UNIVERSIDADE D
COIMBRA

Ana Sofia Almeida Silva Morais

Relatório de Estágio e Monografia intitulada "Organ-on-a-chip: current status and future prospects" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Cláudia Silvestre e da Professora Doutora Carla Sofia Pinheiro Vitorino e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicasde Mestrado Integrado em Ciências Farmacêuticas.

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Coimbra, 28 de outubro de 2020.

Ana Sofia Almeida Silva Morais

(Ana Sofia Almeida Silva Morais)

Agradecimentos

À minha Mãe, ao meu Pai à minha Irmã e ao meu Irmão, por constituírem a casa que será sempre o meu porto de abrigo. Ser-vos-ei sempre grata pelo suporte que foram e sempre serão para mim.

A toda à minha Família! Sempre me apoiaram e acreditaram nos meus sonhos.

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Coimbra, guardar-te-ei sempre comigo!

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Parte I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

Farmácia de Celas

farmáciadecelas

Lista de Abreviaturas

CHUC	Centro Hospitalar e Universitário de Coimbra
FC	Farmácia de Celas
FFUC	Faculdade de Farmácia da Universidade de Coimbra
MICF	Mestrado Integrado em Ciências Farmacêuticas
MNSRM	Medicamentos não sujeitos a receita médica
MSRM	Medicamentos sujeitos a receita médica
PIM	Preparação individualizada da medicação
PSBE	Produtos de saúde e bem-estar
SNS	Sistema Nacional de Saúde
SWOT	*Strengths, Weaknesses, Opportunities, Threats*

1. Introdução

O papel do farmacêutico tem vindo a sofrer algumas mutações ao longo do tempo. Inicialmente, a sua atividade centrava-se na preparação de medicamentos e substâncias medicamentosas, resultando o comum nome de farmácia de oficina. Com a evolução da sociedade, o farmacêutico necessitou de adaptar a sua atividade, centrando-a no utente e na comunidade, surgindo o conceito de farmácia comunitária[1].

A farmácia comunitária é uma das principais áreas de atuação do farmacêutico, tendo esta uma vertente privilegiada pelo seu contacto direto com a população. A farmácia comunitária surge muitas vezes como o ponto de acesso para o Sistema Nacional de Saúde (SNS), onde as pessoas se dirigem primeiramente, na procura de uma resposta para o seu problema, antes mesmo de procurar ajuda médica. Deste modo, o farmacêutico deve promover a literacia em saúde e a consciencialização do uso racional do medicamento, mantendo os seus conhecimentos atualizados[2].

O Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) integra na fase final do seu plano de estudos um estágio curricular completo, diversificado e integrado, onde é permitido ao futuro profissional de saúde experienciar o papel do farmacêutico e o seu impacto na sociedade. Considerando ser uma experiência crucial para a consolidação de conhecimento, onde esta se entrelaça com a comunicação, com a experiência e com a adaptação de comportamento, culminando no desenvolvimento de um profissional mais apto e preparado para os futuros desafios.

Neste âmbito, serve o presente relatório para legitimar a minha atividade ao longo do estágio curricular na Farmácia de Celas, realizado sob a orientação da diretora técnica da farmácia, a Dra. Cláudia Silvestre, com duração total de 810 horas de estágio.

Esta análise tem como objetivo uma avaliação crítica da capacidade de integração dos conhecimentos teóricos adquiridos ao longo dos cinco anos de MICF na atividade prática. Esta encontra-se elaborada sob a forma de um modelo SWOT (que engloba uma dimensão interna, onde se inclui os Pontos Fortes (*Strenghts*) e Pontos Fracos (*Weaknesses*), bem como, uma dimensão externa apresentada pelas Oportunidades (*Opportunities*) e Ameaças (*Threats*)[3].

2. Farmácia de Celas

A Farmácia de Celas (FC) foi fundada em 1957, designando-se inicialmente por "Farmácia Montes Claros" e localizava-se na Rua António José d'Almeida. Em 2002 transferiu as suas instalações para a Av. Armando Gonçalves, com o objetivo de melhorar os serviços prestados aos seus utentes. Atualmente, encontra-se localizada no início da Estrada de Coselhas, situando-se num ponto comum às principais vias de acesso ao Centro Hospitalar e Universitário de Coimbra (CHUC), Hospital Pediátrico, Hospital CUF e Hospital da Luz. Esta localização prestigiada confere à farmácia uma grande heterogeneidade de clientes. A FC tem como missão promover a saúde da comunidade em que está inserida através dos serviços que presta, visando o uso racional do medicamento e produtos de saúde, bem como a melhoria da efetividade e segurança da terapêutica medicamentosa. A valorização, motivação e otimização dos seus colaboradores é a principal força desta instituição[4].

3. Análise *SWOT*

3.1 Pontos Fortes

3.1.1 Dinamismo e proatividade da equipa

A Farmácia de Celas é constituída por uma equipa jovem, qualificada e experiente, que procura estar sempre atualizada, de modo a transmitir informações credíveis e cientificamente sustentadas no momento do aconselhamento aos seus utentes. A enorme recetividade e disponibilidade da equipa para o esclarecimento de quaisquer dúvidas, a partilha de conhecimento e a inclusão em diversos eventos e campanhas dinamizados pela farmácia, foi um dos pontos fortes para a minha completa integração neste grupo de trabalho. Relevo a atitude e a intervenção de cada elemento da equipa durante os meus atendimentos ao público, que contribuíram para a minha autonomia e maior segurança durantes o aconselhamento farmacêutico.

Existe a crescente necessidade da farmácia se tornar num espaço cada vez mais dinâmico e que vá de encontro às necessidades do utente. Neste sentido, observei e participei ativamente na construção e alteração de lineares, para que estes se adequassem a determinadas fases do ano. Destaco a utilização inteligente de estratégias de marketing farmacêutico, cuja a sua utilização permitiu aumentar a rentabilidade de recursos existentes e diminuir o seu desperdício.

3.1.2 Diversidade de serviços farmacêuticos

Atualmente, a venda de Medicamentos sujeitos a receita médica (MSRM) nas farmácias corresponde a uma pequena parte do total de produtos vendidos. Neste sentido, o paradigma das Farmácias Portuguesas tem sofrido profundas alterações ao longo tempo. Atualmente o espaço da farmácia é percecionado como um local de promoção de saúde e bem-estar. A FC apresenta uma grande diversidade de serviços farmacêuticos à disposição do utente. No seu espaço dispõe de três gabinetes de atendimento que são usualmente destinados à medição de parâmetros bioquímicos, consultas de nutrição e nutrição clínica consultas de podologia, *shiatsu*, administração de vacinas, teste de zaragatoa para a pesquisa de *Streptococcus* do grupo A e fotodepilação. Para além destes serviços existem outros dois realizados no laboratório da farmácia - preparação individualizada da medicação (PIM) e preparação de medicamentos manipulados. Neste sentido, saliento a oportunidade que tive de proceder à preparação de medicamentos manipulados, de efetuar a PIM e auxiliar nas consultas de nutrição, reforçando a minha perceção da abrangência do papel do farmacêutico comunitário junto da comunidade. A FC realiza um elevado número de medicamentos manipulados por ano, principalmente para uso pediátrico e veterinário[5]. Estes surgem muitas vezes, devido à falta de opções terapêuticas para um determinado utente devido, por exemplo, à inexistência de uma formulação farmacêutica e/ou dose adequadas ou a alguma intolerância, sendo então necessário adaptar a terapêutica.

Relativamente à PIM, faz parte da função do farmacêutico contribuir para uma correta utilização do medicamento, de forma a garantir a sua segurança e eficaz. Por vezes, doentes com idades avançadas, doenças crónicas associadas e com um elevado número de medicamentos, apresentam alguma dificuldade em tomar todos os seus medicamentos de forma correta. Assim sendo, a PIM surge como um auxílio para garantir a adesão à terapêutica[6]. Apesar de não haver uma grande adesão a este serviço, este era feito quinzenalmente e as estagiárias tiveram a oportunidade de executar esta tarefa.

