eu própria a realizar estas funções. Para além disso, na farmácia Hebel também se administram medicamentos injetáveis e vacinas não incluídas no Plano Nacional de Vacinação. Sendo que para a prestação deste serviço é necessária certificação, apenas tive oportunidade de observar diferentes membros da equipa a proceder à administração de injetáveis. No entanto, foi muito útil fazer este acompanhamento pois, por um lado, permitiu perceber como é feita a abordagem ao utente e adquirir conhecimentos que certamente serão muito úteis no futuro. Por outro lado, tive a oportunidade de aprender a fazer o registo da prestação deste tipo de serviço no programa SIFARMA®.

3.1.3. Medicamentos de Uso Veterinário

A farmácia Hebel está inserida num meio rural e, por isso, muitas pessoas recorrem a esta para obter aconselhamento farmacêutico na área da veterinária, quer para os animais de companhia, quer para os animais de criação.

Apesar do plano curricular do MICF contemplar uma unidade curricular direcionada para medicamentos de uso veterinário, esta não abrange algumas das situações que me foram surgindo. Efetivamente, ao longo de todo o estágio fui confrontada com questões relacionadas com esta área, sendo que por vezes não sabia como aconselhar devidamente, tendo sido crucial o auxílio dos diferentes membros da equipa da farmácia. Assim, o facto de a área veterinária ser um ponto forte desta farmácia, permitiu-me alargar e adquirir novos conhecimentos neste campo.

Complementarmente, a farmácia Hebel é uma farmácia espaço animal, havendo a possibilidade de, perante casos mais específicos, falar com os médicos veterinários do espaço animal de modo a aconselhar o utente de forma personalizada e correta.

3.1.4. Tabelas de indicação e aconselhamento farmacêutico

Apesar de todo o conhecimento adquirido ao longo dos cinco anos do curso, numa fase inicial do estágio, senti alguma dificuldade em fazer um aconselhamento farmacêutico de valor no que se refere a medicamentos não sujeitos a receita médica (MNSRM) ou suplementos alimentares direcionados para afeções menores, muito devido à enorme variedade que existe destes produtos. No entanto, este obstáculo foi sendo ultrapassado, à conta da existência de tabelas de indicação e aconselhamento farmacêutico realizadas pelos farmacêuticos desta farmácia e afixadas no *backoffice*. Estas incluíam, como informação distribuída em diferentes colunas, a afeção em causa, o nome dos produtos disponíveis na farmácia com indicação para essa afeção, a posologia dos mesmos e, quando apropriado, medidas não farmacológicas que deveriam ser referidas aquando da sua dispensa ao utente.

Efetivamente, a existência destas tabelas foi muito útil no decorrer de todo o meu estágio, principalmente para associar os nomes de muitos MNSRM e suplementos alimentares às suas indicações, mas também para relembrar as medidas não farmacológicas que devem ser mencionadas em cada situação e que podem fazer a diferença nestas situações.

3.2. Pontos Fracos

3.2.1. Preparação de medicamentos manipulados

Como consequência da crescente industrialização do medicamento, a prescrição de medicamentos manipulados tem vindo a diminuir, levando a que muitas farmácias tenham vindo a abandonar a preparação deste tipo de medicamentos.

Efetivamente, a farmácia Hebel não dispõe de muitas matérias-primas necessárias à preparação de vários medicamentos manipulados, devido ao facto de ser cada vez mais difícil a sua aquisição, mas também devido ao reduzido número de requisições deste tipo de medicamentos. Portanto, quando surge um utente com uma prescrição de um medicamento manipulado cuja composição inclua matérias-primas que estão em falta na farmácia, esta solicita a preparação do manipulado a outra farmácia. Com efeito, a preparação de medicamentos manipulados na farmácia Hebel não é algo frequente. Na verdade, ao longo de todo o meu estágio surgiram apenas duas prescrições de medicamentos manipulados. Assim, tive a oportunidade de preparar um deles de acordo com a monografia presente no Formulário Galénico Português, nomeadamente, uma solução alcoólica de ácido bórico à saturação,

Em suma, como estagiária considero um ponto fraco não ter tido possibilidade de preparar mais medicamentos pertencentes a esta categoria, uma vez que é uma tarefa que faz parte das funções do farmacêutico, sendo este responsável por assegurar a qualidade e verificar a segurança do medicamento que prepara. Por outro lado, permitiria colocar em prática os conhecimentos adquiridos na unidade curricular Farmácia Galénica.

3.2.2. Área de dermofarmácia e cosmética

Na gestão de uma farmácia comunitária é de extrema importância adaptar o *stock* de produtos existente à população que esta serve, de forma a ir de encontro às suas necessidades e preferências.

Efetivamente, a área de dermofarmácia e cosmética está em constante evolução e crescimento, sendo que, nos dias que correm, há uma crescente preocupação com a imagem por parte das pessoas. Apesar disso, na farmácia Hebel verifica-se uma fraca procura de produtos dermocosméticos e, consequentemente, neste espaço, a aposta nesta área é limitada. Na verdade, são diversos os fatores que contribuem para esta fraca procura por parte dos utentes. Por um lado, o facto de a farmácia estar inserida num meio rural, com uma população envelhecida, onde não existe um grande poder de compra, leva a que não se priorize a imagem e beleza, acabando por ser uma prioridade a aquisição de medicamentos e não de produtos de dermofarmácia e cosmética. Para além disso, nas proximidades existe concorrência no que se refere à venda desta categoria de produtos, quer os espaços de venda de medicamentos não sujeitos a receita médica, quer, para alguns produtos, os hipermercados. Estes tornam-se mais apelativos uma vez que dispõem de preços mais competitivos e possuem uma maior variedade de escolha, com diferentes gamas à disposição.

Deste modo, a farmácia Hebel dispõe de alguns produtos de diferentes marcas como Avene®, La Roche Posay®, Ducray® e Vichy®, contudo, apostando mais nos produtos dos Laboratórios Babé®, apresentando o portefólio completo. Assim, apesar da reduzida variedade, a escolha e gestão destes produtos é feita de forma cuidadosa, garantindo que aqueles existentes em *stock* vão de encontro às necessidades e gostos dos utentes, através da eleição de uma gama de produtos com uma boa relação qualidade-preço.

Assim, considero que a área de dermofarmácia e cosmética foi um dos pontos fracos do meu estágio uma vez que não tive oportunidade de contactar com diferentes gamas e produtos inovadores e de fazer um aconselhamento especializado relativo a esses mesmos produtos.

3.3. Oportunidades

3.3.1. Participação em formações

Tendo em conta que a área das ciências farmacêuticas e médicas está em constante evolução, o farmacêutico deve manter atualizadas as suas capacidades técnicas e científicas com o objetivo de aperfeiçoar constantemente a sua atividade,[3] permitindo prestar o melhor aconselhamento aos utentes.

De facto, desde logo percebi que a Dra. Sara tem este aspeto em consideração, incentivando sempre a atualização de conhecimentos por parte de toda a equipa. Efetivamente, a Dra. Sara incentivou a minha inscrição no site da ANF online, uma vez que através deste é possível aceder a informações relevantes no âmbito da farmácia comunitária, como por exemplo, publicações que abordam temas relacionados com as várias afeções com que somos muitas vezes confrontados na farmácia, notícias e circulares. Adicionalmente, através deste site é possível aceder à plataforma E-learning da Escola de Pós-graduação em Saúde e Gestão, que contempla várias formações online nas quais nos podemos inscrever para participar, ou ainda assistir a gravações de webinares que abordam variadíssimos temas.

Deste modo, tive a oportunidade de participar na formação "Noções básicas de Saúde Animal para um bom aconselhamento na farmácia", que constituiu uma mais-valia na obtenção de conhecimentos relacionados com esta área.

Efetivamente, estas formações são essenciais pois permitem consolidar e atualizar conhecimentos, mas também conhecer melhor alguns produtos, o que se mostra bastante vantajoso no momento de atendimento ao utente e respetivo aconselhamento farmacêutico.

3.3.2. Dispensa de Medicamentos Hospitalares

O surgimento da pandemia de COVID-19 obrigou a adoção de várias medidas de contingência, entre as quais a restrição de deslocações que não fossem estritamente necessárias.

Tendo em consideração que a aquisição de medicamentos hospitalares por parte dos doentes não poderia ser suspensa, e de modo a reduzir significativamente a deslocação de doentes a hospitais apenas para aceder a medicamentos, foi criada a Operação Luz Verde. Esta constitui uma resposta à pandemia, que visa proteger doentes não COVID-19 que necessitem deste tipo de medicação, sendo muitas vezes doentes com um sistema imunitário fragilizado, evitando assim o risco associado à deslocação ao hospital. [4]

Apesar do período do meu estágio coincidir com a altura do levantamento de muitas restrições impostas devido à pandemia, este serviço de dispensa de medicamentos hospitalares na farmácia comunitária manteve-se até então. Assim, tive a possibilidade de acompanhar todo o processo envolvido nesta tarefa, desde a receção dos medicamentos, passando pelo contacto do utente, pela dispensa, aconselhamento e respetivo registo informático, bem como pelo envio mensal do detalhe das dispensas aos serviços farmacêuticos do hospital em causa. Este é um serviço que está a ser prestado por todas as farmácias comunitárias, sem haver qualquer pagamento por parte do Serviço Nacional de Saúde.

3.3.3. Testagem para despiste de infeção por SARS-CoV-2

Numa tentativa de reforçar o controlo da pandemia COVID-19, o Governo adotou medidas com vista à prevenção, contenção e mitigação da transmissão do SARS-CoV-2. Uma dessas medidas consistiu no reforço da realização de testes para deteção deste vírus, através da aplicação de um regime excecional e temporário de comparticipação de testes rápidos de uso profissional a todos os utentes do Serviço Nacional de Saúde. [5, 6] Nesta fase, ao poderem realizar esta testagem, as farmácias comunitárias, tiveram um papel preponderante.

Efetivamente, quando iniciei o estágio, e ao longo de todo o seu período, esta medida ainda se manteve. Tendo a farmácia Hebel aderido à realização destes testes, tive a oportunidade de acompanhar e participar em toda a logística relacionada com este serviço, desde a marcação dos testes, passando pela admissão dos utentes e terminando com a notificação dos resultados dos testes.

Ademais, o facto da farmácia Hebel disponibilizar este serviço aos utentes, fez com que houvesse uma maior afluência a este espaço. De facto, tal situação permitiu um maior contacto

com os utentes e esclarecimento de todas as suas dúvidas, quer relacionadas com o tempo de confinamento, quer relacionadas com contactos de risco, mas também com o uso de medicamentos para o alívio dos sintomas provocados pela infeção por SARS-CoV-2. Na verdade, considero que tudo isto tenha contribuído para um crescimento das minhas competências, sobretudo ao nível da comunicação e interação com o utente.

3.4. Ameaças

3.4.1. Pandemia COVID-19

O surgimento da pandemia COVID-19 veio condicionar o quotidiano de toda a população. Embora na altura em que realizei o estágio em farmácia muitas das medidas de contingência já haviam sido levantadas, a infeção por SARS-CoV-2 era uma ameaça constante, que poderia condicionar a normal realização do estágio, uma vez que caso contraísse a infeção, teria de ficar em isolamento durante 7 dias. Isto traria perturbações por um lado ao nível das horas de estágio que são necessárias realizar para a conclusão do mesmo e, por outro lado, iria levar a uma quebra no ritmo de trabalho e aprendizagem. Felizmente tal situação não se verificou, tendo conseguido terminar o estágio sem qualquer interrupção.

Além disso, o uso obrigatório de máscara e a existência de acrílicos de proteção nos balcões, embora constituíssem uma barreira à propagação do vírus, simultaneamente dificultaram a comunicação com o utente, sobretudo com a população geriátrica.

3.4.2. Prescrição por receita manual

Ao longo de todo o período de estágio, contactei com os diferentes tipos de receituário, nomeadamente, prescrição eletrónica materializada, prescrição eletrónica desmaterializada e prescrição manual.

Efetivamente, fiquei surpreendida com a quantidade de receitas manuais que surgiam na farmácia, uma vez que e menos fluído. esta é apenas permitida em situações excecionais de acordo com a legislação em vigor. [7] De facto, senti que o meu atendimento aos utentes que tinham na sua posse receitas manuais era afetado devido à constante incompreensão do que nelas estava redigido, gerando insegurança no atendimento e, por isso, tendo de recorrer na maioria das vezes aos restantes membros da equipa para tirar as dúvidas. Para além disso, este tipo de prescrição tem de obedecer a regras específicas e bem-definidas, [7] cujo cumprimento tinha de ser averiguado no ato de dispensa dos medicamentos. Assim, este tipo de prescrição tornava o atendimento mais moroso.

4. Considerações Finais

O estágio em farmácia comunitária constituiu uma etapa fundamental no meu percurso académico. Na verdade, este possibilitou, por um lado, visualizar e aplicar na prática diversos conhecimentos adquiridos ao longo dos 5 anos do MICF e, por outro lado, aprofundar e adquirir outros conhecimentos, bem como desenvolver novas competências que certamente terão grande utilidade a nível profissional.

Efetivamente, o estágio em farmácia comunitária permite adquirir uma visão do mundo real, sendo algo desafiante, mas simultaneamente muito gratificante. De facto, considero que este estágio me enriqueceu bastante enquanto futura farmacêutica.

Por fim, realço o papel de toda a equipa, que desde o início me acolheu e que fomentou um ambiente de constante aprendizagem e melhoria. A eles, o meu agradecimento por toda a disponibilidade, pelos conhecimentos transmitidos e pela boa disposição.

5. Casos Práticos

Caso Prático 1

Um senhor deslocou-se à farmácia solicitando uma embalagem de Antigrippine® para uma gripe que julga ter contraído devido ao frio que apanhou há 2 dias atrás. Questionei o utente sobre os sintomas que apresentava, ao qual respondeu que estava com dores no corpo, dor de cabeça e tosse com alguma expetoração.

Estando em plena pandemia de COVID-19, em que a infeção por SARS-CoV-2 pode provocar os sintomas descritos, questionei se já tinha realizado algum teste de despiste à COVID-19, ao que respondeu negativamente. Perante esta resposta, e estando a ser realizados testes de antigénio comparticipados na farmácia, no momento em que o senhor apareceu, aconselhei-o a submeter-se à testagem para despiste de uma possível infeção pelo vírus SARS-CoV-2. Efetivamente, o senhor seguiu o conselho e foi realizar um teste, cujo resultado foi positivo. Este ficou surpreendido pois não tinha conhecimento de ter contactado com alguém positivo. Perante isto, o utente questionou como teria de proceder. Referi que teria de ficar em isolamento durante 7 dias e que, entretanto, a Saúde 24 iria entrar em contacto com ele, sendo que, se em 48h tal contacto não ocorresse, deveria ser este a contactar esta linha.

Além disso, o senhor solicitou que lhe cedesse algo para alívio dos sintomas. Assim, para as dores de cabeça e do corpo recomendei o paracetamol 500 mg de 8h em 8h, durante 3 dias, podendo tomar 2 comprimidos caso 1 não aliviasse a sintomatologia. Adicionalmente,

como o senhor apresentava tosse com alguma expetoração, após certificar-me de que este não era asmático e que não era portador de úlceras gastroduodenais, recomendei a toma de 1 comprimido efervescente por dia de acetilcisteína 600 mg. Para além disso, recomendei beber muita água e, se necessário, elevar a cabeceira da cama durante a noite, de modo a aliviar a tosse.

Caso Prático 2

Uma senhora dirigiu-se à farmácia solicitando algo para a azia que estava a sentir. Perguntei há quanto tempo se sentia assim, respondendo-me que tinha começado depois do almoço. Questionei ainda se era algo que sentia frequentemente, se tinha outros sintomas e se já tinha tomado algo para o alívio desta condição, tendo respondido que não. Posto isto, apresentei alguns medicamentos antiácidos como opção, nomeadamente, Gaviscon®, quer na forma de suspensão oral em saquetas, quer de comprimidos mastigáveis e Rennie® Dual Action. Uma vez que a utente mostrou preferência pelas saquetas, optou por levar Gaviscon® saquetas com sabor a menta, sendo que, antes de ceder este medicamento, certifiquei-me que a senhora não sofria de insuficiência cardíaca congestiva ou insuficiência renal. Assim, indiquei que deveria tomar 1 a 2 saquetas após as refeições e ao deitar, até 4 vezes por dia, tentando espaçar a toma deste medicamento da toma de outros, efetuando um intervalo de 2h entre as tomas. Referi ainda que, caso após 7 dias os sintomas persistissem, deveria consultar o médico pois a azia poderia ser um sintoma de outra patologia mais grave.

Adicionalmente, referi algumas medidas não farmacológicas que poderiam ajudar durante o episódio de azia, nomeadamente, dormir com a cabeceira da cama elevada e evitar alimentos que possam agravar os sintomas, tais como alimentos ácidos, condimentados, picantes, bebidas gaseificadas, álcool, alimentos fritos e com muita gordura. Para além disso, ainda indiquei algumas medidas preventivas, de forma a evitar o surgimento de azia, como comer devagar e mastigar bem os alimentos, fazer várias refeições ligeiras por dia, iniciando a última refeição 3h antes da hora de deitar e evitar roupa muito justa e apertada na zona da cintura.