As consultas de nutrição realizavam-se às terças-feiras e este serviço apresentava uma elevada adesão. Considero um serviço relevante para a melhoria do meu conhecimento, pois a nutricionista apresentou brevemente os produtos dietéticos que existiam na farmácia e em que situações os podia aconselhar.

3.1.3 Foco no utente

O critério fundamental de qualquer atendimento na Farmácia de Celas é a satisfação do utente. Para tal deve-se empregar estratégias que permitam estabelecer um diálogo de confiança entre o farmacêutico e o utente, com vista a esclarecer quaisquer dúvidas

subjacentes ao atendimento ou a outra questão colocada. Deste modo, sempre me foi incutido a dar a máxima atenção ao utente que tinha à minha frente em cada atendimento e certificar-me de que transmiti a mensagem certa. É importante que os utentes percecionem o farmacêutico como um profissional com enorme importância na prestação de cuidados de saúde. Para além disto, a FC dispõe de um sistema personalizado de impressão de etiquetas de posologia, onde constam vários elementos identificativos do utente e do modo de administração dos medicamentos (identificação da farmácia, nome do utente, nome do produto e respetiva dosagem, posologia e, caso se aplique, uma frase de precaução/momento da toma). Este tipo de canal de comunicação com o utente é um fator diferenciador da farmácia pois, adicionalmente ao caráter profissional, torna-se igualmente apelativo dado puder ser possível existir uma etiqueta personalizada para cada utente. Adicionalmente, a possibilidade de criar uma etiqueta individual para cada utente e personalizá-la consoante as suas necessidades, proporcionou uma maior interação entre mim e o utente no momento do atendimento. A empatia, muitas vezes criada, leva a que o utente deposite no farmacêutico uma maior confiança, conduzindo a uma maior valorização do mesmo.

3.1.4 Diversidade de utentes

Inserida num local privilegiado, junto a uma das principais vias de acesso aos principais hospitais e centros clínicos da cidade, a FC abrange uma grande variedade de utentes de diferentes faixas etárias. Para além disso, a variabilidade de motivos que traz um utente à farmácia é evidente, o que diversificou o conjunto de situações clínicas com que me deparei no decorrer do estágio e face às quais fui solicitada a dar o meu aconselhamento. Como tal, procurei sempre adequar e personalizar o meu discurso de acordo com o público-alvo, recorrendo à análise de fatores como a idade, grau de literacia e motivo, que se revelaram preponderantes no momento do atendimento. Todos estes aspetos permitiram o aumento dos meus conhecimentos e desenvolvimento de competências úteis na criação de uma profícua relação farmacêutico-utente.

3.2 Pontos Fracos

3.2.1 Insegurança no aconselhamento de determinadas áreas

Para sermos bons profissionais de saúde temos de saber educar a comunidade em relação ao uso seguro e correto do medicamento, mas também, aconselhar quando somos solicitados pelos utentes. Para tal, é necessário conhecer os produtos, as formas farmacêuticas, os princípios ativos, a sua ação e correta utilização. Na fase inicial do estágio

curricular é difícil conhecer tudo o que existe no mercado e mesmo na farmácia. Para além dos conhecimentos relacionados com o medicamento, também temos de ter uma noção em relação aos produtos de saúde e bem-estar (PSBE) que existem no mercado e quais estão disponíveis na nossa farmácia, como produtos de dermocosmética, de ortopedia, de uso veterinário, entre outros. Apesar da vasta formação que o plano curricular de MICF nos proporciona, considero que por vezes se torna um pouco insuficiente nestas ocasiões, despoletando alguma insegurança no momento do aconselhamento.

Em outras ocasiões em que não me senti confiante no momento de venda foi o desconhecimento e posterior compreensão do sistema de comparticipação do SNS e de complementaridades. Vejo este ponto como algo que está em falta numa disciplina de gestão farmacêutica, que teria como resultado uma maior facilidade no momento da dispensa de qualquer bem de saúde.

3.2.2 Interrupção do estágio curricular

Foi no momento em que estava a sentir-me mais confortável e confiante ao balcão, na interação com os utentes, que vi suspendido o meu estágio por tempo indefinido. Já decorridos dois meses e meio de estágio curricular, foi ativado o estado de emergência, devido à pandemia COVID-19, levando à interrupção deste mesmo, o que acabou por prejudicar a minha aprendizagem como futura profissional de saúde. Quando o estágio foi retomado e regressei à farmácia, senti alguma regressão tanto na autonomia durante o atendimento, como no conhecimento do espaço da farmácia. Considero que confinamento imposto pela situação de pandemia originou alguma frustração e apatia, o que não contribuiu para melhorar os meus conhecimentos, apesar de mais tempo livre.

3.3 Oportunidades

3.3.1 Dinamização dos produtos e espaço físico da farmácia

No decurso do meu estágio foi observando, numa fase inicial, e posteriormente passei a elaborar estratégias de dinamização de produtos tanto no espaço físico da farmácia como nas suas redes sociais. Tive a oportunidade de construir *posters*, vídeos, publicações, entre outros, com o objetivo de divulgar campanhas que decorriam na farmácia ou para melhorar a rotatividade de alguns produtos cujo consumo era mais baixo. O espaço da farmácia foi sofrendo alterações nos lineares e nas gôndolas de forma a tonar o espaço mais coeso, mais organizado, e adequado à época do ano, acabando por resultar numa ajuda tanto para farmacêutico no momento do atendimento como para o utente, acabando por contribuir de

alguma maneira para o aumento das vendas. Deste modo, tive a oportunidade de testar algumas estratégias de marketing, pois não podemos esquecer que a farmácia é uma empresa, e que sem vendas, esta não sobrevive. É necessário desenvolver estratégias capazes de promover os produtos e o espaço.

3.3.2 Formação Contínua

O Desenvolvimento Profissional Contínuo preconizado pela Ordem dos Farmacêuticos prioriza a excelência do exercício profissional farmacêutico, respondendo às necessidades ao nível da saúde e bem-estar dos doentes e dos cidadãos em geral, através da atualização de conhecimentos e formação contínua. Neste âmbito, foi-me concedida diversas oportunidades de participação em formações desde a área da dermocosmética, à nutrição e homeopatia. Consegui absorver conhecimentos que me permitiram melhorar o meu aconselhamento e torná-lo mais personalizado.

3.4 Ameaças

3.4.1 Pandemia COVID-19

Com pandemia do SARS-CoV-2 o modo de funcionamento da FC teve que ser ajustado de forma a cumprir o plano de contingência e garantir a segurança tanto dos colaboradores como dos seus utentes. A equipa passou a trabalhar em espelho, ou seja, metade da equipa trabalhava na parte da manhã, e a outra operava na parte da tarde. Este método surgiu para evitar o contacto máximo entre as duas "sub-equipas" formadas. No entanto, com esta separação surgiram alguns problemas de comunicação e dificuldade em transmitir recados de situações que aconteciam em um dos turnos para o outro. Originando por vezes, alguma falta de coerência no momento do atendimento, quando era questionada por alguma situação que não se tinha sucedido comigo.

Considero a pandemia uma ameaça à minha aprendizagem, pois numa fase inicial, levou a uma ida da população à farmácia abrupta, numa procura desmesurada por equipamentos de proteção individual e álcool, medicamentos de uso crónico e medicamentos não sujeitos a receita médica (MNSRM) indicados em casos gripais ou de constipação. Neste sentido, foi necessário a farmácia adotar um sistema de gestão, que consistia na racionalização da dispensa de medicamentos de uso crónico e no incentivo à consciencialização de que o utente não iria precisar de toda aquela medicação naquele momento.