Caso Prático 3

Uma senhora deslocou-se à farmácia queixando-se de um desconforto a nível vaginal. Ao tentar perceber melhor o tipo de sintomatologia percebi que se poderia tratar de uma candidíase, uma vez que a utente apresentava ardor vaginal, prurido e eritema vulvar e corrimento esbranquiçado sem odor muito forte. Questionei-a sobre a duração dos sintomas

e se era algo frequente, tendo respondido que era a primeira vez que lhe acontecia e que surgiu depois de ter tido uma infeção respiratória. Tendo isto em consideração, perguntei à utente se tinha tomado antibióticos para a infeção, tendo respondido afirmativamente. Assim, expliquei à utente que muito provavelmente seria uma candidíase vaginal e que poderá ter sido provocada pela toma do antibiótico, como consequência de um desequilíbrio ao nível da flora vaginal.

Deste modo, uma vez que a utente apresentava sintomas externos muito incomodativos, optei por aconselhar um antifúngico tópico na forma de creme vaginal, nomeadamente Gino-Canesten® creme vaginal, contendo clotrimazol 1%. Assim, expliquei que deveria fazer a aplicação do creme internamente, com o auxílio de um aplicador, antes de deitar, de modo a tratar a infeção na origem, e aplicar externamente, a nível vulvar, de modo a aliviar o prurido, fazendo este tratamento durante 6 dias. Para além disso, referi a que a existência de suplementos alimentares que auxiliam na recuperação e manutenção da flora vaginal, que poderiam se uma mais-valia nesta situação. Tendo verificado que a utente mostrou interesse neste tipo de produtos, aconselhei a toma de 1 cápsula por dia do suplemento alimentar Advancis® BacilPro Gyno. Este produto contém na sua composição um conjunto de estirpes bacterianas, entre as quais *Lactobacillus sp,* que atuam em sinergia na colonização e consequente proteção do aparelho geniturinário. Para além disso, aconselhei o uso de um gel íntimo suave para higiene diária, sendo que a senhora referiu que já o fazia.

Adicionalmente, referi algumas medidas não farmacológicas de modo a prevenir a ocorrência deste tipo de situação, nomeadamente, após a ida à casa de banho, limpar a área com um movimento de frente para trás, manter a área genital limpa e seca, evitar duches vaginais, utilizar roupa interior de algodão e evitar roupas demasiado justas.

Caso Prático 4

Um utente jovem dirigiu-se à farmácia pedindo algo para um herpes labial que lhe surgira durante a noite. Posto isto, e devido ao facto de o utente estar a usar máscara, questionei se a lesão ainda estava numa fase inicial ou se, pelo contrário, já estava numa fase mais evoluída. O jovem respondeu que apenas sentia um formigueiro e comichão numa zona do lábio, sendo que ainda não era bem visível uma lesão. Tendo isto em conta, recomendei a aplicação do creme Zovirax® Duo 50 mg/g + 10 mg/g uma vez que na sua composição, para além do antivírico aciclovir que trata e impede a progressão do herpes labial provocado pelo vírus *Herpes Simplex*, também contém hidrocortisona, que impede o aparecimento da lesão ulcerosa. Indiquei ainda que deveria iniciar a aplicação o quanto antes, de modo a evitar o

surgimento da lesão ulcerosa, aplicando de 4h em 4h durante 5 dias, lavando sempre bem as mãos antes e depois da aplicação.

Adicionalmente, de modo a prevenir o surgimento deste tipo de situações com frequência, recomendei a hidratação dos lábios com um stick labial com fator de proteção solar, uma vez que a exposição solar pode constituir um fator desencadeante no aparecimento do herpes labial.

Caso Prático 5

Uma rapariga jovem deslocou-se à farmácia com uma receita contendo uma embalagem de fosfomicina 3000 mg, pois havia sido diagnosticada com uma cistite. Esta apresentava-se queixosa, referindo que no decorrente ano já era a segunda vez que ocorrera. Tendo isto em conta, mencionei algumas medidas não farmacológicas que deveria adotar, tais como, beber água com abundância (pelo menos 1,5L por dia), urinar e lavar a vagina após o ato sexual, após a ida à casa de banho limpar a área com um movimento de frente para trás, evitar o uso de pensos diários, não usar roupa íntima sintética, mas de algodão, não deixar de urinar quando tem vontade e esvaziar a bexiga até ao fim.

Adicionalmente, referi a existência de suplementos alimentares que podem ajudar na profilaxia de infeções do trato urinário, contribuindo para a redução da incidência e da recorrência deste tipo de infeções, como por exemplo o produto Cranfort®. Assim, mencionei que caso se tornasse uma situação muito recorrente, poderia optar por iniciar a toma deste tipo de produtos, de modo a tentar evitar o surgimento de novas infeções urinárias.

Finalmente, expliquei também que, no que diz respeito à fosfomicina, deveria dissolver o conteúdo da saqueta num copo com água e ingeri-lo numa só toma com o estômago vazio, ou seja, 2 a 3h após uma refeição e após o esvaziamento da bexiga.

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PARTE II

Relatório de Estágio em Indústria Farmacêutica

Bluepharma Indústria Farmacêutica, S.A.

Departamento de Investigação e Inovação

Sob a orientação da Doutora Ana Catarina Pinto

Lista de Abreviaturas

BLPH- Bluepharma Indústria Farmacêutica, S.A.

EMA- *European Medicines Agency*

FDA- *Food and Drug Administration*

I&I- Investigação e Inovação

MICF- Mestrado Integrado em Ciências Farmacêuticas

SWOT- *Strengths, Weaknesses, Opportunities, Threats*

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) contempla um plano de estudos abrangente e multidisciplinar, permitindo aos estudantes enveredar pelas diversas áreas relevantes na área do medicamento. No âmbito da unidade curricular "Estágio Curricular", onde é feito o acompanhamento tutorial do estudante em ambiente real de trabalho, é dada a possibilidade aos finalistas do MICF de estagiarem noutras áreas, para além de farmácia comunitária, através do estabelecimento de protocolos com diferentes entidades. De facto, esta oportunidade permite aos alunos enriquecerem o seu percurso académico, experienciando atividades relacionadas com as diferentes áreas de atuação do farmacêutico.

O surgimento e crescimento da indústria farmacêutica potenciou a descoberta de novos fármacos e a consequente produção de medicamentos para doenças até então incuráveis, contribuindo assim para enormes ganhos em saúde.[1] De facto, a área de atividade da indústria farmacêutica caracteriza-se pela sua multidisciplinariedade, demonstrando grande impacto na sociedade.

Assim, devido à minha curiosidade relativa a esta área, fomentada ao longo do meu percurso académico, optei por realizar um estágio em indústria farmacêutica. Com efeito, após um processo de candidatura, entrevista e seleção, tive a oportunidade de efetuar este estágio na Bluepharma Indústria Farmacêutica, S.A. (BLPH), mais concretamente no departamento de Investigação e Inovação (I&I). Este estágio decorreu entre os dias 2 de maio e 29 de julho de 2022, sob a orientação da Doutora Ana Catarina Pinto responsável dos Assuntos Científicos, e supervisão do Doutor António Lucas Nunes, Diretor do Departamento.

O presente relatório, elaborado no âmbito da unidade curricular "Estágio Curricular", tem como objetivo fazer uma avaliação global deste estágio, apresentando-se sob a forma de uma análise SWOT, identificativa dos pontos fortes (*Strenghts*), pontos fracos (*Weaknesses*), oportunidades (*Opportunities*) e ameaças (*Threats*).

2. Bluepharma Indústria Farmacêutica, S.A.

A BLPH é um grupo farmacêutico português de capitais privados, sediada em São Martinho do Bispo, Coimbra, que iniciou a sua atividade em 2001, sendo atualmente constituída por 20 empresas inovadoras que abrangem todas as fases da cadeia de valor do medicamento. [2, 3] Atualmente, a BLPH exporta a sua produção para mais de 40 países, tendo aberto escritórios em 4 territórios, nomeadamente, Espanha, Angola, Moçambique e Estados Unidos da América. [4] Esta empresa oferece uma abordagem integrada, desde a investigação e

desenvolvimento, até à produção e comercialização de medicamentos, possuindo um portefólio amplo e diferenciado e regendo-se por altos padrões de qualidade[3]. Efetivamente, a BLPH aposta no investimento contínuo em pessoas, instalações e novos equipamentos com a missão de investigar e desenvolver medicamentos de elevado valor acrescentado. [5]

Na BLPH, o departamento de I&I está inserido no departamento de Parcerias Estratégicas e Desenvolvimento de Produto, segmentando-se em duas unidades que se complementam, nomeadamente, Inovação Tecnológica e Assuntos Científicos.

3. Análise SWOT

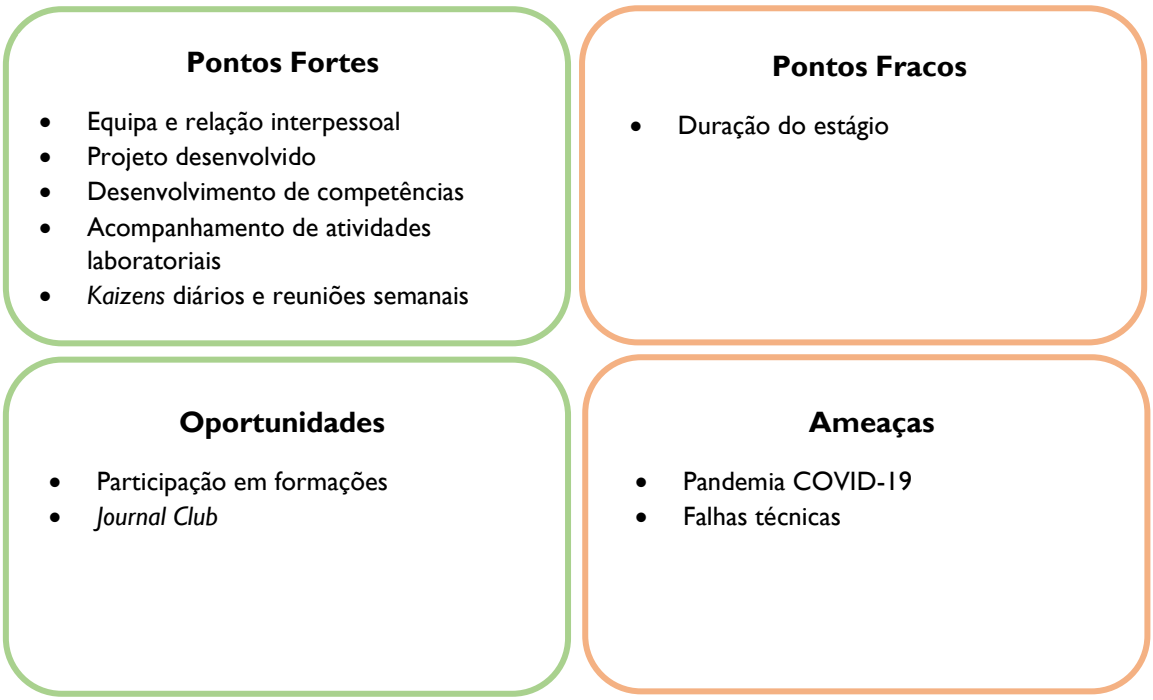

Figura 2- Análise SWOT do estágio realizado na Bluepharma Indústria Farmacêutica, S.A.

3.1. Pontos Fortes

3.1.1. Equipa e Relação Interpessoal

O departamento de I&I é constituído por uma equipa jovem e dinâmica, caracterizada pela multidisciplinariedade e rigor no exercício das suas funções. Os vários colaboradores estão distribuídos pelas duas unidades do departamento, apresentando funções específicas e bem definidas na Matriz Funcional, encontrando-se, no entanto, em constante cooperativismo, sendo notório o espírito de equipa.

O profissionalismo da equipa e bom ambiente vivenciado, aliados à disponibilidade para me auxiliar sempre que necessário e à inclusão das atividades relacionadas com o meu estágio no planeamento da equipa, contribuíram para uma rápida integração, e facilitou todo o processo de aprendizagem e evolução.

3.1.2. Projeto Desenvolvido

No início do estágio na BLPH foi-me proposto pela Doutora Ana Catarina Pinto desenvolver um trabalho de pesquisa abordando o tema "Estudos não clínicos: foco nos medicamentos complexos não biológicos". Efetivamente, este tema foi selecionado tendo em conta o interesse da empresa no desenvolvimento deste tipo de medicamentos, constituindo a fase de estudos não clínicos uma etapa fundamental na sua cadeia de desenvolvimento.

Com efeito, a realização deste trabalho envolveu a elaboração de um relatório e de uma apresentação em PowerPoint em que, numa primeira parte, abordei o tema dos estudos não clínicos, explicando em que consistem, os diferentes tipos de testes que são realizados durante esta fase, bem como os seus principais objetivos e características. Numa segunda parte, expus a temática relativa aos medicamentos complexos não biológicos, primeiramente, definindo-os e, posteriormente, fazendo uma breve referência ao seu enquadramento regulamentar na União Europeia e nos Estados Unidos da América. Finalmente, concluí o relatório apresentando vários casos de estudo. Estes consistiram em investigar, para diferentes produtos considerados medicamentos complexos não biológicos e, quando aplicável, para os seus *follow-on products*, o tipo de estudos não clínicos que foram realizados e apresentados quer à *Food and Drug Administration* (FDA), quer à *European Medicines Agency* (EMA) aquando da submissão da autorização de introdução nos respetivos mercados.

Na verdade, considero que a concretização deste desafio constituiu uma mais-valia no meu estágio, uma vez que consistiu na abordagem de um tema que não é muito versado nas diferentes unidades curriculares que compõem o MICF. Assim, a sua realização permitiu, por um lado, aprofundar conhecimentos relativos aos estudos não clínicos como etapa integrante do desenvolvimento de medicamentos e, por outro lado, obter novos conhecimentos, nomeadamente no que diz respeito ao conceito de medicamentos complexos não biológicos.

3.1.3. Desenvolvimento de Competências

O estágio realizado na BLPH foi muito enriquecedor, permitindo o desenvolvimento de várias competências, nomeadamente a nível de pesquisa, linguístico e de ferramentas informáticas.

O desenvolvimento de competências a nível de pesquisa foi essencial uma vez que a tarefa principal do meu estágio implicava pesquisa e compilação de informação. Assim, foi possível aprofundar conhecimentos relativamente ao uso do motor de busca PubMed, uma ferramenta essencial para a pesquisa de artigos científicos, mas também aprender a pesquisar no website das entidades reguladoras do medicamento, mais concretamente da EMA, FDA e *Medicines and Healthcare products Regulatory Agency*, permitindo encontrar informação fidedigna e atualizada. Complementarmente, fui ganhando competências relativamente à capacidade de síntese e seleção crítica de informação relevante.

Adicionalmente, uma vez que todos os documentos consultados estavam escritos em inglês e o facto de ter de elaborar o relatório e a apresentação, relativos ao tema que me foi atribuído, nessa língua, durante todo o estágio estive em permanente contacto com este idioma. Assim, isto fomentou o desenvolvimento das minhas aptidões ao nível da língua inglesa, essencialmente relacionados com a leitura e escrita.

No que diz respeito ao desenvolvimento de competências ao nível de ferramentas informáticas, este está relacionado com a utilização constante do Microsoft Word, Powerpoint e Excel como ferramentas de trabalho, o que me permitiu adquirir conhecimento de várias funcionalidades que até então desconhecia.

3.1.4. Acompanhamento de Atividades Laboratoriais

Apesar do meu estágio no departamento de I&I da BLPH se prender essencialmente com pesquisa e compilação de informação relativa ao tema que me foi atribuído, tive ainda a oportunidade de acompanhar algumas atividades laboratoriais desenvolvidas pelos diferentes membros da equipa. Por um lado, foi possível acompanhar atividades mais relacionadas com a vertente de desenvolvimento e caracterização de formulação, sobretudo ensaios de *Design of Experiments,* em que se faz variar simultaneamente vários fatores do processo de forma a determinar atributos-chave do processo e quais os fatores que exercem influência sobre a qualidade e estabilidade do produto. Por outro lado, foi também possível acompanhar atividades relacionadas com o desenvolvimento analítico, essencialmente relacionadas com a técnica de cromatografia líquida de alta eficiência, como a preparação de fases móveis e de padrões a serem usados em análises que recorram a este método.

Em suma, a interação com a equipa laboratorial revelou-se bastante útil uma vez que possibilitou o contacto com as tecnologias e técnicas de caracterização desenvolvidas internamente, permitindo entender de forma mais eficaz o trabalho desenvolvido no

departamento de I&I, mas também possibilitando perceber toda a dinâmica do ambiente laboratorial e cumprimento de Boas Práticas.

3.1.5. *Kaizens* Diários e Reuniões Semanais

Com a finalidade de aplicar uma abordagem de melhoria contínua, os diversos departamentos da BLPH aplicam a metodologia *Kaizen*, que significa mudança para melhor. [6] A realização de reuniões diárias em cada departamento, com duração máxima de 15 minutos, onde em cada dia da semana são abordados diferentes temas inerentes ao departamento, faz parte da aplicação desta metodologia. Para além disso, a existência de um quadro, na sala de cada departamento, com informação relevante para a equipa, por exemplo, assiduidade, indicadores, objetivos do departamento e desafios a eles inerentes, comunicações, faz também parte da aplicação da metodologia *Kaizen*.

Efetivamente, desde o início que fui incentivada a participar nas reuniões diárias do departamento de I&I, quer através da atribuição do meu estágio como tema a um dia da semana, em que expunha o ponto de situação relativo às tarefas que desenvolvera na semana anterior, quer através do acompanhamento dos restantes temas, podendo tirar dúvidas, ou participar na discussão de ideias. De facto, estas reuniões foram fundamentais para ficar inteirada do trabalho desenvolvido no departamento de I&I, bem como para ficar a par das diferentes tarefas e desafios de toda a equipa.

Adicionalmente, tive a oportunidade de ter reuniões semanais com a minha orientadora, onde me era dada abertura para contextualizar o trabalho que ia realizando ao longo da semana, bem como esclarecer qualquer dúvida, identificar pontos de melhoria e definir objetivos. Com efeito, estas reuniões constituíram uma mais-valia, permitindo, por um lado, uma maior proximidade com a orientadora, sendo o seu apoio imprescindível ao longo de todo o estágio e, por outro lado, permitindo perceber se o trabalho que ia desenvolvendo estava a ir de encontro ao pretendido.

3.2. Pontos Fracos

3.2.1. Duração do Estágio

O estágio em indústria farmacêutica teve uma duração prevista de 3 meses. Efetivamente, este é o tempo considerado mínimo na empresa para integração dos colaboradores, sendo que apenas depois deste período se começa a adquirir mais autonomia na realização de tarefas, sobretudo na vertente laboratorial. Assim, apesar de ter tido a oportunidade de acompanhar atividades laboratoriais, não tive a possibilidade de desempenhar

tarefas a este nível, muito devido à curta duração do estágio, considerando assim um ponto fraco.

3.3. Oportunidades

3.3.1. Participação em Formações

A BLPH é uma empresa que investe na melhoria contínua e na constante renovação do conhecimento de todos os colaboradores. Assim, estes são frequentemente têm a oportunidade de assistir a uma série de formações, quer por via remota, quer de modo presencial, dinamizadas pelo Departamento de Formação. Na verdade, estas formações também são incluídas no plano dos estagiários, tal como de qualquer outro colaborador da empresa. Efetivamente, a minha primeira semana de estágio foi muito focada na participação em formações iniciais de introdução, integração e contextualização relacionadas com diversos temas da empresa. Tudo isto ocorreu por meio da visualização de formações previamente gravadas, conduzidas por diferentes oradores, mas também através da leitura de normas e procedimentos da BLPH. Posteriormente, ao longo de todo o estágio, tive a oportunidade de participar em formações mais específicas, tendo em consideração o departamento em que estive inserida, assim como o tema do meu trabalho de estágio. De facto, considero que a oportunidade de realizar estas formações foi essencial, quer para ficar inteirada de informações relativas à empresa e ao seu modo de funcionamento, quer para aprofundar e adquirir novos conhecimentos relacionados com tecnologias e métodos usados na BLPH e úteis no contexto do departamento de I&I.

3.3.2. *Journal Club*

De forma a fomentar a aquisição e atualização de conhecimentos dos colaboradores, relativos a diversas áreas científicas, o departamento de I&I tomou a iniciativa de organizar reuniões científicas denominadas de *Journal Club*, realizadas quinzenalmente, por via remota. Para estas reuniões são convidados diferentes oradores, que expõem um tema relacionado com a sua área de atuação, fazendo apresentações de 20 minutos, com espaço para o esclarecimento de dúvidas que possam surgir.

Na verdade, ao longo de todo o estágio, tive a oportunidade de assistir a várias sessões do *Jounal Club*, que abordaram diversos temas e que permitiram aprofundar alguns conhecimentos, mas também adquirir outros, contribuindo, assim, para a minha cultura científica.

3.4. Ameaças

3.4.1. Pandemia COVID-19

Durante o período de estágio na BLPH o número de novas infeções por SARS-CoV-2 já não representava uma grande preocupação a nível nacional, coincidindo com a altura em que o uso de máscara deixou de ser obrigatório na grande maioria das situações. Apesar disso, o facto deste vírus ainda circular entre a população e da infeção por ele causada ainda implicar a realização de um período de isolamento de 7 dias, fez com que a pandemia constituísse uma ameaça à normal realização do estágio.

Na verdade, devido ao facto da principal tarefa do meu estágio se centrar na pesquisa e compilação de informação, tendo a hipótese de levar o computador portátil para casa, poderia haver a possibilidade de continuar o estágio por via remota, passando a laborar em regime de teletrabalho. No entanto, o acompanhamento das atividades laboratoriais iria ficar comprometido durante esse período, bem como a convivência com os colegas da equipa. Felizmente, esta situação acabou por não se verificar, tendo conseguido realizar a totalidade do estágio de forma presencial na empresa.

3.4.2. Falhas Técnicas

Devido ao facto da principal tarefa do meu estágio consistir no desenvolvimento de um trabalho de pesquisa, com a elaboração de um relatório e de uma apresentação, era estritamente necessário ter acesso à *internet* e ter disponível um computador para a concretização deste trabalho. Assim, possíveis falhas no equipamento informático, de energia ou mesmo somente ao nível da *internet* constituíam um entrave à realização desta tarefa. Ao longo de todo o estágio, este tipo de situações ocorreu duas vezes, embora que por breves minutos, impedindo durante esse tempo, de continuar a pesquisa que estava a realizar nesse momento. Deste modo, apesar de falhas técnicas deste tipo não terem sido frequentes nem duradouras quando ocorreram, considero que constituíram uma ameaça à normal realização do meu estágio.

4. Considerações Finais

A profissão farmacêutica possui múltiplos ramos e valências e, consequentemente, um vasto leque de saídas profissionais. Assim, a possibilidade de realizar estágios curriculares em áreas distintas constitui uma oportunidade para experienciar o que mais caracteriza cada uma delas.

Efetivamente, o estágio que realizei na BLPH foi o meu primeiro contacto com a indústria farmacêutica, através do qual constatei a abrangência do papel do farmacêutico neste setor de atividade. Adicionalmente, a realização deste estágio, para além de ter contribuído para enriquecimento dos meus conhecimentos, fomentou também a aquisição e o desenvolvimento de *soft skills*, como por exemplo, a organização, o desenvolvimento de um método de trabalho e a capacidade de gestão do tempo. Efetivamente, tudo isto, aliado ao facto deste estágio me ter obrigado a sair da zona de conforto, terá contribuído para um crescimento a nível profissional.

Durante todo o estágio fui orientada e auxiliada por uma equipa de profissionais que se caracteriza pelo rigor, competência e cooperação, valores que me foram transmitidos desde o início. Termino assim agradecendo a toda a equipa do departamento de I&I da BLPH por todos os ensinamentos e simpatia.

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PARTE III

Monografia

"Blood-brain barrier-on-a-chip for central nervous system disease modeling and drug screening"

Sob a orientação da Professora Doutora Alexandrina Mendes

List of Abbreviations

AC - Astrocyte

Ang2-Liposomes - Angiopep-2 coupled liposomes

ApoE - Apolipoprotein E

BBB - Blood-brain barrier

BCRP- Breast Cancer Resistance Protein

BEC - Brain endothelial cell

BMEC - Brain microvascular endothelial cell

CNS - Central Nervous System

EC - Endothelial cell

ECM - Extracellular matrix

FITC- Fluorescein isothiocyanate

GLUT-1- Glucose transporter 1

HBMEC - Human brain microvascular endothelial cell

HUVEC - Human umbilical vein endothelial cell

ICAM-1- Intercellular Adhesion Molecule 1

IL-6 - Interleukin-6

iPSC - Induced pluripotent stem cell

PC - Pericyte

PDGFRβ - Platelet-derived growth factor receptor β

PDMS - Polydimethylsiloxane

PECAM-1- Platelet endothelial cell adhesion molecule-1

P-gp - p-glycoprotein

TEER - Transendothelial electrical resistance

TJ - Tight junction

TNF α -Tumor Necrosis factor α

TOM20- Translocase of outer membrane 20

VE- Vascular endothelial

ZO-1- Zonula occludens-1

α-SMA- α-Smooth muscle actin

αSyn - Alpha-synuclein

Resumo

A barreira hematoencefálica é uma estrutura complexa e dinâmica que regula as moléculas que passam do sangue para o sistema nervoso central, mantendo a homeostase cerebral. No entanto, esta também constitui um obstáculo à entrega de fármacos. Adicionalmente, a disrupção da barreira hematoencefálica está associada a várias doenças neurológicas. Assim, modelos *in vitro* da barreira hematoencefálica que permitam o estudo da função e disfunção desta barreira são essenciais para investigação na área biomédica e no desenvolvimento de fármacos que tenham como alvo o sistema nervoso central. Nesse sentido, *BBB-on-chips* são modelos *in vitro* promissores que replicam mais fisiologicamente o ambiente *in vivo* da barreira hematoencefálica, permitindo obter resultados mais representativos e transponíveis para a situação clínica. Nesta revisão, serão descritos os diferentes componentes dos *BBB-on-chips* e as estratégias que têm sido utilizadas para os otimizar ao longo dos últimos anos.

Palvaras-chave: *BBB-on-a-chip*; Barreira hematoencefálica; *Screening* de fármacos; Modelação de doença; Microfluídica.

Abstract

Blood-brain barrier (BBB) is a complex dynamic structure that regulates the molecules that pass from the blood to the central nervous system (CNS), maintaining brain homeostasis. However, it also constitutes an obstacle to drug delivery. In addition, BBB disruption is associated with various neurologic diseases. Thus, *in vitro* BBB models that allow the study of BBB function and dysfunction are essential for biomedical research and the development of drugs targeting the CNS. In that regard, BBB-on-chips are promising *in vitro* models that replicate the *in vivo* BBB microenvironment more physiologically, allowing more representative and transferable results to the clinical situation. In this review, the different components of BBB-on-chips and the strategies that have been applied to optimize them over the last few years will be described.

Keywords: BBB-on-a-chip; Blood-brain barrier; Drug Screening; Disease Modeling; Microfluidic.

1. Introduction

Disorders of the central nervous system (CNS) have a considerable impact on society, being associated with high economic and social costs.[1] Despite constant discoveries on CNS physiology, the development of new drugs for this kind of disorder constitutes a significant challenge. Success rates in CNS drug development are lower than for other drug classes due to several factors, such as an insufficient understanding of the pathophysiology of complex CNS conditions and the existence of the blood-brain barrier (BBB). [2]

BBB is the term applied to the CNS microvasculature, comprised of brain endothelial cells (BECs) and its basement membrane. These cells are surrounded on their abluminal side by pericytes (PCs) and astrocytic perivascular endfeet, which also contribute to the formation and maintenance of the barrier that separates blood vessels from the brain tissue (Figure 1). [3, 4, 5] The cellular complex of BECs, PCs, astrocytes (ACs), microglia, and neurons and their functional interactions and signaling form the neurovascular unit. [3, 6, 7] The BBB is a complex dynamic, and highly organized structure, being the interface between the bloodstream and the brain, regulating molecules that pass from the blood to the CNS. Thus, BBB protects the CNS from potentially harmful elements and maintains cerebral homeostasis. Therefore, on the one hand, the restrictive nature of this structure constitutes an obstacle for drug delivery to the CNS since it inhibits the translocation of drugs from the bloodstream to neuronal tissue, impacting the success rate of new drugs. On the other hand, BBB dysfunction has been associated with several central nervous system diseases.[6] BBB disruption contributes to some neurological pathologies, for instance, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. In contrast, other CNS pathologies, such as trauma and stroke, may lead to BBB. [8, 9, 10, 11] Therefore, developing BBB models that closely mimic *in vivo* physiology is essential to understanding the pathological mechanisms underlying CNS diseases and enabling more efficient drug development for these disorders.

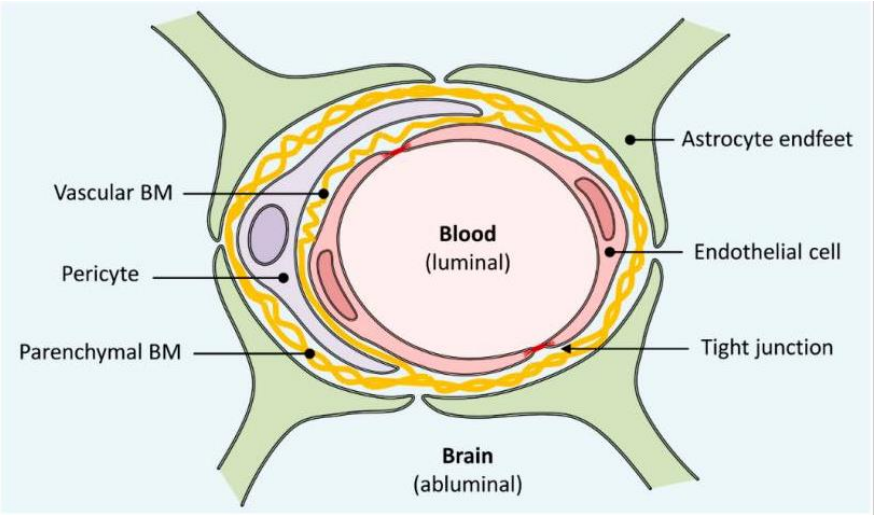

Figure 1- Schematic illustration of the blood-brain barrier (BBB). The complex BBB network consists of a monolayer of endothelial cells joined together by tight junction proteins, forming a blood vessel surrounded by pericytes, a vascular basement membrane (BM), astrocyte endfeet, and a parenchymal BM. Reproduced from Neumaier, Zlatopolskiy and Neumaier. [5]

So far, progress towards understanding human BBB pathophysiology and drug development has been achieved using *in vitro* studies and animal models. [12] *In vivo* animal models are considered the gold standard for studies on the BBB since they mimic more closely the complexity of the microenvironment found in this structure. [12, 13, 14] With these models, it is possible to monitor the effect of drugs at the cellular, tissue, organ, and systemic level. Furthermore, animal models allow studying pharmacodynamic, pharmacokinetic, and immunologic responses. [12] The most common animals employed in *in vivo* BBB models are rodents, appealing due to their availability, easy handling, low cost, and relatively short breeding time. [15] However, although animal models have the significant advantage of an intact BBB with the whole complexity of the brain, there are several disadvantages. Firstly, physiological differences between humans and animals, such as at the cellular, genetic, and immunological levels, result in many discrepancies in disease development mechanisms and in physiological responses that occur when a drug compound is administered, becoming problematic to translate the results obtained using animals to the human context. [16] In addition, other drawbacks of using animal models include costs, ethical issues, low- throughput, and time-consuming protocols. [17]

To bypass the concerns regarding the use of animals, *in vitro* cell-based models to study BBB have prospered. These models can study the permeability and transport of compounds across the BBB and perform toxicological, pathophysiological, and immunological studies. [18, 19, 20] Higher throughput capacity, simplicity, ease of maintenance, few or no animals needed, lower cost, the possibility of assaying compounds directly in physiological buffer, and the ability to

identify early signs of cell toxicity are the main advantages of *in vitro* models compared to animal models. [21] However, due to their simplicity, conventional cell culture systems fail to mimic critical aspects of human physiology, being difficult to answer complex research questions. [22] Cell-based Transwell assays have been one of the most common techniques adopted to mimic the BBB *in vitro*.

This system consists of two compartments simulating the blood and brain sides. These compartments are separated by a microporous semipermeable membrane on which cells are seeded, forming a cell monolayer that contacts with different culture media in each compartment. [23] BECs from various origins, such as mouse, rat, pig, bovine, and human, have been incorporated into these models in monoculture, co-culture, or triple co-culture configurations with astrocytes and pericytes (Figure 2). [23,-28]

Despite many of these models generated during the past years having provided information on the physiology and pathophysiology of the BBB and drug screening, they have some limitations. In fact, Transwell cultures lack the complex architecture and tridimensional cell-cell interactions of the BBB and reflect a static environment, failing to replicate correct shear stress from blood flow and, therefore, the dynamic mechanical BBB microenvironment. [23, 29]

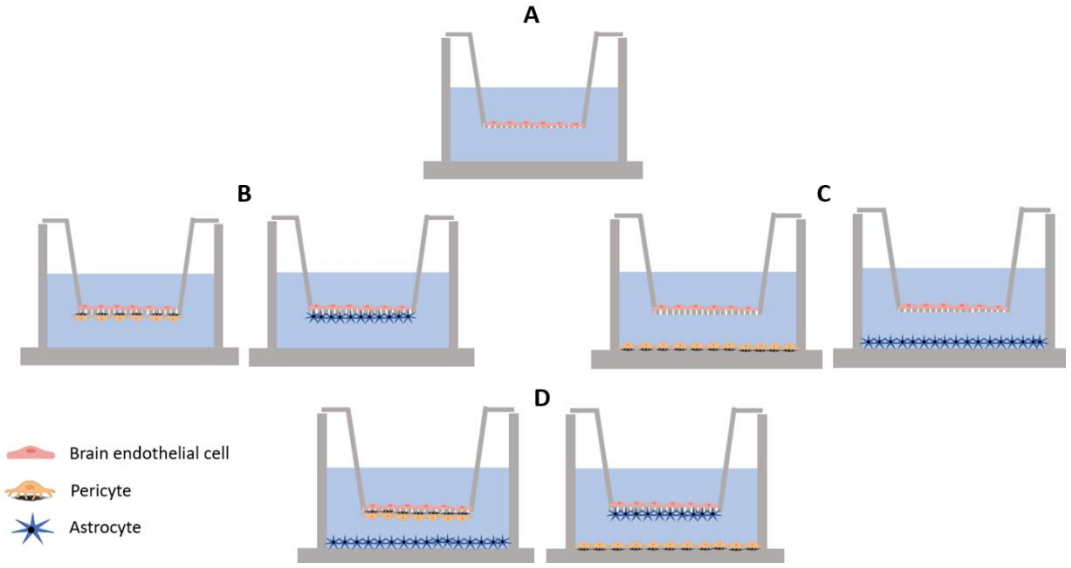

Figure 2- *In vitro* Transwell models of blood- brain barrier (BBB). (A) Monoculture of brain endothelial cells (BECs). (B) Contact co-culture of BECs with pericytes (PCs) or astrocytes (ACs). (C) Non-contact co-culture of BECs with PCs or ACs. (D) Triple co-culture of BECs, ACs and PCs.