Numa fase mais tardia, e após a retoma do estágio, a farmácia teve pouca procura, as pessoas evitavam sair à rua, e os aconselhamentos que me pediam eram quase sempre

relativos à situação de pandemia e de como é que deveriam de se comportar. Confesso que foi difícil transmitir informações que estavam em constante mudança. Todos os dias tinha a preocupação de me atualizar sobre novas normas e diretrizes sobre a situação de pandemia e como proceder, mas nem sempre o que transmitia num dia era verdadeiro para o dia seguinte. No entanto, tinha que manter a credibilidade da informação que partilhava.

4. Caso Clínico

Utente, do sexo masculino, com cerca de 43 anos dirige-se à farmácia com uma prescrição médica de Nolotil® e questiona se há algo mais que lhe possa aliviar as dores advindas de uma contractura muscular na perna direita, já diagnosticada no CHUC[7].

Com o intuito de validar a informação dada pelo utente, questionei-o acerca de quando e como surgiu a dor e se tinha introduzido alguma medicação nova. Este referiu que a dor surgiu como uma pontada aguda enquanto andava de mota e que após este momento o "músculo ficou preso", não tenho iniciado nenhuma medicação.

As lesões músculo-esqueléticas são uma das principais causas de dor e classificam-se como agudas ou crónicas. Estas podem afetar músculos, ligamentos, articulações ossos ou cartilagem. Surgem normalmente de atividades diárias (ex. exercício físico), da adoção de posturas incorretas ou sedentarismo. Quando causadas por um trauma acidental, pioram com o movimento e cedem ao repouso. As lesões músculo-esqueléticas agudas mais comuns são: cãibras, contusões distensões, contracturas e entorses. Estão geralmente associadas a dor e edema, podendo ocorrer hematoma e dificuldade de movimentos. Por outro lado, as lesões músculo-esqueléticas crónicas mais comuns são a osteoartrite e as lombalgias.

Nestes sentido, aconselhei o uso de um anti-inflamatório tópico, Voltaren Emulgel® (diclofenac), que deverá ser aplicado com uma massagem suave na zona dolorosa três a quatro vezes por dia[8]. Propôs, também, um suplemento com magnésio indicado para a manutenção muscular, visto que este catião possui impacto na contração muscular. O magnésio desempenha um papel antagonista comparativamente com a função desempenhada pelo ião cálcio (cálcio: responsável pela contração; magnésio: responsável pelo relaxamento).

Por fim aconselhei a evitar qualquer posição que favorecesse o agravamento da lesão ou que pudesse vir a desencadear outra e incentivei à correção de postura, mesmo durante o sono. Como outra medida não farmacológica de conforto sugeri a utilização uma botija de água quente na zona da contractura, com o intuito de contribuir para o relaxamento muscular.

Avisei o utente que em caso de não sentir melhoras durante os sete dias seguintes para contactar novamente o médico.

5. Considerações Finais

Concluo esta etapa do meu percurso académico com um enorme sentimento de gratidão. A Farmácia de Celas permitiu moldar e aperfeiçoar a profissional de saúde que se foi construindo ao longo destes cinco anos de MICF. Os conhecimentos adquiridos são a base fundamental, mas a comunicação, a experiência e toda a prática são essenciais.

O estágio curricular realizado na FC ao longo de seis meses permitiu-me adquirir uma visão global de todas as tarefas inerentes à prática farmacêutica, aplicando e consolidando conhecimentos teóricos previamente adquiridos ao longo do meu percurso académico.

Este não foi o estágio que idealizei, foram tempos de muitas incertezas, resiliências e acima de tudo, muito desafiantes. Os farmacêuticos estiveram e estão todos os dias na linha da frente, esta nova realidade obrigou a uma reorganização da estrutura das farmácias, exigiu uma adaptação muito rápida quer dos profissionais quer do espaço. O farmacêutico é desde sempre um elemento fundamental na educação para a saúde, na promoção de estilo de vida saudável, na adesão à terapêutica, no incentivo ao uso racional do medicamento, na prestação de cuidados farmacêutico e preservação da Saúde Pública. É o rosto mais próximo que o utente tem do SNS, encontrando-se numa posição singular para colocar em prática um serviço com qualidade e de confiança, capaz de responder às necessidades e particularidades de cada utente.

Hoje, prestes a ser farmacêutica, sei que toda esta nova realidade me permitiu ganhar muitas mais valências, graças à equipa excelente que tive como suporte, uma equipa motivada e preocupada, que contribuiu desde o início para a minha segurança e bem-estar, mas que acima de tudo sempre me apoiou e permitiu que eu crescesse enquanto profissional.

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Parte II

MONOGRAFIA

"Organ-on-a-chip: current status and future prospects"

List of Abbreviations

ADME	Absorption, distribution, metabolism and excretion
BBB	Blood-brain barrier
DDTs	Drug Development Tools
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
FDA	Food and Drug Administration
FSS	Fluid shear stress
IL-2	Interleukin-2
iPSC	Induced pluripotent stem cell lines
MPS	Microphysiological systems
Multi-OOAC	Multi-organs-on-a-chip
NCE	New chemical entities
OOAC	Organ-on-a-chip
PDMS	Polydimethylsiloxane

Resumo

Organs-on-a-chip, também conhecidos como sistemas microfisiológicos (MPS), são microssistemas de bioengenharia com capacidade de recriar aspetos da fisiologia e da função de órgãos humanos. Surgem como uma ferramenta *in vitro* com inúmeras aplicações na descoberta e desenvolvimento de fármacos, inspirados na natureza complexa e hierárquica do corpo humano.

A capacidade de mimetizar tecidos humanos em ambientes multicelulares tridimensionais fisiologicamente relevantes permite a sua aplicação em diversas etapas do processo de desenvolvimento do medicamento. Estas incluem a avaliação da segurança e da toxicidade de potenciais moléculas terapêuticas durante a fase inicial do desenvolvimento pré-clínico, a avaliação *in vitro* da eficácia do fármaco e a modelação de doenças, permitindo simular a fisiopatologia de subpopulações específicas e até mesmo de um indivíduo em particular, alcançando assim, a medicina personalizada.

Nesta monografia são explorados o desenvolvimento e a evolução das plataformas microfluídicas que permitiram a transição de células em chips para órgãos em chips e algumas das oportunidades e aplicações oferecidas pela tecnologia *organ-on-a-chip*. Adicionalmente, são apresentados o potencial atual e as perspetivas futuras destes sistemas microfisiológicos, bem como e os desafios que esta tecnologia ainda enfrenta.

Palavras-chave: *Organ-on-a-chip*; Microfluídica; Microambiente celular; Modelação de doenças; Avaliação de medicamentos; Medicina personalizada.

Abstract

Organs-on-a-chip, also known as microphysiological systems (MPS), are bioengineering microsystems capable of recreating aspects of the physiology and function of human organs. They are inspired by the complex and hierarchical nature of the human body, thus arising as an *in vitro* tool with numerous applications in the discovery and development of drugs.

The ability to recapitulate human tissues in physiologically relevant three-dimensional multicellular environments allows their applications in various stages of the drug development process. In particular, they may be employed in the evaluation of the safety and toxicity of potential therapeutic molecules during the initial phase of pre-clinical development, in the *in vitro* assessment of the efficacy of the drug and in the modelling of human diseases. This will allow the simulation of the pathophysiology of specific subpopulations and even of a particular individual, thus reaching personalized medicine.

In this monograph, the development and evolution of microfluidic platforms that have allowed the transition from cells on chips to organs on chips and some of the opportunities and applications offered by the organ-on-a-chip technology are presented. Also, the current potential and future perspectives of these microphysiological systems and the challenges that this technology still faces are discussed.

Keywords: Organ-on-a-chip; Microfluidic; Cellular microenvironment; Disease modelling; Drug testing; Personalized medicine.