Advances in microfluidic technology and their convergence with microengineering allowed the development of a new generation of *in vitro* platforms that simulate complex *in vivo* physiology, named organs-on-a-chip. [30, 31] Organ-on-a-chip is a microfluidic cell culture device created with microchip manufacturing methods in which cells are cultured in an

environment that is engineered to replicate the *in vivo* microenvironment of a tissue. [22, 30] In these devices, living cells are cultured in continuously perfused micrometer-sized chambers to closely mimic the dynamic conditions and the *in vivo* microstructure of a tissue. [30] Thus, microfluidic culture devices with relevant multicellular tridimensional architecture, dynamic perfusion, and physicochemical microenvironment can mimic structural tissue arrangements and functional complexity of living organs. [30, 31] With organ-on-a-chip technology, it is possible to model different organs or tissues. [32, 33] In this context, this review will focus on microfluidic BBB-on-a-chip systems. These models are suitable for co-culturing different cells and enable the incorporation of sensors that provide real-time monitoring of genetic, metabolic, and biochemical activities. [30] Moreover, the use of BBB-on-a-chip can improve BBB modeling by incorporating fluid flow and shear stress on BECs, which are critical contributors to BEC structure and function, mimicking the conditions present in the human brain endothelium, and by having more realistic geometries and dimensions. [30, 34] Thus, these models recapitulate the physical structure and the specific biochemical and mechanical microenvironment of the human BBB better than conventional *in vitro* models. On the other hand, microfluidics BBB-on-a-chip are cell cultures devices that need low reagent volumes, reducing reagents consumption, such as culture media and drug compounds, and the number of cells required, thus potentially decreasing the final costs. [35, 36] In short, BBB-o-a-chip systems combine several advantages of *in vivo* and conventional *in vitro* models, potentially overcoming most of their limitations. Thereby, due to the improved reliability of these models, microfluidic-based BBB-on-a-chip is a powerful approach to disease modeling and high-throughput screening of new drugs targeting CNS disorders. [37, 38]

This review presents the various types of BBB-on-a-chip devices and discusses their advantages and limitations. Firstly, the parameters to consider when developing a BBB-on-a-chip will be addressed. Secondly, the main analyses performed to characterize BBB-on-a-chip models are mentioned. Then, several applications of BBB-on-chips, focusing on drug screening and disease modeling, will be addressed. Finally, challenges and future perspectives on the use of these models will be discussed.

2. Parameters to consider when developing a BBB-on-a-chip

When developing a BBB-on-a-chip, some parameters are to consider, including the cell types that will be cultured and their source and the device design and dimensions. Furthermore, fluid flow and shear stress are other aspects that must be considered when designing and testing these sophisticated cell cultures.

2.1. Cell types and sources for BBB-on-a-chip

Existing *in vitro* BBB-on-a-chip models consist of monocultures of BECs, [39] co-cultures of BECs with ACs [40] or PCs, [41] or triple co-cultures of BECs, PCs, and ACs. [42]

2.1.1. Brain Endothelial Cells

BECs are simple squamous epithelial cells of mesoderm origin and are the primary component of the BBB, forming the wall of blood vessels. These cells are phenotypically unique compared with endothelial cells (ECs) in other tissues, which makes them ideal for permeability regulation. In fact, BECs are tightly sealed by adherens and tight junction (TJ) proteins that minimize paracellular transport and are characterized by the presence of specialized transcellular transport mechanisms that selectively control the transport of ions, molecules, and cells to the brain. [6, 7]

It is essential that BECs cultured in BBB-on-chips exhibit physiological characteristics typical of BBB *in vivo*. Therefore, in those *in vitro* models, BECs have to form a tight barrier monolayer and express key markers seen *in vivo*, such as TJ proteins (Zonula occludens-1 (ZO-1), claudin-5, and occludin), EC-specific markers (Platelet endothelial cell adhesion molecule-1 (PECAM-1) and Vascular endothelial (VE)-cadherin) and membrane transporters (P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), glucose transporter 1 (GLUT-1)). [43] BBB-on-chips have been developed with BECs of different sources, namely immortalized EC lines, [44, 45] primary, [42, 46] and stem-cell-derived ECs, [41, 47] and from different species, including mouse, [40, 46] rat, [37, 48] and human. [49, 50]

Human (hCMEC/D3, hBMEC, TY10, HBMEC/ci18) and murine (bEnd.3, RBE4) immortalized EC lines have been employed in several BBB-on-a-chip cultures. [37, 48, 49, 51, 52, 53, 54] In a study comparing four immortalized human brain capillary EC lines, hBMEC proved to be the most promising cell line for human *in vitro* BBB models due to the barrier tightness, since the highest transendothelial electrical resistance (TEER) values and lowest paracellular permeability for Lucifer Yellow and sodium fluorescein were obtained with mono-cultures of this cell line. [55] However, the hCMEC/D3 cell line is the most used in the development of BBB-on-chips, [45, 56, 57, 58, 59] probably because it is the best characterized human BBB cell line, representing a stable and easily grown population that expresses specific BEC markers and the most important junctional proteins. Furthermore, the hCMEC/D3 cell line is commercially available. [56, 58, 60, 61] Otherwise, murine cell lines have also been reported for BBB-on-a-chip cultures. [37, 62] However, due to genetic differences between those animals and humans, human-derived cells should be the first option when the objective is to mimic the human BBB. [63]

Immortalized EC lines have advantages such as ease of use, low cost, scalability, and the retention of BBB transporter expression after multiple passages. [55, 64] However, immortalized human and animal BECs exhibit a loss of TJ proteins and low baseline TEER compared with physiological values (1,500– 8,000 Ω cm^2). [65, 66] Therefore, immortalized EC lines are not suitable for permeability studies.

Primary ECs are another source of BECs to be applied in BBB-on-a-chip cultures, which are obtained from microvessels isolated from cerebral tissue by surgical removal. [67] Primary ECs from different origins, such as rat, [29] mouse, [46, 68] and human, [42, 69] have been cultured in BBB-on-a-chip devices. Although those primary cultures resemble the BBB phenotype *in vivo* better than immortalized cell lines, they do not achieve physiological TEER levels and may lose many of their BBB characteristics quickly in culture. [70, 71] Further, the process of cell extraction and purification is labor-intensive, and a limited number of cells are obtained, leading to problems of scalability. [67, 72, 73] Indeed, primary human BECs are challenging to obtain in sufficient quantities for drug screening and disease models. [66]

Recent advances in the field of induced pluripotent stem cells (iPSCs) have allowed the culture of BECs generated via the differentiation of these cells in BBB-on-chips. [41, 43, 47, 74, 75] Effectively, human iPSCs display the capacity to differentiate virtually into any cell type allowing large numbers of differentiated cells to be obtained. [76] First, to generate iPSCs, somatic cells are reprogrammed through the introduction of reprogramming factors into these cells. Several methods for delivering reprogramming factors have been applied, including viral and, more recently, non-viral methods. Then, the generated iPSCs can be differentiated into specialized cells. [76, 77 78] For differentiation of iPSCs into BEC-like cells, some protocols have been applied. However, they are complex and time-consuming. [79] Nonetheless, iPSCs-derived BECs have a huge potential for studying BBB since they mimic many of BECs hallmarks such as TJ proteins and efflux transporters, permeability, and TEER values. [39, 71] Additionally, they have the potential to generate large quantities of specialized human cells, being a robust, scalable and renewable source of human BECs. [66] In general, iPSCs- derived BECs have the potential to overcome the limitations of primary and immortalized cells and therefore seem to be the best option to recapitulate BBB *in vivo*.

In addition, some BBB-on-a-chip developed so far have used human umbilical vein endothelial cells (HUVECs) to mimic CNS microvasculature *in vitro*. [50, 80-64, 81-84] Nevertheless, to date, HUVECs have not been shown to form a tight barrier. Uwamori *et al.* compared the performance of hBMECs and HUVECs in terms of microvascular formation and barrier functions. They concluded that despite no differences in microvascular diameter and the

number of pericytes peripherally associated with the microvasculature, HUVECS expressed significantly lower levels of TJ proteins, and the permeability coefficients were significantly higher than those of hBMECs. [80] Thereby, HUVECs are not the best option for modeling the BBB *in vitro*.

2.1.2. Pericytes

PCs of the BBB are contractile mural cells embedded within the basement membrane between BECs and AC end-feet, being in direct physical contact with the brain endothelium. [41, 85] These cells are essential in preserving a functional BBB and regulating cerebral blood flow and capillary diameter. [86, 87] Identification of these cells is challenging due to the lack of specific markers. NG2, platelet-derived growth factor receptor β (PDGFRβ), and α-Smooth muscle actin (α-SMA) are commonly used as PC markers, but their expression is not specific to PCs. [80, 87, 88]

With the objective of better mimicking *in vivo* environment, PCs have been cultured in several BBB-on-a-chip models developed [42, 52, 89] or, alternatively, BECs are cultured in the presence of PC conditioned medium. [90] Regarding cell sources of PCs, different options are available. Primary PCs are commercially available and are widely used in these microfluidic BBB models. These cells have been isolated from human, [47, 91] bovine [92], and rat [48] brain tissue. Further, immortalized and iPSC-derived PCs are also an option. [41, 59]

Herland *et al.* analyzed the individual contribution of PCs and ACs to the barrier function. Through a permeability test with 3 kDa fluorescent dextran, they concluded that the integrity of the endothelium depends on the presence of PCs when cultured in a 3D microenvironment. [89] Equally, Campisi *et al.* showed that the presence of PCs enhanced BBB like-properties. Effectively, co-culture of iPSC-ECs with primary human PCs formed smaller and more highly branched vessels, expressed higher levels of TJ proteins, and had lower permeability than iPSC-ECs cultured alone. Furthermore, they proved that direct contact between BECs and PCs facilitated endothelial organization since, for iPSC-ECs cultured only with PC conditioned medium, no difference in vascular networks was observed in relation to iPSC-ECs cultured alone [47]. On the contrary, the findings of Jamieson *et al.* suggest that PCs are not essential for establishing barrier function in healthy iPSC-derived brain microvascular endothelial cells (BMEC) monolayers, showing no influence on the TEER values. However, co-culture with iPSC- derived PCs was able to rescue barrier function in stressed iPSC-derived BMEC monolayers. [41]

2.1.3. Astrocytes

ACs are the most abundant cell type in the brain, which, by their end-feet, cover 99% of the microvasculature surface in the brain. [93] They serve as physical contact between neurons and BECs. In addition, they play a critical role in the maintenance of the BBB or modulation of its integrity through the secretion of chemical factors that regulate TJ proteins expression in BECs. [49, 85, 93] In addition, astrocytes react sensitively in response to injury and disease such as trauma, infection, stroke or neurodegenerative disorders, a process defined as a reactive gliosis. This state involves morphological changes, with ACs changing from a star-like shape to an expanded shape, but also molecular alterations, such as upregulation of the glial fibrillary acidic protein. On the one hand, reactive astrocytes help to restore homeostasis and modulate neurogenesis. [94, 95] However, astrogliosis in pathological conditions can also be harmful to BBB, leading to its disruption via secreted proteins, such as vascular endothelial growth factor. [95, 96] Therefore, to appropriately model the BBB, several studies have incorporated ACs [29, 83, 84] or AC conditioned medium [37, 81, 97] into their BBB-on-a-chip cultures.

As for previous cells, there are several sources of ACs. In microfluidic BBB devices, primary human ACs have mainly been used. [58, 74, 75, 98] However, primary mouse [46, 68] and rat [99, 100] ACs were also incorporated in these cultures, as well as immortalized cell lines [40, 51, 101, 102] and iPSC-derived ACs. [87] For the latter, several protocols have been published to generate iPSC-derived ACs. [102-105]

The findings of Campisi *et al.* demonstrate that incorporating ACs in a co-culture of iPSC-ECs and PCs leads to the development of a complex architecture similar to native vasculatures. [47] Xu *et al.* also show that the addition of ACs to BECs improved the BBB properties by increasing the expression of TJ proteins (ZO-1 and Claudin-5) and VE-cadherin, P-gp, and GLUT-1 and by significantly diminishing the diffusion of sodium fluorescein tracer, comparing to BECs cultured alone. [29] The fact that incorporating ACs increases the integrity of the endothelial monolayer in BBB-on-a-chip devices where the design does not allow direct contact between ACs and BECs, leads to the belief that this is possible due to the secretion of soluble factors by ACs. [51] Thus, some groups developed BBB-on-a-chip cultures with AC conditioned media. [37, 81, 84] Effectively, Tang and collaborators found no significant differences in barrier permeability, TEER, or ZO-1 expression, when BECs were cocultured with AC conditioned medium compared with BECs cocultured with ACs. [97]

2.2. Device design and dimensions

2.2.1. 2D

2D BBB-on-a-chip models were differentiated from static 2D Transwell models, including devices with two compartments separated by a permeable membrane. [44 45, 90 106] They consist of devices having BECs cultured in 2D in one compartment, which acts as the vascular channel, with fluid flow to mimic shear stress along the capillaries, and a secondary compartment often containing supporting cell types. [48, 51] Usually, these devices have a sandwich design, also known as top-bottom design, with the porous membrane sandwiched between upper and bottom polydimethylsiloxane (PDMS) channels. Commonly, BECs are cultured in the upper channel on top of the membrane and supporting cells in the bottom channel, which does not represent the anatomical organization of brain microvasculature. [40, 101] Despite the relatively simple geometry, the incorporation of fluid flow in these microfluidic devices is an essential upgrade over the standard Transwell models since it more closely mimics the physiological conditions of the BBB. Some examples of 2D microfluidic BBB models are represented in figure 3.

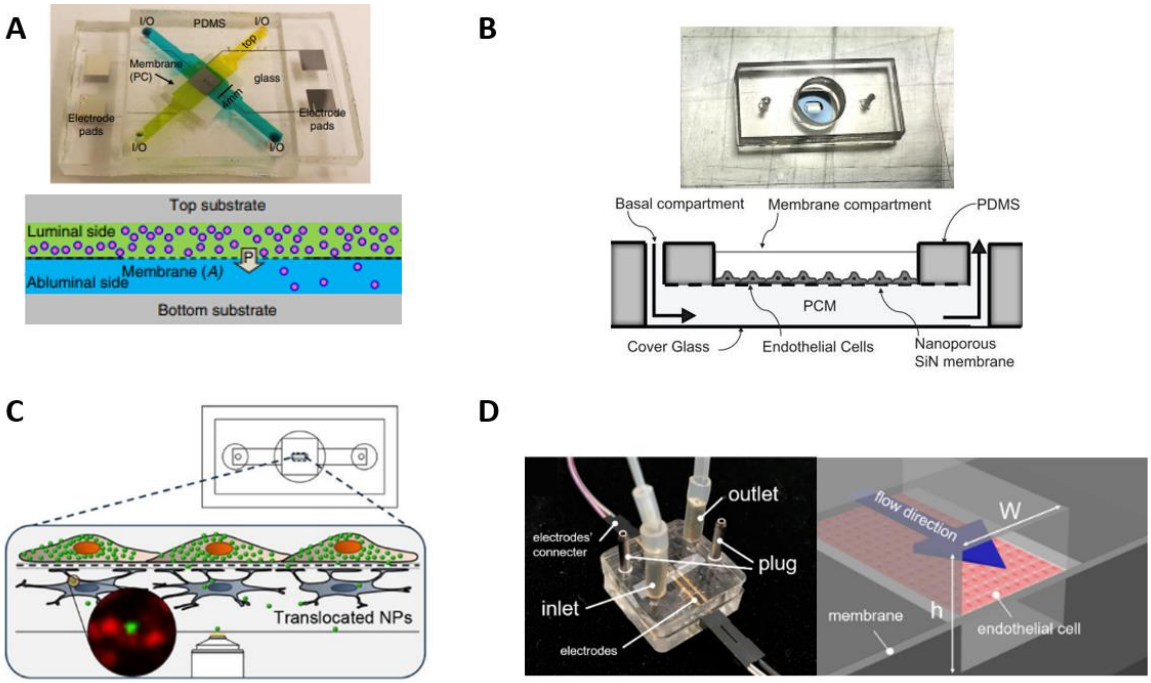

Figure 3- 2D Blood-brain barrier (BBB)-on-a-chip devices. **(A)** A microfluidic BBB model comprising two crossing channels separated by a porous polycarbonate membrane for permeability analysis of neuroactive drugs. Reproduced from Booth and Kim. [101] **(B)** A dual-chamber device divided by a nanoporous silicon nitride membrane with high permeability and optical transparency allows the study of T-cell migration across the BBB. Reproduced from Mossu *et al.* [90] **(C)** A BBB model consisting of a dual-chamber platform that features human brain endothelial cells and primary astrocytes grown on opposite sides of an ultrathin silicon nitride membrane. The BBB-on-a-chip device enables high-resolution imaging of nanoparticle interactions with endothelial cells. Reproduced from Hudecz *et al.* [125] **(D)** A BBB-on-a-chip model with custom-made integrated electrodes for online real-time TEER monitoring. To simulate physiological shear stress of *in vivo* capillaries, fluids were continuously infused through the vascular channel. Reproduced from Tu *et al.* [54]

2.2.2. 2.5D

2.5D BBB-on-a-chip devices are more sophisticated *in vitro* models to mimic the BBB. There is an increase in complexity from 2D BBB-on-a-chip models to the 2.5D, requiring a greater degree of technical expertise and specialized equipment for device fabrication.[107] They consist of a BEC monolayer seeded in a rectangular channel (BEC compartment) and a 3D extracellular matrix (ECM) compartment, often with ACs and/ or PCs, usually separated by PDMS pillars to create distinctions between the different channels. Usually, these devices have a planar parallel configuration, where the two channels are horizontally aligned. [37, 56, 97] In this design, an artificial membrane is not required, allowing direct cell-to-cell contact. In addition to allowing the incorporation of fluid flow and shear stress into de BBB-on-a-chip model, the distinct advantage of 2.5D is the capacity to enable more physiologically relevant cellular interactions, thus, allowing its use in more complex applications, such as the study of the

extravasation of metastatic tumor cells. [29] Figure 4 denotes some examples of 2.5D BBB-on-chips.