1. Introduction

The development of improved models drives biological and biomedical research. These systems aim to mimic the human physiology and pathology from the molecular level to the tissue and organ complexity, generating relevant information on the etiology of the disease, therapy and prevention. The human body can be understood as an organized set of cellular and non-cellular materials, which interact in a highly orderly manner. Its complex nature inspires and prompts the recreation of various levels of the human being and the elaboration of biological models, which can consist of various types of cells to similar organ systems.

Conventionally, animal models are often used to recapitulate human physiology in several areas of biomedical research. However, they do not have the ability to faithfully mimic human responses due to the vast number of variables and differences between animal and human biology. Simplistic models, such as two-dimensional (2D) monocultures of cells on plate, have their merit in application for the study of biological processes, but these formats usually lack cell-cell and matrix-cell interactions, essential for maintaining and defining specific phenotypes of cells. In this way, they are unable to resemble the cellular functions and intercellular communication that exist in tissues or organs. Three-dimensional (3D) cell aggregates and spheroid cultures demonstrate better functional capabilities than 2D models. However, they still need to be improved in several critical aspects in order to simulate the development and function of organs, such as space-time biochemical signals, vascular perfusion, mechanical signals or cellular co-cultures. Most current models are still far from being fully capable of reconstituting all the functions that exist at the cell, tissue and organ levels. Notwithstanding, it is essential to develop biological systems that can increasingly address specific scientific issues in biomedical research.

Considerable advances in microfabrication and microfluidics technology have led to enhanced cell culture capacity in a complex and highly controlled microenvironment (space-time), more faithfully imitating the *in vivo* microenvironment (CHIN *et al.*, 2016). The combination of microfabrication and microfluidic technology allows precise control of the dynamic flow of fluid, enabling to create cellular microenvironments in which the cells are subjected to relevant chemical gradients. These new features have driven forward the development of alternative models based on *in vitro* cells, which better simulate the complex structures and functional complexity of living organs, called organs-on-a-chip. The organ-on-a-chip systems combine bioengineering and cellular biology with microfluidics, which enable the study of various biological and pathophysiological mechanisms of the human body, which

otherwise was not possible with animal models and conventional 2D and 3D cell culture models (BHATIA and INGBER, 2014).

In this monography, it will be addressed in more detail the evolution of cell culture models and the microfluidic culture system, as the basis for the development of dynamic cell cultures with improved resources. Also, design considerations and the main components indispensable to recreate the cellular microenvironment using microfluidic chips will be described. Some examples of organ-on-a-chip models and their transition from cells-on-a-chip to organs-on-a-chip will be presented, with the demonstration of possible applications in the health area, envisioning to improve clinical practice. Finally, some regulatory aspects will be exposed that will culminate in the commercialization and practical application of these models.

2. Progression from 2D cell culture to organ-on-a-chip

2.1 Limitations on drug development

The traditional approach to pharmaceutical development presents some limitations in what concerns the screening to identify molecule(s) with real therapeutic benefit. The lengthy steps considered prior to the molecule is available for marketing, subject to approval by regulatory authorities, which on average last between 10 to 12 years, constitute one of the critical challenges in the development of a new drug and its translation to the clinical practice (WARE and KHETANI, 2017; ZHANG *et al.*, 2017).

Millions of deaths occur every year due to the rapid progression of certain diseases, such as cancer. In addition, several specific genetic mutations resulting from multiple factors can trigger a new disease. Therefore, there is an urgent need for the development of tests and methods of analysis able to provide a rapid and accurate evaluation of the efficacy and safety of both new or existing molecules and, as such, shorten the time spent during the development of the medicinal product (WARE and KHETANI, 2017).

The development of safe and effective drugs has been slowed down in recent years mainly due to the inability to develop models that represent the phenotype, function and intercellular signalling of human cells with *in vitro* precision. Animal models are widely regarded as the gold standard in pre-clinical studies for testing new medicines. However, these models do not accurately reflect human drug metabolism, thus resulting in insufficient predictive capacity for human outcomes (MESSNER *et al.*, 2013). Significant efforts have been made to develop cell culture systems based on microengineering, microfabrication,

bioprinting and microfluidic technologies (LIU *et al.*, 2018) that focus on the development of models that allow the creation of an *in vitro* controlled environment of 3D tissue systems (HUH *et al.*, 2011).

For a complete understanding of how tissues develop and function, as well as their physiopathology, the study of how cells and tissues behave as an integral part of entire living organs is of extreme importance. These are composed of various types of dynamic and variable tissues in terms of 3D structure, biochemical microenvironment and mechanical properties. Unfortunately, most studies on cell and tissue regulation are based on procedures with cell growth in 2D cell culture models, which do not have the ability to reconstitute the cellular microenvironment *in vivo* and, as such, does not demonstrate its differentiated functions (HUH *et al.*, 2011).

2.2 Evolution of 2D cell culture and addition of a third dimension to the system

In 2D cultures, the cells are cultivated in a monolayer on a flat surface. This methodology has been used for many years, making it an noteworthy tool for drug screening, toxicity assessment studies, and cellular and molecular analysis of mechanisms of numerous diseases, such as Parkinson's Disease (SINGH *et al.*, 2017), diabetes (WALLET *et al.*, 2017), among others. It is due to its simplicity, stability, ease of handling and high performance features that 2D cell culture continues to be widely used. Gene editing technology has been applied in 2D systems as a tool for obtaining several induced pluripotent stem cell lines (iPSC), where cells from a single donor are derived to give rise to multiple tissues (SINGH *et al.*, 2017).

Despite the wide use of 2D cell culture systems, the limitations of this model prevent an accurate mimicking of human physiology. Plate-cultured cells reveal inconsistent properties when compared to the source tissue. In addition, it is evidenced the loss of normal tissue architecture and the disappearance of biophysical forces and the 3D heterotypic environment or cell niche. This corresponds to the environment where cells normally reside *in vivo* and have the ability to interact dynamically and reciprocally with other cell types, inserted within the highly organized structure of the extracellular matrix (ECM) (JIANG *et al.*, 2018). These unnatural characteristics can induce a significant change in the phenotypic properties of cells, which are forced to adapt to the new conditions. These are limiting factors that hamper the accurate simulation of physiological manifestations *in vitro*. This incompatibility becomes evident when the *in vitro* drug diffusion kinetics that does not

correspond to *in vivo* environmental function is evaluated and where effective doses in 2D cultures are, in most cases, ineffective when sized for animals and patients.

Several efforts have been made to minimize these limitations, which has led to the development of 3D cell culture models in which cells are grown inside ECM gels (HUH *et al.*, 2011). This approach improves the expression of differentiated functions and tissue organization. However, it still does not have the ability to reconstruct the characteristics of organs *in vivo*, which are critical to their primary function, such as the tissue-tissue interface (vascular epithelium-endothelium), spatio-temporal gradients of chemicals and oxygen and a mechanically active microenvironment (movements of inhalation and exhalation - respiration).

3D culture systems also demonstrate some technical challenges, such as the evaluation of physiological diffusion gradients (transport of ions in the kidney) or collection of samples of cellular products secreted (bile flow in the liver). It is due to the several limitations previously presented for 2D and 3D cell culture systems, that the analysis of normal and pathological cell processes is permanently dependent on studies with animal models that are expensive and time consuming (OLIVEIRA and REIS, 2020).

However, 2D and 3D cell cultures have their own advantages and disadvantages, which must be taken into account for the intended application (Table 1).

2.3 Organoids

The term organoids corresponds to cultures of three-dimensional cells, which normally derive from stem cells and self-organise to form a tissue and/or organ in a simplistic model. Accordingly, different cell types and ECM are arranged in pre-production chambers with microfluidic channels - allowing fluid to flow through the cells - and cultivated under different conditions, favouring the mimicking of tissue complexity. The process described above corresponds to the development of microphysiological systems (MPS), such as organs-on-a-chip. These models allow precise control over the architecture and composition of the tissue to be formed, adding the ability to pre-programme specific oxygenation and nutrient diffusion profiles. These improvements in the system have promoted a significant increase in the viability of the chip, which can function from several weeks to months (TANATAWEETHUM *et al.*, 2018). In order to promote differentiation, cell maturation, and data acquisition regarding tissue function, MPS can be applied, which allows cell-cell interactions, specific tissue interactions and biophysical forces (shear stress, mechanical tension, electrical forces, peristalsis and respiratory movements) that make them more

reliable than the 2D culture systems discussed above. Another advantage of MPS is the possibility of incorporating several biosensors, which enable genetic sequencing, reading of analytical parameters, real-time monitoring, sample recovery, among others.