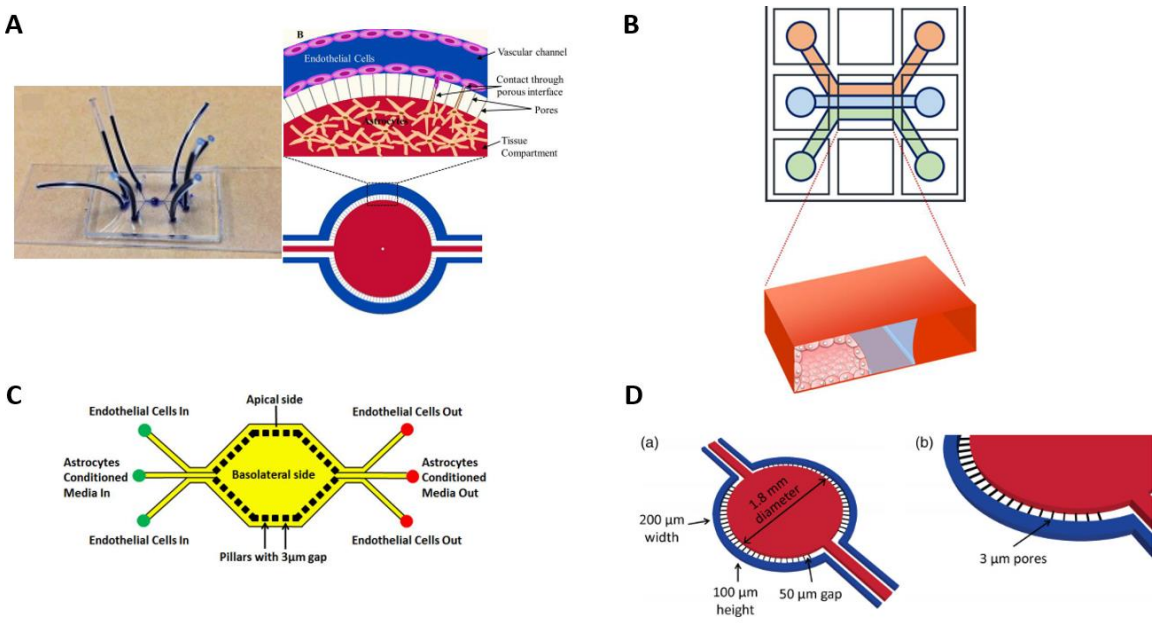

Figure 4- 2.5D Blood-brain barrier (BBB)-on-a-chip devices. **(A)** A neonatal BBB-on-a-chip device that comprises a tissue compartment and vascular channels placed side-by-side designed to study neonatal neural pathologies and the development of appropriate therapeutics. Reproduced from Deosarkar *et al.* [99] **(B)** A BBB-on-a-chip device developed using a MIMETAS OrganoPlate® 3-lane plate with human induced pluripotent stem cell -derived brain endothelial cells (BECs) cultured in the vascular channel under continuous flow. Reproduced from Kurosawa *et al.* [43] **(C)** A BBB-on-a-chip platform comprising apical and basolateral compartments placed side-by-side with rat BECs and astrocyte conditioned media, respectively. Reproduced from Prabhakarpandian *et al.* [37] **(D)** A BBB-on-chip platform developed using a commercially available microfluidic chip from SynVivo Inc. as a scaffold. It consists of a central disk-shaped basolateral compartment surrounded by vascular channels. Reproduced from Brown *et al.* [56]

2.2.3. 3D

To address the limitations of 2D models in mimicking the BBB's architecture and function, 3D BBB-on-a-chip models have been developed. These devices consist of a perfusable circular cross-section representing the vascular channel, surrounded by a 3D matrix. This is an improvement in BBB-on-a-chip technology since the geometry of microchannels with rectangular cross-sections results in nonuniform shear stress profiles, which can cause differences in the morphology and behavior of the ECs. [108] A common approach that has been applied to create BBB-on-a-chip with tube-like 3D vessel structures includes using SU-8 molds [100, 109] or other systems such as microneedles [110], wires, [71, 111] tubing, [39] and rods [41] with the channel features. In short, these cylindrical templates are usually embedded within collagen gel or PDMS. Then, the molds are removed to leave a hollow that mimics the vascular channels after collagen gelification or PDMS polymerization. [41, 100, 110] Patterned vasculature BBB-on-a-

chip devices easily enable imaging and capture of live cell events, such as cellular interactions or molecule transport. Nevertheless, patterned vascular vessel diameters are larger than the *in vivo* brain microvasculature. [107]

Other approaches to generate 3D BBB-on-a-chip models are the *in vitro* vasculogenesis and angiogenesis strategies. [47, 74, 80] Angiogenesis, in a strict sense, describes the formation of new vessels from the existing vasculature. [112, 113] On the other hand, vasculogenesis is the *de novo* formation of blood vessels. [112] While the common vasculature develops via vasculogenesis, CNS vasculature develops via angiogenesis instead. The development of vasculogenesis and angiogenesis based models require optimization and compliance of ECM composition to enable EC invasion and ECM degradation while maintaining structural stability. [107] These strategies are particularly advantageous since they allow the obtention of smaller *in vitro* BBB vessel diameters and may better mimic the cell's interactions between BCEs and ACs or PCs. [47, 74] Lee and colleagues compared the results of a vasculogenesis-like assay with those of an angiogenesis-based protocol. After coculturing with the same ratio of PCs and ACs, a notable difference in perivascular coverage along the microvessels was observed between the two methods of reconstituting CNS vasculature. Effectively, using a vasculogenesis-like protocol resulted in a random alignment of PCs, while using the angiogenesis-based protocol, PCs were aligned more parallel to the microvessels. [82] Uwamori *et al*. also reported a BBB-on-a-chip culture developed via vasculogenesis with BMECS or HUVECs to test if there are differences in the formation process and endothelial barrier functions. They concluded that the formation process, endothelial barrier functions, and fundamental morphology differed depending on EC origin. Effectively, despite both BMECs and HUVECs have formed continuous lumens with no significant differences in terms of vessel diameter and the number of pericytes covering microvasculatures, more extensive networks, in terms of microvascular length and branch points, were observed in HUVECs microvasculature. On the other hand, lower permeability coefficients and significantly higher expression of ZO-1 and occludin were found for BMEC microvasculatures, indicating a more robust barrier function. Thus, these results suggest that the BECs origin has to be considered when developing a BBB-on-a-chip model. [80] Some examples of 3D microfluidic BBB models are represented in figure 5.

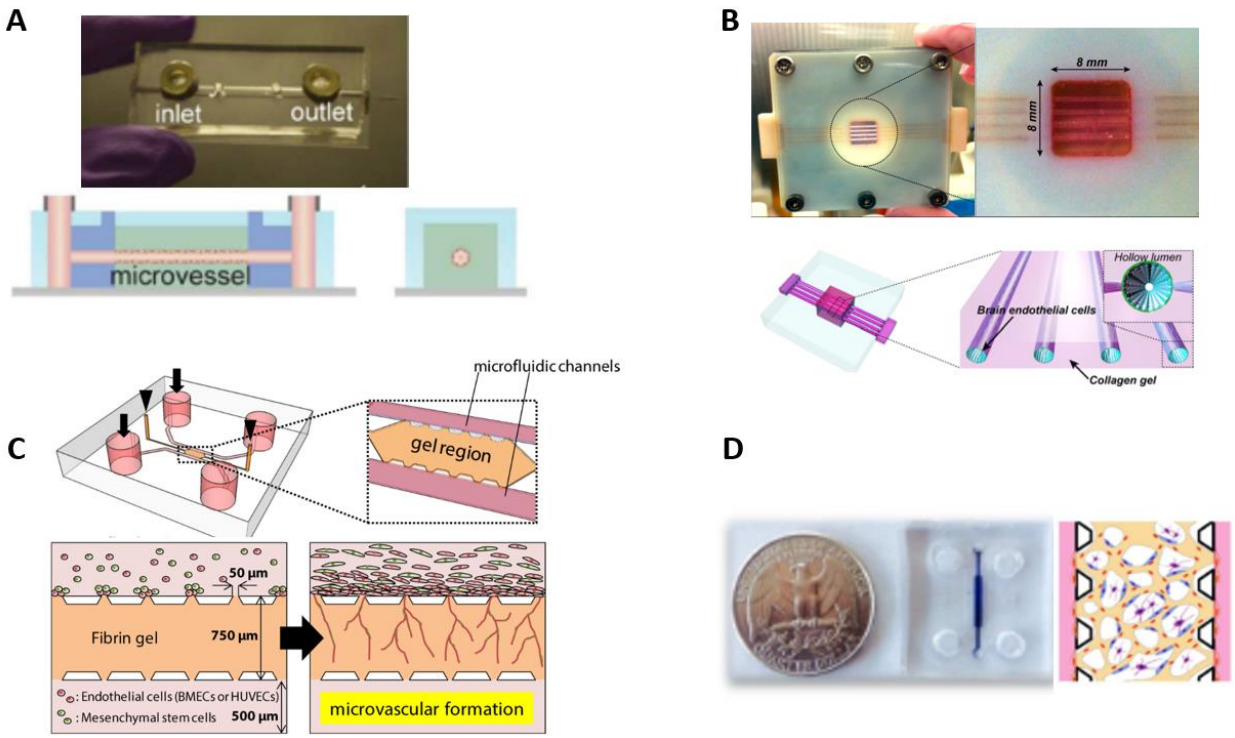

Figure 5- 3D Blood-brain barrier (BBB)-on-a-chip devices. **(A)** A microfluidic device that mimics human brain post-capillary venules using human induced pluripotent stem cell (iPSC) derived- brain endothelial cells (BECs). For the microvessel fabrication, type I collagen and agarose were gelled around a suspended wire. Reproduced from Linville *et al.* [71] **(B)** A microfluidic platform recapitulating in vitro brain microvasculature. Mouse BECs were cultured on the luminal surface of the collagen microchannels. Reproduced from Kim *et al.* [110] **(C)** A BBB-on-a-chip device developed via angiogenesis by coculturing BECS and mesenchymal stem cells. Reproduced from Uwamori *et al.* [80] **(D)** A microfluidic system that replicates the BBB microvascular network developed via vasculogenesis. This BBB-on-a-chip includes human iPSC- derived ECs, brain pericytes and astrocytes. Reproduced from Campisi *et al.* [47]

2.3. Fluid flow and shear stress

In vivo, fluid flow along microvasculature allows the delivery of nutrients, the removal of metabolic wastes and provides mechanical signaling to cells. In addition, fluid flow in vessels results in shear stress on the endothelial lining of the vascular wall. Shear stress regulates EC morphology and function, shear-stress responsive genes, and mediates signaling and transport processes between the vascular system and surrounding tissue. [114]

The channel geometry influences shear stress distribution on the cells. While in a tube with a circular cross-section the shear stress will be equal along the cylindrical wall, in a rectangular channel, the wall shear stress will not be uniform. [115] For brain capillaries, physiological shear stress levels between 3 and 23 dyne/cm^2 (0.3-2.3 Pa) have been reported. [49, 115] While some BBB-on-a-chip models developed until now incorporated shear stress under

physiological levels, [40, 41, 49, 54, 106] many applied shear stresses in orders of magnitude lower than what is considered physiologically relevant. [45, 59, 89, 90, 116] Ahn *et al.* and Jeong *et al.* evaluated the effect of different shear stress conditions applied on their custom-made BBB-on-a-chip cultures and concluded that physiological shear stress levels induced higher TEER values and ZO-1 expression than non-physiological values. [52, 68]

To generate fluid flow in BBB-on-a-chip, different approaches have been followed. Most devices use pumping systems, such as a syringe [53, 97, 109, 116] or a peristaltic pump [40, 49, 106, 117] which allow constant flow rates to obtain constant shear stress levels. Furthermore, some devices utilized gravity as the driving force. [41, 70, 100] With this strategy, it is more challenging to obtain constant shear stress levels; however, by eliminating the need for external pumps and tubing, there is no risk for air bubbles formation, and the design of the system is simplified, facilitating a possible scale-up. Effectively, Wang and colleagues used a rocker platform to deliver the fluids to the luminal compartment via gravity-driven flow. Thus, they were able to run multiple BBB-on-a-chip units in parallel on a single rocking platform. [70]

The positive effect of fluid flow on BBB properties has already been demonstrated in several BBB-on-a-chip models. Winkelman and colleagues developed brain microvascular networks through both angiogenic and vasculogenic processes to highlight the importance of interstitial flow for *in vitro* brain microvascular network development. They found that interstitial flow enhanced BECs angiogenesis, brain microvascular network morphology and longevity, and basal lamina protein production in microvessels. Moreover, it increased astrocyte coverage of microvessels and decreased microvessel permeability. [98] Xu *et al.* also showed that permeability of BMECs layer to sodium fluorescein tracer diminished, and TEER of BMECs monoculture increased by more than 4-fold in the presence of dynamic flow. Additionally, although exposure to dynamic flow did not alter the expression of the transporters -glycoprotein and GLUT-1, it significantly increased the expression of the adhesive protein VE-Cadherin and endothelial TJ proteins in BMECs. [29] Similarly, several other studies indicate that incorporating shear stress in BBB-on-a-chip models increases TEER values [51, 57] and the expression of TJ proteins, [54, 57, 118] thus exhibiting improved vascular morphological features as well as enhanced endothelial barrier function. Nevertheless, several transwell-based BBB models with static conditions have also presented TEER values within the range of *in vivo* levels, suggesting that applying shear stress in BBB models is not required to mimic *in vivo* barrier tightness. [119, 120] Taking this into account, to test the necessity of applying shear stress in establishing *in vivo*-like barrier properties in BBB-on-a-chip models, Wang, Abaci and Shuler designed a microfluidic platform that allows physiologically relevant perfusion while

minimizing shear stress on the cell surface. The wall shear stress was minimized by introducing a "step chamber" design that increased the distance between the cell plane and the perfusion layer. Their results illustrate that the BBB-on-a-chip developed achieved significant barrier integrity, with continuous TJ proteins (claudin-5 and ZO-1), TEER levels above 2000 Ω·cm2, and extremely low permeabilities to fluorescein isothiocyanate (FITC)-dextrans, comparable to observations reported *in vivo*. Thus, they concluded that high levels of shear stress are not required to develop an effective BBB-on-a-chip model with *in vivo*-like properties. [70] Interestingly, Lippmann *et al.* and Wang and colleagues used iPSC-derived BECs in their culture systems, which can be the main reason for having achieved significant barrier integrity, even without high levels of shear stress since, as referred previously, iPSC-derived BECs intrinsically mimic many of BECs hallmarks such as TJ proteins and efflux transporters, permeability, and TEER values. It is probably that these results would not be achieved by performing the same experiments with immortalized or primary BECs. In fact, the positive effect of shear stress on barrier properties can be more noticeable in barriers formed by immortalized or primary BECs with lower baseline TEER values, where a variation will be more relevant. [70, 119, 120]

In addition, fluid flow and shear stress influence EC phenotype and nanoparticle interactions with these cells. To evaluate this behavior, Papademetriou and co-workers developed a BBB-on-a-chip model to evaluate, in static and flow conditions, the binding and internalization by BECs, and BBB penetration of Angiopep-2 coupled liposomes (Ang2-Liposomes), designed to facilitate delivery of drugs to the brain. Ang2-Liposomes They found that the binding of Ang2-Liposome to brain ECs was efficient in static fluid or at low shear stress (1 dyne/cm^2). However, the binding was inhibited at higher shear stress levels (6 dyne/cm^2), corresponding to physiological values. On the other hand, Ang2-Liposomes' penetration on the BBB model was enhanced in the presence of flow relative to static conditions, probably due to the opening of the paracellular route resulting from shear stress application. In summary, this study demonstrated that fluid flow modulates Ang2-functionalized nanoparticles' binding to BECs and their penetration through the BBB. [44] Thus, it is essential to perform more studies focusing on tuning nanoparticle characteristics to enable the binding to brain ECs at physiological shear stress levels while maximizing BBB penetration. Concluding, applying physiological levels of shear stress in BBB-on-chips is essential to mimic the *in vivo* BBB, which is crucial to evaluating the efficacy of new drug delivery strategies to the CNS.

3. Assessment of BBB-on-a-chip characteristics

To assess BBB-on-a-chip characteristics and validate the fabricated system, several analyses have been performed. Cell imaging, gene expression analyses, TEER, and permeability measurements are the most common. [47, 56, 121] Cell imaging allows for analyzing cell distribution and morphology and, when a Live/Dead assay is performed, assessing the viability of cultured cells. [51, 52, 121] Furthermore, immunocytochemistry is a commonly used method to investigate the presence of tight and adherent junctions and transporters highly expressed at the BBB *in vivo*. Additionally, gene expression levels of such proteins are also analyzed in some studies. [38, 43, 52]

The integrity of the barrier created in the microfluidic device is usually assessed by TEER measurements and permeability studies. [44, 45, 51, 52, 71, 110, 121] In fact, TJ proteins are a fundamental feature of endothelial barriers *in vivo,* which is reflected in high electrical resistance and low passive permeability for hydrophilic compounds. [48]

Annex I summarizes the key features of several studies dedicated to developing BBB-on-a-chip devices and assessing biological processes related to the barrier function.

3.1. TEER measurements

The BBB has tightly sealed cell-to-cell contacts resulting in a measurable electrical resistance [10]. TEER measurements correlate electrical properties of an epithelial layer with morphology, cell layer confluency and TJs formation, being a very sensitive method to confirm the integrity of the monolayer. Moreover, it enables non-invasive, real-time monitoring of barrier integrity. [122, 123] To measure TEER values in BBB-on-a-chip devices, one electrode is placed in the vascular compartment and the other in the adjacent compartment. Devices with integrated electrodes on either side of the porous membrane have been developed. [57] Nevertheless, these electrodes often make visual inspection difficult. Thus, optically transparent integrated electrodes are a better option [48]. Alternatively, an electrode compartment can be created outside the vascular channel, and, for electrical resistance measurements, electrodes are inserted into that compartment and in the tissue compartment. [99]

TEER values measured *in vivo* have been reported to be 1500– 8000 Ω cm^2. [65] To date, few developed BBB-on-a-chip reached physiological values. In fact, while devices cultured with human iPSC-derived ECs can achieve values similar to those verified *in vivo*,[70, 71] BBB-on-a-chip with immortalized or primary BEC cultures do not. [48, 51, 54, 106]

Although TEER measurement is very useful to monitor barrier formation, some groups do not perform such TEER measurements. [43, 45, 110] Effectively, assessing barrier formation with TEER in ECM-based 3D models is challenging due to device incompatibility since it is difficult to introduce electrodes on opposite sides of the gel. [89] Furthermore, TEER measurements can differ across studies with similar setups due to differences in electrode characteristics. [49]

3.2. Permeability measurements

Another assay that has been widely used to assess BBB integrity is based on the permeability of the barrier to paracellular tracer compounds of various molecular weights. [122] Several markers have been used to access barrier function in BBB-on-a-chip devices. FITC-labeled dextrans with different molecular weights are largely used. [74, 75, 82, 98, 124] However, smaller molecule dyes have also been reported, such as Lucifer Yellow [43, 92] and sodium fluorescein [39, 118]. The measured flux of the selected tracer across the cell layer allows for the determination of the endothelial permeability coefficient. [122] A significant advantage relative to TEER measurements is that fluorescent tracer permeability can be measured in nearly all BBB-on-a-chip designs. [73, 125, 126]

4. Applications of BBB-on-a-chip

Besides the applications of BBB-on-chips for disease modeling [29, 83, 100] and drug screening [45, 81, 101] already mentioned, these devices have several other applications, including evaluation of techniques to temporarily permeabilize the BBB, [62, 118, 127] cell migration [58] and antibody transcytosis. [124] The key features of BBB-on-chips developed for these purposes are summarized in annex II.

4.1. Drug screening

BBB-on-a-chip models are promising cell cultures that can facilitate early screening of drugs candidates targeting the CNS. In that regard, Shao and colleagues fabricated a microfluidic BBB-on-a-chip to evaluate drug permeability across the BBB and anti-tumor activity of sunitinib on cerebral tumor cells. First, to ensure the integrity of the BBB model, immunostaining of tight-junction proteins and permeability assays with sodium fluorescein and FITC-dextran 70 kDa were performed. Then, the authors conducted a permeability assay with sunitinib where they found that apparent permeability coefficient was consistent with literature reported values, being highly permeable. Finally, to study sunitinib anti-tumor activity, U251 cells, a human malignant glioma cell line, were applied in the chambers. After

exposure to sunitinib for 0, 24 and 48 h, the 3D-cultured U251 cells were stained with a Live/ Dead assay. The results showed that after 48h of treatment the U251 cell viability was reduced to 69.7%. [45]

Nanomedicines have been widely investigated to overcome the limits that BBB poses for drugs targeting the CNS. [128] Thus, several groups developed BBB-on-a-chip platforms to study the interaction between nanoparticles and the BBB. [44, 52, 116, 126] Falanga and co-workers developed a BBB microfluidic device with a sandwich design to investigate if gH625, a membranotropic peptide, enhances the transport of nanoparticles across the BBB. Effectively, they found that the functionalization of nanoparticles with the gH625 peptide contributed to enhancing the BBB crossing. [116] In the same way, Hudecz *et al.* fabricated a BBB-on-a-chip with an ultrathin silicon nitride membrane that facilitates imaging of nanoparticle interactions with BECs. Three types of nanoparticles were used: 40 nm PS-COOH, 100 nm PS-COOH, and apolipoprotein E (ApoE)-conjugated 100 nm SiO2. The apical side of the BBB co-culture model was exposed to nanoparticles for 10 min and then the translocation was assessed at different time intervals. In the end, the authors concluded that ApoE plays an important role in the nanoparticle- mediated transport across the BBB, since the number of translocation events at all time points was significantly increased for ApoE-nanoparticles. [126]

Annex III summarizes and compares different studies that used BBB-on-chips for drug screening applications.

4.2. Disease Modeling

BBB-on-chips are very promising for disease modeling applications since they recapitulate important features that can be involved in pathologic mechanisms, such as the physical structure and the specific biochemical and mechanical microenvironment of the human BBB. In fact, several groups have developed BBB-on-a-chip cultures that somehow recapitulate aspects involved in disease. [29, 57, 83, 97]

Neuroinflammation is associated with several CNS disorders, such as neurodegenerative diseases and traumatic brain injury. [129, 130] Neuroinflammatory mechanisms involve various cytokines, including Tumor Necrosis factor α (TNF-α), one of the major mediators of the neuroinflammation associated with neurodegeneration and, consequently, being implicated in various inflammatory diseases of the central nervous system. [131] Thus, a neuroinflammatory stimulus with TNF-α was applied by various groups in their BBB-on-a-chip cultures. [57, 71, 89 100] Some of these studies demonstrated that exposure to TNF-α induces BBB disruption, decreasing TEER values [57, 100] and reducing occludin gene expression. [100] Further,

the production of interleukin-6 (IL-6) was increased in response to the inflammatory stimulus of TNF-α. [89, 100] On the other hand, Linville *et al.* found that TNF-α activation resulted in no change in barrier function. This discrepancy in the results from different studies is probably due to TEER values in the devices developed. In fact, BBB-on-a-chip cultures developed by Griep *et al.* [57] and Yu *et al.* [100] exhibited TEER values bellow physiological levels, which can make them more susceptible to disruptive changes in barrier function. On the contratry, Linville *et al.* developed a BBB-on-a-chip with physiological TEER (2260 ± 39 Ω cm^2). Thus, these results indicate that TNF-α stimulation alone, without other conditions associated with an immune response, is insufficient to induce BBB disruption *in vivo*. [71]

BBB disruption can either be a cause or consequence of some neurodegenerative diseases, initiating and/or contributing to a vicious cycle of disease progression. [132, 133] Therefore, some groups have developed BBB-on-a-chip devices that enable the study of the effects of neurodegenerative diseases pathological microenvironments on BBB function. Pediaditakis *et al.* created a BBB-on-a-chip device reproducing several key aspects of Parkinson's disease, which consisted of two microfluidic channels separated by a thin PDMS membrane: a brain channel, representative of the substantia nigra area, and a vascular channel. While in the brain channel human iPSC-derived dopaminergic neurons, as well as human primary brain ACs, microglia and PCs were cultured, in the vascular channel human iPSC-derived BMECs were seeded. Since Parkinson's disease is characterized by the abnormal accumulation of alpha-synuclein (αSyn) aggregates, to reproduce Parkinson's disease characteristics in their model, they added human recombinant αSyn fibrils to the culture medium of the brain channel under continuous flow and performed permeability assays. The results showed that there was a significant permeability to 160 kDa, immunoglobulin G, 3kDa dextran, and 0.5 kDa lucifer. Additionally, exposure to αSyn fibrils induced ZO-1 damage in endothelial cells, which contributes to the enhanced permeability. Further, to study the mechanisms attributable to BBB impairment, the authors examined changes in the expression of translocase of outer membrane 20 (TOM20), a mitochondrial import receptor subunit which prevents αSyn-induced mithochondrial dysfuntion, and intercellular adhesion molecule 1(ICAM-1), involved in inflammatory process in ECs. The results suggested a possible role for αSyn fibrils in BBB breakdown correlated with mitochondria dysfunction and vasculature inflammation, since TOM20 showed attenuated expression while ICAM-1 exhibited increased expression. Finally, through transcriptomic analyses of BBB endothelium, they also demonstrated that αSyn fibrils lead to transcriptomic changes in these cells, altering the expression of genes implicated in cellular processes associated with Parkinson's disease, such

as mitochondrial function, oxidative stress, and inflammation. Taken together, these result show that the model developed by Pediaditakis and colleagues allows the study of BBB dysfunction dynamics in Parkinson's disease. [134] Additionally, Shin *et al.* created a microfluidic platform mimicking key events in Alzheimer's Disease pathogenesis and having a BEC monolayer with a BBB-like phenotype. To recapitulate Alzheimer's Disease pathology, they cultured in the device ReNcell VM human neural progenitors, an immortalized cell line, expressing mutations in the Amyloid Precursor Protein (ReN-GA cells) and Presenilin 1 (ReN-mGAP cells) genes representative of those found in Alzheimer's disease patients. The microfluidic platform consists of five parallel channels where a central microchannel separates the BBB chamber and the brain chamber. The brain chamber consists of two microchannels, one that contains a neural cell medium and the other that includes the ReN cell culture. In turn, the BBB chamber consists of one microchannel containing a collagen scaffold and another microchannel cultured with BECs. The central microchannel was filled with a hydrogel to allow the interaction between the BBB and brain chambers. The authors also created a device where wild-type ReN cells were cultured as a control. First, by measuring BBB permeability using 3 kDa and 40 kDa dextrans, Shin an collegues tested if the model developed mimicked the BBB breakdown observed in Alzheimer's Disease. The results indicated that the permeability of the BEC barrier was significantly increased in the disease model as compared to the control model. Through the screening of adherens junctions and TJs gene expression in the BECs, they also found that the levels of Claudin-1, Claudin-5, and VE-cadherin were significantly decreased in the disease model, which suggests that, at least in part, the increase of BBB permeability is induced by reduced expression of tight and adherens junction proteins. Further, the disease model developed mimics several other alterations observed in Alzheimer's Disease patients, namely, increased levels of matrix-metalloproteinase-2 and intracellular reactive oxygen species in the BECs. In addition, the authors also explored whether reducing Aβ generation improved BBB impairment. For that, ReN-GA and ReN-mGAP cells were treated with a Beta-secretase 1 inhibitor (LY2886721), which prevents the production of the amyloid peptide, Aβ, founding that the levels of Aβ40 and Aβ42 secreted from ReN-GA and ReN-mGAP cells were dramatically reduced compared to vehicle and that the treatment with LY2886721 significantly decreased BBB permeability and increased the level of claudin-5 expression in BECs. Shin and co-workers also explored the possibility that circulating neurotoxins enter the brain through the impaired BBB and exacerbate Alzheimer's Disease progression. For that purpose, they introduced thrombin, a neurotoxic serine protease, within the channel cultured with BECs. The authors demonstrated that cell death increased after treatment in the disease model, suggesting that thrombin crossed through the disrupted BBB, reached the ReN-GA and ReN-

mGAP cell culture, and exacerbated neuronal death. Therefore, this microfluidic device has the potential to be a useful tool in the study of BBB biology in Alzheimer's Disease. [38] In the same context, Vatine *et al.* cultured iPSCs derived from a Huntington's disease patient in a BBB-on-a-chip device to investigate the BBB dysfunction in this disease. They also found that BBB permeability was increased for dextran of several molecular sizes, suggesting the disruption of this structure. [135]

BBB-on-a-chip models also allow evaluation of the brain metastases process. In that regard, Xu and co-workers designed a BBB microfluidic device co-culturing primary rat BMECs and astrocytes and infused various cancer cell types through the vascular compartment to assess malignant cell extravasation across the BBB. They found that while the liver cancer cell line, BEL-7402, did not demonstrate any migration across the BBB over 72 hours, lung cancer (A549), breast cancer (MDA-MB-231) and melanoma (M624) cells showed various degrees of migration over the same period. Additionally, the authors also investigated the ability of brain tumor cells (U87 glioma cells) to cross the BBB from the brain to the vascular compartment. The results show that U87 cells were unable to cross the BBB, being in line with the clinical finding that glioma rarely metastasizes out of the cerebral spinal fluidic space. [29]

Annex IV summarizes several BBB-on-chips developed to study BBB function when exposed to pathologic mechanisms of some CNS diseases.

5. Challenges and future perspectives

Advances in the development of BBB-on-a-chip devices provide opportunities to answer questions about the role of BBB pathophysiology in neurologic conditions and for efficient drug development for these disorders. However, several challenges in the field of BBB-on-a-chip devices must be considered in the fabrication of these platforms to use them as reliable models of the human BBB.

First, in various BBB-on-a-chip, the geometry and dimensions of the vascular compartment do not truly mimic cerebral blood vessels *in vivo*. Effectively, some BBB-on-a-chip devices have a rectangular cross-section, contrasting with the circular cross-section found in living blood vessels. [56, 57, 75, 102] In addition, the vessels diameters vary between the different chips [110, 111, 116] and are generally higher than those that have been reported *in vivo* (7–10μm). [136] However, some groups have already fabricated BBB-on-a-chip devices containing blood vessels with diameters closely or within the physiologic range. Those were developed via vasculogenesis or angiogenesis. [47, 69, 80]

Additionally, most BBB-on-a-chip devices created represent a single blood vessel, which allows the study of mechanisms inherent to basic blood vessels biology but does not reproduce disease phenotypes involved in more complex vascular networks.

Furthermore, interactions between the different cell types are a key aspect when mimicking a BBB model. Nevertheless, physical cell contact can be compromised by the presence of membranes, commonly used in the top-bottom design. [68, 75, 92, 121] Thus, in that regard developing 2.5D or 3D BBB-on-a-chip devices can overcome this problem since they allow for direct cell-cell interactions.

In addition, one remaining major technical challenge is using PDMS in BBB-on-a-chip devices fabrication. In fact, most BBB-on-chips developed until now are made from PDMS. [47, 56, 57, 62, 69, 100, 127] This material is very used due to attractive physical and mechanical properties, such as biocompatibility, low cost, ease of fabrication, oxygen permeability and optical transparency. [137, 138] However, this material tends to exhibit significant adsorption of hydrophobic drugs, biasing drug screening. Thus, it is of interest to replace the fabrication with PDMS for other types of biocompatible polymers less interactive with molecules, such as poly(methyl methacrylate), polycarbonate or polystyrene. [137]

Moreover, a key challenge in BBB-on-a-chip development is the lack of standardization which limits the direct comparison of the results obtained between the different devices developed. In fact, chip materials, biologic coating agents, device designs, cell types and culture protocols, and reagents used differ significantly among the microfluidic BBB-on-a-chip developed. Additionally, quantification of parameters such as TEER measurements, barrier permeability and shear stress also need to be standardized to enable results comparison.

It is likely that, after overcoming standardization issues, BBB-on-a-chip platforms will be implemented and accepted by industry and regulatory agencies to enable the obtention of more reliable *in vitro* results.