2.4 Organ-on-a-chip vs. Organoid: Synergistic potential

Unlike the organ-on-a-chip development, which is stimulated by specific design and production characteristics, the organoid formation is dominated by stochastic principles of self-organization, where cells group together to form masses that reproduce the normal organization and functioning characteristics of *in vivo* tissues (LOU and LEUNG, 2018). In order to achieve the various types of human tissues, induced pluripotent stem cells have been widely used. As they differentiate *in vitro*, as response to specific inductive stimuli, they acquire their own 3D structural arrangement that contains different cell types in spatial configurations similar to those recorded in organs during normal *in vivo* development (DUTTA *et al.*, 2017).

However, although organoids have a complex architecture similar to native tissues, the time required to develop is extremely high when compared to partial tissue formation. This technology is also associated to other disadvantages, such as the poor reproducibility of the models, the complexity of the sample collection process, especially in what concerns the impossibility of reading and analysing relevant tissue development parameters and the difficulty in assessing these cultures, which hampers their real time monitoring. Moreover, the lack of perfusion of these 3D structures is pointed out as a conditioning factor in the sufficient transport of oxygen and in the supply of nutrients to the innermost cells of these constructions. This condition may trigger the development of a necrotic nucleus in the cell mass formed, leading to decreased viability and reduced life span of these cultures (OLIVEIRA and REIS, 2020).

A critical appraisal of tissue-on-a-chip technology and organoids is summed-up in Table 1.

Aiming at combining the best of both approaches, a new 3D *in vitro* model was developed - synergic engineering (TAKEBE *et al.*, 2017). Generally speaking, the design goal for this new model is to take advantage of the design precision of bioengineering, which is characteristic of the production of an organ-on-a-chip together with the formation and organization at random of the common development of organoids. This joint potentiality may be useful for the development of a culture medium that has the capacity to supress all the needs of different types of connected tissues, resembling blood in the multi-organs system (TAKEBE *et al.*, 2015; ZHANG *et al.*, 2016).

3. Organ-on-a-chip: fundamentals

For the development of reliable cell cultures based on microfluidics, it is indispensable to reproduce the cell microenvironment observed *in vivo*. To produce an *in vitro* biomimetic cell microenvironment, complex, multi-purpose designs are required to combine microproduced substrates with microfluidic technology and cell biology. The cells and the ECM generate several biochemical and mechanical signals, similar to the *in vivo* microenvironment. For the construction of a functional and coordinated tissue, it is crucial the presence of these stimuli that serve as guidance for tissue organization and growth, establishing cell polarization and migration, promoting a balance between growth and apoptosis, and regulating cell behavior and the functional expression of proteins. Cell-cell communications inserted in the cellular microenvironment present several characteristics, such as the reduced communication distance between cells and other stimuli, the continuous supply of nutrients and removal of waste, and the synergistic actions of all cells to external stimuli.

Microfluidic systems arise as a useful tool for the reconstruction of the cellular microenvironment, allowing precise control of intercellular communication and the application of biochemical and biophysical stimuli necessary for the formation and maturation of tissues or organs (WIKSWO, 2015).

Organ-on-a-chip (OOAC) is a three-dimensional cell culture system that combines microfluidic technology with tissue engineering, in which cells are carefully arranged and cultivated within chambers and channels uninterruptedly perfused, with the aim of reiterating the physiology of tissues and biological organs in realistic models (Figure 1). This platform demonstrates the ability to mimic the functionalities of an organ, reproducing its complex multicellular architecture and human physiology, including crucial aspects such as cell-cell and matrix-cell interaction, physicochemical microenvironment, vascular system and electrical stimulation, which are not possible to replicate in 2D and 3D *in vitro* culture static systems (EL-ALI *et al.*, 2006). These platforms can be applied in disease modeling, drug screening and in the identification of patient subgroups that may have greater benefit with a given clinical treatment. The OOAC technology enables data real-time monitoring from a variety of cell and tissue specific responses in a non-invasive way. To this end, continuous monitoring of the tissue produced is required to evaluate its functionality and response to environmental stimuli (presence of pathogens, drugs or toxic compounds) (BALIJEPALLI and SIVARAMAKRISHAN, 2017; XU *et al.*, 2013).

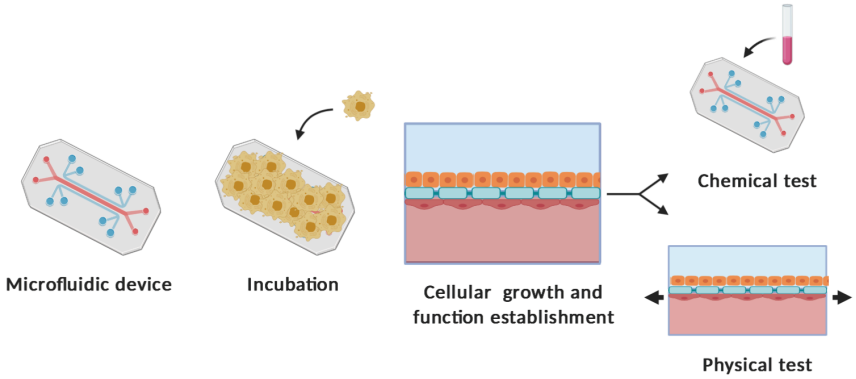

Figure 1. Key steps involved in the manufacturing process of an OOAC. The process to produce different OOAC is in principle, the same, taking into account its application. First, the design of the platform must be in line with the properties that the system intends to address. Second, different cells must be incubated in the device. Third, cell growth, differentiation and function are established so that the chip functions as an organ. Fourth, the data are obtained through tests that allow detecting changes in the system. [Adapted from (SOSA-HERNÁNDEZ *et al.*, 2018)]

Initially, analysis techniques were incorporated into microfluidic platforms, such as the enzyme immunoabsorption assay - ELISA; electrical transepithelial resistance - TEER and multiple electrode matrices - MEA (HENRY *et al.*, 2017; KOUTSOURAs *et al.*, 2017; MAOZ *et al.*, 2017). Currently, several techniques are applied simultaneously on a single organ-on-a-chip platform. These platforms have physical (temperature, pH detection), chemical (immunobiosensors to monitor secreted biomarkers or to assess oxygen levels) and optical (sensors to observe the neuromuscular contractile capacity) sensors, which allow continuous measurements of the environment and cellular behavior. However, to ensure the long-term stability of the embedded sensors, technical improvements should be made to certify that no loss of accuracy or precision of the method occurs during prolonged cell culture times. Advances in this technology indicate the increasing utility of MPS in various contexts for the development of cutting-edge technology that can provide detailed information on cellular functions that have never been achieved on any other platform (RIAHI *et al.*, 2016).

3.1 Key Components

The development of organ-on-a-chip platforms essentially requires four main components, which include microfluidics (fluid which provides all the components necessary for cell maintenance and balance, and which allows the removal of the residual liquid with cell surpluses); the living cell component (2D or 3D cell structure developed in an organized way and often associated with biomaterials such as hydrogels); the physicochemical stimulation (application of stimuli in order to improve the mimetization of the microenvironment *in vivo*

- may correspond to the addition of a drug to be evaluated); the detector (addition of sensors and analysis technologies at critical points of the system that allow the collection and processing of relevant data from the microphysiological device - incorporation of electrodes, microscopy, chromatography.