A promising approach in the field of BBB-on-a-chip models is using iPSCs derived from patients with neurologic diseases as an efficient tool in precision medicine. Effectively, developing individual BBB-on-a-chip devices may allow to address interindividual variability, both genotypical and phenotypical features, which may cause differences in patient drugs response. Thus, with individual BBB-on-chip models, it can be possible to apply personalized drug screening to optimize the therapeutic response of each patient. [135]

6. Concluding Remarks

Understanding the role of BBB dysfunction in pathological disease mechanisms and developing new drugs for CNS disorders still constitutes a considerable challenge because of the lack of *in vitro* and *in vivo* models that mimic the human BBB characteristics.

The convergence of microfluidic technology and microengineering techniques allowed the emergence of organs-on-a-chip that can model different organs or tissues *in vitro*. Particularly, BBB-on-a-chip cultures have been widely developed.

Throughout this review, the factors to consider when developing a BBB-on-a-chip have been highlighted, and the applications for which these models have been used. In fact, the most important considerations when developing a microfluidic BBB-on-a-chip are the cell type used, the device design and configuration, and the incorporation of shear stress. Effectively, these parameters will influence not only the BBB-on-a-chip quality but also its applicability, since some features might be more important than others, depending on the intended application.

Considerable advances in that field have been made in the last decade, with many groups developing new BBB-on-a-chip platforms for several applications. Although these models still have some limitations, they have shown capability in modeling critical aspects of the human BBB.

In summary, after overcoming issues related to standardization and validation of BBB-on-chips, these sophisticated *in vitro* BBB models may significantly impact biomedical science, drug development and precision medicine.

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Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier.

Device dimensions	Endothelial cells	Co-cultured cells	TJ protein immunostaining	Permeability test	TEER	Other details	External stimuli	Shear stress	Findings	Year	Ref.
2D	Immortalized mouse BECs (b.End3 cell line)	Immortalized mouse ACs (C8D1A cell line)	ZO-1	4 kDa FITC-dextran, 20 kDa FITC-dextran, 70 kDa FITC-dextran	↓	BBB-on-a-chip results were compared with static models results	Histamine, high pH (>10)	ND	Histamine and high pH induced BBB disruption	2012	[121]
2.5D	Immortalized rat BECs (RBE4 cell line)	NA (ACM)	ZO-1 Claudin-1	3 kDa FITC-dextran, 5 kDa FITC-dextran	NA	RBE4 cells were cultured in BBB-on-a-chip and Transwell model in the presence and absence of ACM and the results were compared	R123, Verapamil	↓	1. The barrier permeability is reduced by the use of the ACM 2. The presence of ACM in BBB-on-a-chip allowed significant uptake and increased efflux compared with the RBE4 cells in the absence of ACM	2013	[37]
2.5D	Primary neonatal rat BECs	Primary neonatal rat astrocytes	ZO-1	40 kDa FITC-dextran	NA	The device allows endfeet-like astrocyte-endothelial cell interactions	NA	↓	1. ZO-1 expression increased with the presence of ACM or ACs and with fluid flow in the vascular channels 2. Permeability of fluorescent 40 kDa dextran	2015	[99]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

									decrease in the presence of ACs or ACM 3. Electrical resistance increases in the presence of ACM		
2D	Immortalized mouse BECs (bEnd.3 cell line)	Immortalized mouse ACs (C8D1A cell line)	Claudine-5	70 kDa FITC-dextran	NA	PTFE membrane was integrated into the BBB-on-a-chip and the tests results were compared with devices containing PE membrane	NA	=	PTFE membrane has a better performance than polycarbonate membrane under physiologically shear stress	2015	[40]
3D	Immortalized mouse BECs (bEnd.3 cell line)	Primary mouse ACs	ZO-1	40 kDa FITC-dextran	NA	Treatment with mannitol was applied to assess BBB disruption	Mannitol	ND	Longer temporal exposure to mannitol affects barrier function recovery	2015	[110]
2D	1. Immortalized human BECs (hCMEC/D3 cell line) 2. Primary rat brain endothelial cells	1. Primary rat ACs 2. Primary rat brain PCs	ZO-1	NaF, 4.4 kDa FITC-dextran, Evans blue	↓	1. The same device can be used for modeling three different types of barrier models 2. BBB-on-a-chip results were compared with static models results	NA	↓	The primary cell-based tri-culture model exhibited better BBB properties than hCMEC/D3 cell line monoculture model	2016	[48]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

2D	Immortalized mouse BECs (bEnd.3 cell line)	1. Immortalized mouse PCs 2. Immortalized mouse ACs (C8D1A cell line)	NA	[14C]-mannitol, [14C]-urea	↓	Co-culture (BECs+PCs) and triculture (BECs + PCs +ACs) models of the BBB were developed, and the results were compared	Dexamethasone (to assess functional expression of P-gp)	↓	P-gp efflux pump increased with the increase in number of days in culture and has an enhanced activity in the triculture model	2016	[51]
2D	hiPSC-derived BECs	Primary rat ACs	ZO-1, claudin-5	4 kDa FITC-dextran, 20 kDa FITC-dextran, 70 kDa FITC-dextran, Caffeine, Cimetidine, Doxorubicin	=	The hypothesis on the necessity of applying shear stress in establishing *in vivo* like barrier properties in microfluidic BBB models was tested	NA	↓	High levels of unidirectional laminar shear stress are not required to develop an effective BBB-on-a-chip	2017	[70]
3D	Immortalized human BECs (hCMEC/D3 cell line)	Normal human ACs	ZO-1	4 kDa FITC-dextran	=	BBB-on-a-chip was developed to investigate the mechanical stimuli exerted by blood flow on BBB permeability and waste transport	TNF-α	↓	1. Shear stress and cyclic strain improve the overall BBB integrity 2. Vessel wall pulsation provides a convective force that facilitates retrograde transport along the basement membrane of the cerebral microvasculature	2017	[109]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

2D	Primary mouse BECs	Primary mouse ACs	ZO-1	3 kDa dextran, 10 kDa dextran, 70 kDa FITC-dextran	↓	16 BBB units on a single chip, allowing the conduction of up to 16 different assays in parallel	Histamine	=	1. Use of Matrigel as basement membrane results in tighter barrier formation than fibronectin 2. Interactions between BECs and ACs led to tighter barrier formation	2018	[46]
3D	hiPSC-derived BECs	1. Primary human brain PCs 2. Primary human brain ACs	ZO-1, Occludin, Claudin-5	10 kDa-FITC, 40 kDa-FITC	NA	BBB model developed via vasculogenesis	NA	NA	Culturing hiPSC-derived BECs with ACs and PCs improves the BBB integrity	2018	[47]
3D	1. Primary human BMECs 2. Primary HUVECs	MSCs	ZO-1, Occludin	70 kDa FITC-dextran	NA	Comparison of organ-specific ECs	Thrombin	NA	The organ source of ECs influences the properties of microvasculature	2019	[80]
3D	hiPSC-derived BECs	NA	Claudin-5	Lucifer Yellow, 10 kDa FITC-dextran	NA	1. Directed differentiation of human BMECs from two fluorescently labeled hiPSCs 2. Cryopreservation of hiPSC-derived BECs	NA	↓	Cryopreserved human BMECs display similar barrier function to fresh human BMECs in 3D models	2019	[111]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

3D	1. hiPSC-derived BECs 2. Primary HUVECs	NA	Occludin, Claudin-5	NaF	ND	Study of hiPSC-derived BECs capability to form a confluent barrier	R123 (P-gp substrate), Cyclosporin A (P-gp inhibitor), H2DCFDA (MRP substrate), MK-571 (MRP inhibitor)	↓	1. hiPSC-derived BECs showed better barrier function than HUVECs 2. Efflux transporter activity was maintained over 3 weeks	2019	[39]
3D	hiPSC-derived BMECs	hiPSC-derived PCs	NA	Lucifer Yellow	NA	Effects of PCs on BEC barrier function	NA	=	hiPSC-derived PCs do not influenced barrier function	2019	[41]
2.5D	HUVECs	Human ACs	ZO-1	40 kDa FITC-dextran	↓	The BBB-on-a-chip was obtained by chemical surface modification	NA	↓	The polydopamine-coated surface is a cost-effective ECM alternative for cell culture inside microfluidic devices	2019	[50]
2.5D	Immortalized human BECs (hCMEC/D3 cell line)	Primary human ACs	ZO-1, Claudin-5	10 kDa FITC-dextran, 70 kDa FITC-dextran	NA	BBB model leverages a commercially available chip	NA	=	Exposing endothelium to physiologically shear stress has positive effects on barrier properties	2019	[56]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

2.5D	hiPSC-derived BECs	1. Primary human PCs 2. Primary human ACs	ZO-1, Claudin-5	3 kDa dextran, 10 kDa dextran, 70 kDa dextran, Cetuximab	NA	1. hiPSC-derived BECs were cultured under hypoxic conditions to generate more highly differentiated BMECs 2. Delivery of Cetuximab by reversible osmotic opening of the human BBB in vitro	R123, DiOC2, Citalopram, Doxorubicin, Verapamil (P-gp inhibitor), MK571 (MRPs inhibitor), Ko143 (BCRP inhibitor), $CoCl_2$, DMOG, Mannitol	=	1. Hypoxia-enhanced BBB-on-a-chip sustained levels of low barrier permeability for more than 2 weeks, similar to those observed in the human brain 2. The enhanced BBB-on-a-chip was able to mimic transporter-mediated drug efflux, including appropriate substrate specificity	2019	[75]
3D	Primary human BECs HUVECs	1. Primary human PCs 2. Primary human ACs 3. Primary human lung fibroblasts	ZO-1, Claudin-5, Occludin	10 kDa FITC-dextran, 70 kDa FITC-dextran	NA	1. BBB model developed via angiogenesis 2. Efflux transport system was assessed	Calcein, valspodar (p-gp inhibitor), elacridar (p-gp/BCRP inhibitor)	ND	1. Tri-culture condition exhibited the highest expression level of ZO-1 2. Under inhibitor treatment, a lower proportion of fluorescent molecules was effluxed	2020	[82]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

2D	Immortalized human BECs (hCMEC/D3 cell line)	Human ACs	ZO-1	4 kDa FITC-dextran	NA	1. Multiplexed chip allowing eight parallel experiments in a single chip 2. Two variants of the multiplexed platform were developed: a one-layer device (monoculture of BECs) and a two-layer device (co-culture of BECs+ACs)	NA	↓	The permeability assay showed a size-dependent trend for permeability coefficients	2020	[53]
2D/3D	Immortalized human BECs (hCMEC/D3 cell line)	Normal human ACs	ZO-1	4 kDa FITC-dextran, 20 kDa-FITC dextran, 120 kDa FITC-dextran	NA	The BBB-on-a-chip developed allows for customization in design, cellular composition, cellular orientation, and physiologically-relevant fluid dynamics	TNF-α, VEGF, Verapamil, Calcein AM	=	The BBB-on-a-chip developed is responsive to biochemical (vasodilator cytokines) and mechanical (shear stress) cues	2020	[49]
3D	Immortalized human BECs (TY10 cell line)	NA	ZO-1, Occludin, Claudin-5	10 kDa FITC-dextran, hmAb AF568 (fluorescently labeled recombinant monoclonal human IgG1 antibody)	NA	BBB-on-a-chip with an open design	NA	ND	BBB-on-a-chip cultured with BECs established a significant barrier to antibody diffusion	2020	[73]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

2D	Human ECs derived from hematopoietic stem cells	Bovine brain PCs	Claudin-5, ZO-1	Lucifer Yellow, Evans blue	↓	The impact of fluid flow on glycocalyx-related genes and EC surface charge was assessed	NA	↓	1. Fluid flow and co-culture with PCs decreased the negative surface charge of ECs 2. Fluid flow upregulated glycocalyx-related genes	2021	[92]
2D	Primary mouse BECs	Primary mouse ACs	ZO-1	NA	NA	A numerical approach-based simulation model was proposed to accurately predict the *in vivo* level shear stress	NA	Variable under study	Shear stress increased with a decrease in the dimension of the microfluidic channel and a decrease in the porosity of the polycarbonate membrane	2021	[68]
2D	Immortalized human BECs (hCMEC/D3 cell line)	ACs	ZO-1	NA	↓	Home-made electrodes were placed in the BBB-on-a-chip to measure TEER	NA	=	Shear stress enhances BBB function	2021	[54]
3D	hiPSC-derived BECs	1. Primary human brain PCs 2. Primary human brain ACs	NA	10 kDa FITC-dextran	NA	Commercial chip from AIM Biotech	NA	NA	NA	2021	[91]
3D	Primary human BMECs	1. Primary human PCs 2. Primary human ACs	ZO-1	10 kDa- FITC dextran, 70 kDa FITC-dextran	NA	1. Analyzing the role of human PCs, human ACs and human bone marrow-derived	NA	NA	BBB-on-a-chip cultured with human bone marrow-derived	2021	[69]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

		3. Human bone marrow-derived MSCs 4. Primary human lung fibroblasts				MSCs on the architecture of human BEC-derived microvasculature- on-a-chip			MSCs exhibited better BBB properties than cultured with human PCs		
2D	1. Immortalized human BECs (HBMEC/ci18 cell line) 2. Primary human BECs	NA	ZO-1	Caffeine, Lucifer yellow	↓	Gene expression of BBB markers was compared between BBB-on-a-chip cultured with HBMEC/ci18 cells and BBB-on-a-chip cultured with primary human BECs	R123	↓ OR =	1. HBMEC/ci18 and primary human BECs exhibited similar responses to fluid shear stress 2. The optimized flow condition is 0.3 dyn/cm2	2022	[106]
2.5D	hiPSC-derived BECs	NA	Claudin-5, ZO-1	Lucifer Yellow	NA	1. Commercially available MIMETAS OrganoPlate® 3-lane 2. Passive diffusion evaluation 3. Evaluation of ABC and SLC Transporter-Mediated Transport	[^{3}H] L-arginine (CAT1 substrate), [3H] L-glutamate(GLAST substrate), [14C] L-lactate (MCT1 substrate), AZD3965 (MCTs inhibitor), Gabapentin (LAT1 substrate), JPH203 (LAT1 inhibitor)	↓	Functional expression was observed for BCRP, MCT1, and LAT1 transporters but not for P-gp	2022	[43]

Annex I- Summary of key features of BBB-on-a-chip devices assessing biological processes related to the blood-brain barrier. (Continuation)

3D	1. hiPS-derived BECs 2. Primary human BECs	1. Primary human brain PCs 2. Primary human brain Acs	ZO-1, Claudin 5	10 kDa FITC-dextran, 40 kDa FITC-dextran	ND	Models of hiPS-BECs + PCs + ACs and primary BECs + PCs+ ACs were employed to compare expression levels of 22 BBB-relevant genes via qRT-PCR	NA	NA	In appropriate culture conditions, hiPS-BECs adopt gene expression profiles that closely match those of human primary brain ECs	2022	[74]
3D	Primary human BECs	1. Primary human PCs 2. Primary human Acs	ZO-1	70 kDa dextran	NA	Static and flow conditions were created in the BBB-on-a-chip to study the effect of interstitial flow on the human brain MVN formation	VEGF, Aprotinin	↓	Under flow conditions, microvessels exhibited improved vascular morphological features and enhanced endothelial barrier function	2022	[98]

Legend: ↓- Below physiological levels; =- Physiological levels; ABC- ATP-binding cassette; AC- Astrocyte; ACM- Astrocyte conditioned media; BBB- Blood-brain barrier; BCRP- Breast cancer–resistance protein; BEC- Brain endothelial cell; BMEC- Brain microvascular endothelial cell; CAT1- Cationic amino acid transporter 1; CoCl2 - Cobalt chloride; DiOC2 - 3,3′-diethyloxacarbocyanine Iodide; DMOG- dimethyloxalylglycine; EC- Endothelial cell; ECM- Extracellular matrix; FITC- Fluorescein isothiocyanate ; GLAST- Glutamate/aspartate transporter; hiPSC- Human induced pluripotent stem-cell; HUVEC- Human umbilical vein endothelial cell; LAT1- L-type amino acid transporter 1; MCT- Monocarboxylate transporter; MRP- Multidrug resistance protein; MSC- Mesenchymal stem cell; NA- Not applicable; NaF- Sodium fluorescein; ND- Not defined; PC- Pericyte; P-gp- P-glycoprotein; PTFE- Polytetrafluoroethylene; R123- Rhodamine 123; SLC- Solute carrier; TEER- Transendothelial electrical resistance; TJ- Tight junction; TNF α -Tumor Necrosis factor α; VEGF- Vascular endothelial growth factor; ZO-1- Zonula occludens-1.