3.2 Microfluidic technology

The advance of microfluidic technology has attracted the interest of researchers from various fields of knowledge, due to its ability to develop systems with structures designed to assess the physical relevance of the mechanism and transport of fluids. There has been a boost in the development and improvement of several aspects of microfluidic devices, specifically involving the manufacturing, the materials used as surface and the integration of several operations and techniques (OLIVEIRA and REIS, 2020).

Microfluidics is the science that manipulates and processes the fluid with high precision on a micro-scale using a system of pre-defined channels in a device, which can vary its size up to hundreds of micrometers. The ultimate goal of its current application is as an integral part of a device in reconstituting the physiological microenvironment of tissues and/or organs *in vivo*, in a normal or disease situation, which takes advantage of its ability to control the fluid and its spatial-temporal physical characteristics (JUSOH *et al.*, 2015). Despite initially employed as biochemical analysis and detection tools, more recently, microfluidic or lab-on-a-chip platforms are being applied to diagnosis. These devices allow the study of various parameters, conditions and biological mechanisms *in vitro* consuming reduced quantities of reagents and cells and obtaining results with high sensitivity in a short period of time, which distinguishes them from current cell cultures (WHITESIDES, 2006). Microfluidic systems have allied themselves with *in vivo* strategies and demonstrated their value in the development of new drugs and biological research, due to the possibility of the technology being used for the evaluation and development of new biocompatible materials (BARATA *et al.*, 2016).

In microfluidics, the fluids are forced to flow in a microchannel with the aid of a pressure applied to the reservoir where this same channel is connected. In microfluidic devices the flow is characterized by a low number of Reynolds, which means that the flow is laminar. The number of Reynolds corresponds to the ratio between the inertia forces and the flow viscosity forces (DU *et al.*, 2016).

3.3 Manufacturing components

Microfluidic culture devices are usually produced using the soft lithography technique using polydimethylsiloxane (PDMS) (MCDONALD and WHITESIDES, 2002). This process starts from a mold usually produced with photoresistent silicon, which contains the negative image of the intended channel pattern and where the material that will give rise to the chip shape is poured together with a cross-linking agent. After cooling and separation of the parts, the newly formed block can be reversibly or irreversibly sealed, depending on the final purpose of use. This method allows the production of multilayer microfluidic devices with flexible microstructure configurations. Thus, soft lithography is a rapid prototyping technique being applied to build micro and nanostructures with a defined channel pattern, usually using a low-cost polymer. This process corresponds to the evolution of previously used procedures along with the incorporation of microelectromechanical system techniques (AHMED *et al.* 2018; ZHANG *et al.*, 2013).

However, the high time and cost associated with the soft lithography process makes it unattractive to mass production. The chip development process involves multiple steps with several restrictions, such as the difficulty of complete automation of some phases of the process (e.g. assembly and gluing). This technique produces a device with a much lower structural complexity when compared to that observed in *in vivo* tissues. In this way, 3D printing emerged as one of the new technologies used to produce microfluidic devices. The 3D printer makes it possible to print complex and highly realistic structures for a short period of time and at low cost. 3D printing enables the production of microfluidic systems with the desired configuration in a single step and uses only one device, where the digital data are converted into the desired structure. This technology allows the standardization of the procedure and results in the production of more reproducible devices when compared to the previous method (BHATTACHARJEE *et al.*, 2016).

Similarly, it is highlighted the possibility of bioprinting microfluidic models *in vitro*, i.e. to directly bioprint 3D artificial tissues on microfluidic platforms. The production of tissues with complex microstructures allows the proper investigation of several stimuli, including dynamic mechanical cues (rigidity and fluid flow) and chemical cues (chemotaxis and concentration gradients)(YI *et al.*, 2017).

3.4 Biomaterials - application in microfluidic systems

The remarkable evolution of biomaterials is promoting their application in *in vitro* microenvironments, with the ability to simulate cell niches *in vivo*, as well as their usefulness

in the regeneration and replication of various types of normal and diseased tissues in tissue engineering. The growing development of 3D culture models using biomaterials has demonstrated its relevance in the conception of biological models for the study of complex biological processes, which are not possible to evaluate with traditional 2D models (PRADHAN *et al.*, 2016).

PDMS is a synthetic polymer with high optical transparency which allows to observe the behavior of cells and to detect the expression of cell molecules responsible for various pathophysiological mechanisms, through the incorporated use of a brightfield or fluorescence microscope. The high flexibility of the polymer allows the integration of microvalves that act in the cellular manipulation of the chip. The main advantage of PDMS is the biocompatibility with the cellular material which is essential for the long-term viability of *in vitro* cell culture in microchannels or microchambers, as well as gas permeability, which is seen as a beneficial characteristic for cell development (GAO *et al.*, 2009; SHI *et al.*, 2010; WEN *et al.*, 2015). However, due to PDMS hydrophobic characteristics, its application in these systems is not beneficial to cell adhesion. Alternatively, other biomaterials available for the production of configurable microstructures for the development of microfluidic cell cultures are natural polymers such as collagen, fibrin, agarose and synthetic polymers, such as polyethylene glycol - PEG, both of which can be used for the production of hydrogels. These materials have demonstrated good ability to mimic conditions and to develop cell structures similar to those observed *in vivo* in ECM (BERTHIER *et al.*, 2012; CHOI *et al.*, 2011; SHI *et al.*, 2015; TOH *et al.*, 2009).

3.5 Application of dynamic systems in 3D culture models

The microfluidic systems when applied to cell culture, initially corresponded to a single cell layer aligned in microchannels or microchambers with a medium perfused in a controlled way. The evolution of these systems allowed their use in the study of cell growth, proliferation, differentiation and screening of drugs. The gradual alteration of these analytical structures made possible the development of *in vitro* perfunded 3D cell cultures, which represents an enormous step towards the recapitulation of *in vivo* dynamics, since the existence of flow influences the structural complexity of *in vitro* cells (CHUNG *et al.*, 2012; WALKER *et al.*, 2004). Microfluidic 3D cell culture exhibits numerous advantages, such as cell size regulation, tissue reproduction and ease of manipulation of different hydrogels (YAMADA *et al.*, 2015). In addition, these platforms demonstrate a great potential for efficient high performance experimentation (SACKMANN *et al.*, 2014).

Natural products are one of the main sources of new chemical entities (NCE), but they are also increasingly in demand by the world population, as shown by the remarkable increase in the growth rate of the world market. Microfluidic systems can be useful in understanding and assessing the impact of these natural products by identifying the physiological action of a given compound present in an original complex matrix, thus helping identifying potential drug candidates (SHEN, 2015).

As demonstrated, microfluidic devices offer new models for different areas, such as the study of biological mechanisms of diseases, identification of new targets, screening of new drugs and development of new biomaterials. All the evolution and effort employed to create models that better reflect the *in vivo* situation has given rise to the technology that is now known as organ-on-a-chip (BROWN *et al.*, 2015; WANG *et al.*, 2017).

3.6 Design concepts

3.6.1 Cell – Extracellular matrix Interaction

The process of tissue formation is mediated by extracellular matrix proteins that are secreted by various cell types, responsible for providing physical support to the structure. These proteins have the ability to promote cell-cell and matrix-cell interactions, directing the development and behavior of the cells and stimulating several intracellular signaling pathways. MPS exhibit the ability to incorporate the ECM in order to promote gradients of several parameters for the correct simulation of the *in vivo* environment. Several studies demonstrate the impact of ECM proteins on cell proliferation and cell phenotype in response to exposure to various conditioning factors such as oxygen gradients, nutrients and soluble factors (evaluate effects of collagen on growth and differentiation of mesenchymal stem cells; study of the impact of polyornithine and laminin on proliferation and phenotypic differentiation of rat neural progenitor cells). As an example, a microfluidic device was planned to mimic the microenvironment of neuronal cones through the association of gradients of soluble guidance cues with surface-bound guidance signals. The surface-bound laminin gradient enabled to tune the polarity of the neuronal growth cone in response to gradients of neurotrophic factors. This model revealed the impact of knowledge and understanding of the mechanism of tissue formation and progression (BAKER *et al.*, 2013; WANG *et al.*, 2008).

3.6.2 Cell – Cell interaction

Cell-cell interaction influences morphogenesis and cell development, and promotes tissue healing, as inferred from its impact on the organization of the human body which requires a complex, ordered and synergistic arrangement among the various cell types to form organized structures with functional interactions. Interactions between cells may occur by direct contact (cell-cell junction) or by indirect contact (diffusion of soluble factors) under normal physiological conditions. Some OOAC devices were built in order to study the impact of interactions between different cell types, since this technology allows the manipulation and cultivation of different cell types within a microchamber, demonstrating the importance of communication between cells in the adjustment of functions and migration of metastatic cells (ZHANG *et al.*, 2013).

3.7 Control of the biochemical environment

3.7.1 Concentration gradients

The various cellular behaviors such as growth and differentiation, migration and angiogenesis are influenced not only by cellular interactions, but also by biochemical factors that have an important regulatory action and they are present in the microenvironment of the tissue, forming gradients of soluble concentrations. These gradients are physiologically relevant, however, difficult to simulate in 2D and 3D cell cultures. OOAC can overcome this limitation because they have the ability to generate chemical gradients that mimic those existing *in vivo* and thus enable the study of their impact on the orientation of cell behavior and the evaluation of their importance in the variability of biological responses. Microfluidic cell culture platforms have been developed with the intention of investigating angiogenesis in the presence of growth factor gradients and to validate chemo-attraction of leukocytes exposed to different concentrations of inflammatory stimuli (SHIN *et al.*, 2011).

Oxygen gradients have shown to be an important factor in the performance and maintenance of homeostasis in specific tissues, presenting an action promoting angiogenesis and inducing an acute cellular response in inflammatory situations (CHUNG *et al.*, 2006).

3.8 Control of the biophysical environment

3.8.1 Fluid flow – induced stress

The fluid flow has as function the mass transport (administration of nutrients), the distribution of soluble factors and the collection of cellular surpluses that are present throughout the human body - blood vessels, lymphatic vessels. Depending on the organ, the

flow may have different velocity values that vary throughout the body. Different fluid flow rates may have the ability to induce various responses in different cell types. The organ-on-a-chip systems allows the generation and simulation of fluid shear stress (FSS) in microchannels involved in the activation of cellular surface molecules and associated signaling cascades, so as to evaluate their effect on cellular adhesion, growth, morphology and protein expression (MEER *et al.*, 2009). The simulation of these stresses in models representative of human physiology is of extreme relevance, which may improve the investigation of their effect at the level of regulation of specific tissues. Some studies have demonstrated the impact of the FSS on the modulation of cell behavior through the reorganization of the cytoskeleton and its effect on the modeling of angiogenesis associated with tumor biology (SONG and MUNN, 2011).

3.8.2 Tissue mechanics

Under physiological and pathological conditions, the cells are exposed to mechanical stimuli specific to each organ, such as traction and compression forces, in addition to the FSS. For simulation and interpretation of these forces a multilayer microfluidic device was developed to simultaneously evaluate the effects of solid mechanical and surface tension stress induced by cyclic wall elongation and the reproduction of the air-liquid interface in the alveoli of the lung (DOUVILLE *et al.*, 2011). In studies of intestinal absorption and metabolism, a microfluidic chip was created in which the intestinal epithelial cells suffered physiological mechanical deformation (drip flow and cyclic mechanical distortion). These mechanical stimuli induced the cells to spontaneously form robust structures of intestinal villi and promoted their differentiation into four different cell lines of the small intestine. Mechanical stimulation is a key determinant of differentiation during the physiological process (KIM *et al.*, 2012; KIM and INGBER, 2013).

In general, these platforms present a great potential in the advancement of studies correlated with tissue engineering, resembling the organ physiology and analyzing healthy and sick tissues, as well as their etiology. This technology has demonstrated a wide potential in the investigation of specific molecular mechanisms, efficient drug screening, toxicity and predictive medicine, which are considered critical points in the current drug development process (WARE and KHETANi, 2017).

In the next sections, it will be presented some organ-on-a-chip models directed to the following organs: liver, lung and heart and multi-organ-on-a-chip platforms that aim at screening, efficacy and evaluation of drug toxicity.

4. Cell resources for developing an organ-on-a-chip

Cell models should be able to reproduce some functions of the tissue or organ they represent and should include a correct ratio between the various cell types that make up the structure. To improve the analogy between OOAC and human tissues, a correct selection of biological resources is necessary. Immortalized human cell lines and primary cells are commonly used for the recapitulation and study of human biology, as they are easy to cultivate, economical and have biological characteristics similar to their *in vivo* equivalents (WORKMAN *et al.*, 2018).

Immortalized cell lines, achieved through genetic alterations, can be continuously cultivated without presenting any phenotypic and genotypic variations. These cell lines have the ability to proliferate rapidly under relatively simple culture conditions and can be applied in processes of optimization of several organ-on-a-chip system parameters at an early stage of development. However, immortalized cell lines are not sufficient to accurately evaluate human physiology (metabolic activities, efficacy and toxicity).

Primary cells can be isolated from human biopsies or obtained from discarded tissues. Their use on OOAC platforms for the production of a given organ can be advantageous, since it has the capacity to generate pharmacologically reliable results regarding the toxicological response triggered by xenobiotics. Its ability to create different microenvironments, specific to the organ to be reproduced *in vitro*, favors the behavioural similarity between cells. Although primary cells produce an enhanced model of human physiology when compared to the model of immortalized cell lines, their ability to simulate the level of complexity of an organ is low due to the lack of cell proliferation and source of human tissue (LUNI *et al.*, 2014).

4.1 Stem cell engineering

In order to overcome the limitations mentioned above, stem cells have been the new target of biomimetic models for the accurate prediction of human responses to drug treatment. These cells present by definition the capacity of self-renewal and controllable differentiation, under specific microenvironments, in various types of specialized cells or tissues, becoming a powerful source of biological tissue for the production of an organ-on-a-chip. The most common stem cell types include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells (ASCs). Mesenchymal stem cells (MSCs) are the most common type of ASC cells that can be extracted from adult tissue, such as bone marrow or fat tissue. However, they are of little application in the development of microphysiological systems due

to their limited ability to differentiate, lack of consistent cell shunt protocols and unclear biological responses (WAGNER and HO, 2007). ESCs come from blastocysts or from internal embryo cells. Despite their pluripotent nature, this type of cells trigger numerous ethical questions, leading to specific regulations and restrictions (MUSHAHARY *et al.*, 2018). The iPSCs are generated through reprogramming with specific transcription factors in somatic cells from adult tissue, originating immature cells from mature ones, which acquire the ability to differentiate into different cell types (SAYED *et al.*, 2016). No significant differences were observed between ESCs and iPSCs with the same genetic background regarding the levels of gene expression, surface marker expression and morphology (NARSINH *et al.*, 2011; WAGNER *et al.*, 2006).

Induced pluripotent stem cell derived tissue models have demonstrated great applicability in large-scale studies and their uniformity over time. This has led to the development of different iPSC differentiation protocols on chips (GERAILI *et al.*, 2018) and the updating of existing Good Cell Culture Practice guidelines, which reinforces the importance of accuracy and quality among OOAC systems. Cell line libraries are being created with iPSCs of individuals without a characterized disease, with different genetic origins and from different ethnicities, as well as from individuals with several genetic pathologies. It is expected that these cell lines may be available for the study of many diseases, allowing to recapitulate the physiology outside the patient, arising as an alternative to reduce costs and develop customized tissue models (PAMIES *et al.*, 2018).