Annex II- Summary of key features of BBB-on-a-chips developed for other applications than drug screening and disease modeling. (Continuation)

Device dimensions	Endothelial cells	Co-cultured cells	TJ protein immunostaining	Permeability test	TEER	Other details	External stimuli	Shear stress	Findings	Year	Ref.
2D	Immortalized mouse BECs (bEnd.3 cell line)	NA	NA	Propidium iodide, 4 kDa FITC-dextran	NA	Study of BBB permeabilization by PEFs	PEFs	ND	1. Most of the cells that were electroporated with 10 pulses recovered, while electroporation with 30 and 90 pulses was mostly irreversible	2016	[62]
2D	Human cerebral microcapillary Ecs	NA	NA	NaF, 70 kDa-FITC-dextran	NA	Study of BBB permeabilization by PEFs	PEFs	NA	PEFs can temporarily or permanently disrupt the BBB	2017	[118]
2.5D	Immortalized human BECs (TY10 cell line)	1. Human brain PCs (hBPCT cell line) 2. Human ACs (hAst cell line)	Claudin -5	20 kDa FITC-dextran	NA	Study of antibody transcytosis	NA	↓	1. TY10 cells cultured under perfusion show much tighter barrier formation 2. The BBB-on-a-chip developed is sensitive to differences in antibody penetration	2018	[124]

Annex II- Summary of key features of BBB-on-a-chips for other applications than drug screening and disease modeling (continuation)

2D	Immortalized human BECs (hCMEC/D3 cell line)	Primary Human ACs	NA	NA	NA	T cell extravasation through the BBB was assessed	TNF-α, CD4+ T cells, Jurkat T cells	↓	1. In the infiltration process, CD4+ cells reached longer track length, with increased directionality, and higher speed compared to Jurkat cells 2. Under the inflammatory condition, Jurkat cells and CD 4+ T cells infiltrate through the BBB	2019	[58]
2D	Human cerebral microcapillary Ecs	NA	NA	NaF	NA	Study of BBB disruption by PEFs	PEFs	NA	Lower voltages (300-900 V/cm) induced reversible BBB disruption Higher voltages (1620 – 2700 V/cm) induced irreversible BBB disruption	2021	[127]

Legend: ↓- Below physiological levels; AC- Astrocyte; BBB- Blood-brain barrier; BEC- Brain endothelial cell; EC- Endothelial cell; FITC- Fluorescein isothiocyanate; NA- Not applicable; NaF- sodium fluorescein; ND- Not defined; PCs- Pericyte; PEFs- Pulsed electric fiels; TEER- Transendothelial electrical resistance; TJ- Tight junction; TNF α -Tumor Necrosis factor-α

Annex III- Summary of key features of BBB-on-a-chips developed for drug screening applications.

Device dimensions	Endothelial cells	Co-cultured cells	TJ protein immunostaining	Permeability test	TEER	Other details	External stimuli	Shear stress	Findings	Year	Ref.
3D	HUVECs	NA (ACM)	ZO-1	Evans blue dye, 4 kDa FITC-dextran, 40 kDa FITC-dextran, 70 kDa-dextran	NA	1. Drug screening 2. Effect of hydrogen peroxide on the transendothelial permeability	Propranolol Antipyrine, Carbamazepine, Verapamil, Atenolol, Hydrogen peroxide	↓ Or =	1. Incubation with ACM significantly lowered drug permeability 2. High dose of hydrogen peroxide instantly increased the permeability for dextran	2012	[81]
2D	Immortalized mouse BECs (bEnd.3 cell line)	Immortalized rat glial cells (cell line C6)	ZO-1	NA	↓	1. BBB-on-a-chip results for monoculture and co-culture were compared with Transwell results 2. Drug screening	Ethosuximide, Gabapentin, Sertraline, Sunitinib, Traxoprodil, Varenicline, PF-3084014	=	1. Higher TEER and lower permeability were obtained for all drugs in dynamic and co-culture models 2. Correlation of the resultant logPe values with in vivo brain/plasma ratios showed linear correlation (R2> 0.85)	2014	[101]

Annex III- Summary of key features of BBB-on-a-chips developed for drug screening applications (continuation)

2D	immortalized human BECs (hCMEC/D3 cell line)	Human glioma (U251)	NA	NaF, 70 kDa FITC-dextran	NA	Evaluation of drug permeability across the BBB and drug cytotoxicity on cerebral tumor cells	Sunitinib	↓	The efficacy of sunitinib in BBB-on-a-chip was better predictive of *in vivo* cerebral events compared to the simplified 96-well plates 2D cultures	2016	[45]
2D	Immortalized mouse BECs (bEnd.3 cell line)	NA	Claudin-5	FITC-BSA	ND	Study of the capability of the membranotropic peptide gH625 to enhance NP transport across the BBB	gH625-funcionalized NPs	↓	gH625 increased the transport of NPs through the BBB compared to blank NPs	2017	[116]
2D	Immortalized mouse BECs (bEnd.3 cell line)	NA	Claudin-5	4 kDa FITC-dextran, 20 kDa FITC-dextran, 500 kDa FITC-dextran	↓	Effect of acute flow on Ang2-functionalized NPs binding to BECs and penetration through BBB	NA	↓	Fluid shear stress impacted the binding and BBB penetration of Ang2-functionalized NPs	2018	[44]

Annex III- Summary of key features of BBB-on-a-chips developed for drug screening applications (continuation)

2D	Immortalized human BECs (hCMEC/D3 cell line)	Primary human normal ACs	Claudin-5	4 kDa FITC-dextran	NA	Introduction of an ultrathin silicon nitride membrane in the BBB-o-a-chip that allows for high-resolution imaging of NP interactions with BECs and the capture of rare NP translocation events	40 nm PS-COOH NPs 100 nm PS-COOH NPs 100 nm ApoE-SiO2	NA	1. NP translocation is size-dependent 2. ApoE-SiO2 NPs have a higher number of translocation events	2020	[126]
2D	Immortalized human BECs (hCMEC/D3cell line)	1. Primary human brain PCs 2. Primary human Acs	ZO-1, Occludin	4 kDa FITC-dextran, 40 kDa FITC-dextran	↓	BBB-on-a-chip developed allows for the monitoring of the interactions between cells and NPs and enables the quantification of NPs distribution in vascular and perivascular spaces	IL-1β	=	HDL-mimetic NPs with apolipoprotein A1 are a potential CNS drug delivery system	2020	[52]

Legend: ↓- Below physiological levels; =- Physiological levels; AC- Astrocyte; ACM- Astrocyte conditioned media; Ang2- Angiopep-2; ApoE- Apolipoprotein E; BBB- Blood-brain barrier; BEC- Brain endothelial cell; BSA- Bovine serum albumin; FITC- Fluorescein isothiocyanate; HDL- High-density lipoprotein; HUVEC- Human umbilical vein endothelial cell; IL-1β- Interleukin 1 beta; NA- Not applicable; ND- Not defined; NP- nanoparticle; PC- Pericyte; TEER- Transendothelial electrical resistance**;** TJ- Tight junction; ZO-1- Zonula occludens-1

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications.

Device dimensions	Endothelial cells	Co-cultured cells	TJ protein immunostaining	Permeability test	TEER	Other details	External stimuli	Shear Stress	Findings	Year	Ref.
2D	Immortalized human BECs (hCMEC/D3 cell line)	NA	ZO-1	NA	↓	BBB-on-a-chip results were compared with static models results	TNF-α	=	1. Shear stress increases BBB integrity 2. TNF-α induces BBB disruption	2013	[57]
2.5D	Immortalized Rat BECs (RBE4 cell line)	NA	ZO-1	40 kDa FITC-dextran	NA	1. Assessment of neutrophil chemotactic transmigration 2. BBB-on-a-chip was treated with TNF-α to simulate neuroinflammation and with OGD procedure to simulate an ischemia model	Human neutrophils, IL-8, TNF-α, OGD procedure, Edaravone (antioxidant), Y-27632 (Rho Kinase inhibitor)	NA	1. Treatment with TNF-α elevated the release of several cytokines and reduced the expression of ZO-1 protein 2. OGD procedure led to activation of ROS and Rho Kinase by the oxidative stress 3. Edaravone and Y-27632 showed limited protective effects	2015	[125]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications. (Continuation).

2D	Primary Human BMECs	1. Primary human PCs 2. Primary human ACs 3. hiPSC-derived cortical neurons	ZO-1, Claudin-5	10 kDa-FITC dextran**n**	↓	Assessment of BBB responses to inflammatory stimulation	LPS, TNF-α, IL-1β, MCP1,2	ND	Initial exposure to LPS and cytokine cocktail increased dextran diffusion and TEER and reduced TJs expression	2016	[139]
2.5D	Primary rat BMECs	Primary rat ACs	ZO-1, Claudin-5	NaF	=	1. Modeling of malignant cell extravasation in brain metastases 2. Investigation of the process of brain metastases 3. Drug screening	Lung cancer cells (A549), Breast cancer cells (MDA-MB-231), Melanoma cells (M624), Liver cancer cells (BEL-7402), U87 glioma, 8 therapeutic agents (TMZ, CBP, DDP, 5-Fu, NDP, GEM, IFO, FTO)	↓	1. Liver cancer cells did not demonstrate any migration across the BBB 2. Lung cancer, breast cancer and melanoma cells showed various degrees of migration 3. U87 cells were unable to cross the BBB to the vascular compartment 4. Only TMZ passes through BBB and induces apoptosis in glioma cells	2016	[29]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications. (Continuation).

3D	Primary human BECs	1. Primary human brain PCs 2. Primary human brain Acs	ZO-1	3 kDa fluorescent dextran	NA	1. BBB-on-a-chip results were compared with static models results 2. Viscous fingering method was performed to generate lumens in collagen gels	TNF-α	↓	1. Apparent permeability was lower for BBB-on-a-chip than for the Transwell model 2. Secretion levels of G-CSF, IL-6 and IL-8, induced by TNF-α, were significantly higher in the microfluidic BBB chip compared to Transwell cultures	2016	[89]
2.5D	HUVEC	1. Rat brain ACs (CTX-TNA2 cell line) 2. Met-1 metastatic murine breast cancer cells- BTB	NA	3 kDa Dextran, 70 kDa Dextran, Sulforhodamine 101	NA	The authors developed two models: BBB (reference) and BTB models	R123, Verapamil (P-gp inhibitor), Cyclosporine A (P-gp inhibitor)	↓	The function of P-gp remained intact in both models	2017	[84]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications. (Continuation).

2.5D	Human BMECs	NA (ACM)	ZO-1	40 kDa dextran	↓	Contribution of PKCδ in neuroinflammation was assessed	TNF-α, PKCδ inhibitor, neutrophils	ND	PKCδ inhibition prevented activation of BECs, protected BBB structure integrity and attenuated neutrophil adhesion and migration	2018	[97]
2.5D	Immortalized human BECs (hCMEC/D3 cell line)	ReNcell VM human neural progenitors (ReN-GA cells, ReN-mGAP cells, ReN-WT)	ZO-1, Claudin-5	3 kDa dextran 40 kDa dextran	NA	A BBB-on-a-chip that recapitulates several key aspects of BBB dysfunction observed in Alzheimer's disease was developed	LY2886721 (BACE1 inhibitor)	NA	1. In Alzheimer's disease model, BBB permeability was increased, accompanied by reduced expression of TJs 2. Amyloid-β deposition was evident in the Alzheimer's disease model 3. Reducing amyloid-β generation with LY2886721 decreased BBB permeability	2019	[38]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications. (Continuation).

3D	hiPSC-derived BECs	NA	ZO-1, Claudin-5, Occludin	Lucifer Yellow, 10 kDa FITC-dextran	=	1. P-gp inhibition 2. Hyperosmolar blood-brain barrier opening 3. Inflammation induction	Tariquidar (p-gp inhibitor), R123, Mannitol, TNF-α	=	1. Tariquidar increased permeability of R123 2. Mannitol disrupted BBB 3. TNF-α exposure resulted in upregulation of ICAM-1 and VCAM-1 4. Microvessels exposed to TNF-α showed higher numbers of adherent peripheral blood mononuclear cells	2019	[71]
2D	Stem cell-derived brain-like endothelial cells	NA (pericyte-conditioned medium)	Claudin-5, ZO-1	Lucifer yellow	NA	1. human T-cells interacting with a human BBB model 2. Nanoporous silicon nitride membrane was used in the BBB-on-a-chip	TNF-α, Human Th1 cells	↓	TNF-α stimulation favored interactions between T-cells and brain-like endothelial cells	2019	[90]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications (continuation)

2D	hiPSC-derived BECs	hiPSC-derived ACs	ZO-1, Occludin, Claudin-5	NaF	=	Two types of BBB-on-chips were developed: devices with porous membrane with either 0.4 or 8.0 μm pore size for studying the transport of molecules or cells, respectively	Calcein AM (P-gp and MRP1 substrate), Verapamil (P-gp and MRP1 inhibitor), TGF-β1	ND	Treatment with TGF-β1 induces astrocytes activation	2019	[93]
2.5D	Immortalized human BECs (hCMEC/D3 cell line)	1. Human Primary ACs 2. Immortalized human GBM cell line U87	NA	10 kDa-FITC dextran**n**	↓	Modeling of the brain tumor microenvironment	Tumor spheroids (with U87 B cells), Ab-Nut-NLCs	ND	When treated with Ab-Nut-NLCs, about 70% of the GBM cells resulted to be positive for ethidium homodimer-1	2020	[140]
3D	HUVEC	Primary human ACs	NA	NA	NA	1. Investigation of BBB dysfunction by INPM 2.Microfluidic chips by AIM Biotech	INPM, vitamin C	ND	1. High exposure of INPM (40 μg/ml) significantly increased ROS levels 2. Vitamin C treatment attenuated the total ROS concentrations in the 40 μg/ml INPM exposure group	2020	[83]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications. (Continuation).

3D	Neonatal primary rat BECs	1. Neonatal primary rat brain PCs 2. Neonatal primary rat brain ACs	ZO-1	40 kDa FITC-dextran	↓	Fluid flow was controlled by a pump-free strategy	TNF-α, Dexamethasone	ND	1. TNF-α reduces the gene expression of occludin and increase the production of IL-6 and CINC-1 2. Dexamethasone restricts the production of IL-6 and CINC-1	2020	[100]
2.5D	Immortalized human BECs (hCMEC/D3 cell line)	1. Immortalized human PCs 2. Immortalized human ACs 3. Immortalized human GBM cell line U87	ZO-1	10 kDa FITC-dextran, Nitrofurantoin, Sucrose, Caffeine, D-glucose, Alanine-L	NA	1. Chip channels were coated and functionalized with a photocrosslinkable copolymer 2. Cellular uptake and BBB permeability of porous silicon NPs were evaluated	R123, Elacridar	↓	1. Surface functionalization with photocrosslinkable copolymer allows the formation of a stable and evenly distributed ECM coating 2. GBM model was able to predict the permeabilities of nanomaterials across the BBB	2020	[59]

Annex IV- Summary of key features of BBB-on-a-chips developed for disease modeling applications (continuation)

2.5D	hiPSC-derived BECs	1. Primary human PCs 2. Primary human brain ACs 3. iPSC-derived dopaminergic neurons 4. Primary human microglia	Claudin-1, Claudin-5, Occludin, ZO-1	3 kDa dextran, Lucifer Yellow, 160 kDa Immunoglobulins	NA	Assessment of BBB disruption in αSyn-associated Parkinson's disease pathology	αSyn fibrils, Trehalose	ND	1. Exposure to αSyn fibrils induced TJs derangement and BBB endothelium gene expression alterations associated with distinct biological processes implicated in Parkinson's disease 2. Trehalose rescued the derangement of the TJs	2021	[134]
2D	Primary human BMECs	1. Primary human brain PCs 2. Primay human ACs	ZO-1	70 kDa FITC-dextran	NA	1. Characterization of neuroimmune interactions after systemic inflammation 2. Testing the efficacy of a clinically relevant omega-3 fatty acid emulsion	IL-1β, Omega-3 fatty acid emulsion	NA	1. IL-1β induced overexpression of biomarkers linked to neuroinflammation and impaired permeability 2. Omega-3 fatty acids eliminated the IL-1β, induced VCAM-1 overexpression and inhibited IL-1β-induced intercellular gaps	2022	[42]

Legend: ↓- Below physiological levels; =- Physiological levels; 5-Fu- 5- Fluorouracil; Ab-Nut-NLCs- antibody- functionalized nutlin-loaded nanostructured lipid carriers; AC- Astrocyte; ACM- Astrocyte conditioned media; BACE1 - Beta-Secretase 1; BBB- Blood-brain barrier; BEC- Brain endothelial cell; BMEC- Brain microvascular endothelial cell; BTB- blood-tumor barrier; CINC-1- Cytokine-induced neutrophil chemoattractant 1; ECM- Extracellular matrix; FITC- Fluorescein isothiocyanate; GBM- Glioblastoma; G-CSF- Granulocyte colony-stimulating factor; hiPSC- human induced pluripotent stem-cell; HUVEC- Human umbilical vein endothelial cell; ICAM-1- Intercellular Adhesion Molecule 1; IL- Interleukin; INPM- indoor nanoscale particulate matter; LPS- Lipopolysaccharide; MCP- Monocyte chemotactic protein; MRP- Multidrug resistance protein; NA- Not applicable; NaF- Sodium fluorescein; ND- Not defined; NP- Nanoparticle; OGD- oxygen-glucose deprivation; PC- Pericyte; P-gp- P-glycoprotein; PKCδ- Protein kinase C delta; R123- Rhodamine 123; SS- Shear stress; TEER- Transendothelial electrical resistance; .TGF-β1- Transforming growth factor beta 1; TJ- Tight junction; TMZ- Temozolomide; TNF α -Tumor Necrosis factor α ; VICAM-1- vascular cell adhesion molecule-1; WT- Wild type; ZO-1- Zonula occludens-1; αSyn- alpha-synuclein