To make their application in OOAC feasible and profitable, it is essential to improve the efficiency and reproducibility of the iPSC differentiation process. Their conversion into the desired cell type relies on the exposition to determined chemical and physical stimuli. Note that for cell maturation and aging occur, it is necessary to promote mechanical, electrical and others stimuli that reflect the *in vivo* environment, where the tissue is originally inserted. Therefore, most systems with iPSCs still reflect a profile of embryonic gene expression, and improved development is required to achieve an adult phenotype.

Thus, iPSCs have attracted attention in the development of customized OOAC models, because they offer patient induced pluripotent stem cells from patient's somatic cells. This enables to produce normal and disease customized platforms that simulate patient specific physiology more effectively when compared to animal models and allows their use in personalized drug screening studies (ZHAO *et al.*, 2011). This excludes the need for primary tissue isolation of patients, which is usually an invasive and difficult procedure. The reduction of pre-clinical research time and the possibility of drug development based on genetic maps

and diseases of individual patients during the safety and toxicity testing of new drug candidates demonstrate the importance of introducing iPSCs together with OOAC in the evaluation of the pharmaceutical pipeline (ESCH *et al.*, 2015; SCOTT *et al.*, 2013).

The application of iPSCs in OOAC will allow comparing individual physiological responses of several patients to drugs and developing models for the study of genetic diseases, whose responsible mutation is not yet known. However, and despite the enormous potential presented by these cells, there are still numerous limitations regarding their precise ability to reiterate human organs in OOAC. Therefore, it is necessary to implement systematic experimental methods for the study of individual cell response to external physical stimuli and ECM changes during the screening of new drugs and toxicity assessment, based on patient and disease specific iPSCs (ESCH *et al.*, 2015).

5. Bioengineering organs-on-a-chip devices

5.1 Liver-on-a-chip

The liver is one of the most important organs of the human body, being responsible for numerous functions, such as protein synthesis, digestion and metabolization of xenobiotics. In order to simulate the complex *in vivo* microenvironment of the liver, several liver-on-a-chip devices have been developed (BHISE *et al.*, 2016; NO *et al.*, 2015). To imitate the functional unit of this organ, LEE and collaborators elaborated an artificial microdevice inspired in the biological hepatic structure. In this system, a barrier capable of performing the function of transport analogous to the hepatic acino was constructed. This model was used to evaluate the hepatotoxicity of diclofenac (LEE *et al.*, 2007). With advances in microfabrication technology, extremely organized hepatic microtissues capable of simulating the structural and physiological functions of the liver *in vivo* were created, which contrasts with conventional approaches, that use random co-culture methods (HO *et al.*, 2013). Despite the simplicity of 2D culture systems, hepatocytes quickly lose their ability to proliferate, form differentiated structures and their specific functions, when compared to the microenvironment observed in 3D culture. 3D culture models can promote hepatocyte functions and maintain differentiated properties for extended periods of time *in vitro*, in addition to reconstructing *in vivo* tissue architecture as similar as possible (GIESECK *et al.*, 2014; MALINEN *et al.*, 2014). Several methods, based on microfluidic technology, have been developed in order to recapitulate the 3D liver tissue, and demonstrated to be more

effective, because they offer a convenient and direct platform for the formation of 3D structures of uniform shape and size.

Continuous perfusion is an important factor in 3D cell culture systems with significant impact on the maintenance of hepatocyte function and viability in the long term. In this way, microfluidic technology allows the precise control of the medium perfused in the culture and its chemical composition, which is not possible in traditional systems. The fluid flow has promoted and maintained the 3D tissue-like architecture and the specific functions of human primary hepatic cells, as well as the differentiation of stem cells into hepatocytes. DASH *et al.* (2013) reported hepatocytes with higher metabolism capacity by the specific enzymes of P450 cytochrome in the flow system, when compared to that analyzed in the non-flow system (DASH *et al.*, 2013). In another model, hepatocytes and fibroblasts were co-cultivated in a mixed format in order to evaluate rifampicin toxicity. This device was also used to study the invasion and aggregation of breast cancer cells (TRIETSCH *et al.*, 2013). Recently, a low-cost cell culture device was developed with the ability to produce 3D liver microtissues. Several cell types were included in this system, including primary hepatocytes, star cells, fibroblasts and Kupffer cells under specific fluid flow conditions. The hepatic microtissues showed good enzymatic activity and developed response to bacterial lipoprotein (ESCH *et al.*, 2015). Finally, the evolution of 3D hepatic microsystems represents an important step towards the adoption of OOAC and demonstrates the interest of the scientific community in this type of technology for disease modeling and development of new drugs.

5.2 Lung-on-a-chip

The lung is the main organ responsible for gas exchange in the human body, serving as a gateway for drugs, toxins, pathogens and other xenobiotics. It allows the exchange of gases from the airways to the circulatory system. Several biomedical researches have been dedicated to this subject in order to improve lung health, preventing lung diseases and somehow saving lives. The alveolar epithelium - endothelium interface is the fundamental functional unit of the living lung. There are, currently, some improved methods that reproduce the architecture of this pulmonary interface based on the culture of endothelial cells and epithelial cells on opposite sides of a thin and porous membrane. However, despite the advance they demonstrate in relation to traditional cell culture, these models are unable to simulate the pulmonary mechanical microenvironment, characteristic of respiratory movement, observed *in vivo*. These mechanical stresses are reported to have a crucial role in monitoring pulmonary epithelial cell functions, such as growth, migration, apoptosis, ECM

protein secretion and fluid transport and defense action (DORYAB *et al.*, 2016; TAVANA *et al.*, 2011).

HUH *et al.* (2007) developed a biomimetic microsystem of a lung-on-a-chip, which reconstitutes the functional interface pulmonary-endothelium epithelium. This microfluidic device consists of two layers of PDMS, which form two sterile microchannels, separated by a thin membrane (10 μm), flexible and porous PDMS (HUH *et al.*, 2007). With the intention of simulating the largest number of biological properties in the system, other authors designed a multifunctional device that presents the main structural, mechanical and functional properties of the pulmonary interface. In order to reproduce physiological respiration in this system, human alveolar epithelial cells and pulmonary capillary endothelial cells were cultivated on opposite sides of the PDMS membrane, and the pulmonary physiological respiratory movement was achieved through compression and expansion of the system (HUH *et al.*, 2010). These changes in the system were created using two microchannels strategically positioned at the ends of the membrane, which act as vacuum chambers. This movement promoted, besides the capture of nanoparticles by the epithelial and endothelial cells, the transport of particles to the underlying vascular channel, stimulated by the mechanical restrictions of the air imposed on the system, demonstrating the impact of mechanical tension on the permeability of the pulmonary epithelial barrier of these devices. In order to simulate the innate cellular response to bacterial pulmonary infection, the authors introduced inflammatory cytokines and bacteria into the air of the system and human immune cells into the fluid of the vascular canal. Such approach enabled to measure the passage of immune cells from the endothelial canal to the alveolar canal, at the same time as the liquid overflow in the same direction. This model demonstrated the worsening of pulmonary dysfunction experienced in lung diseases.

Using the same lung microsystem on a chip, another model of lung disease was elaborated, in order to simulate the development and progression of pulmonary edema induced by interleukin-2 (IL-2) administration (HUH *et al.*, 2012). As in the previous device, air was introduced into the upper alveolar canal and fluid was inserted into the lower vascular canal. This dynamic model demonstrated the ability of IL-2 to diffuse from the vascular canal to the alveolar canal.

With the continuous evolution of these systems, a lung chip capable of reproducing the lung parenchyma was designed (STUCKI *et al.*, 2015). For the development of this system, a human bronchial epithelial cell line of a patient was used, which allowed to represent the alveolar barrier and include a cyclic tension that simulates the movement of the diaphragm in

the breathing. This device reinforced the impact of mechanical stress on alveolar barrier permeability and led to an improvement in dynamic cell culture compared to the static culture model.

The group of HUMAYUN *et al.* (2018) developed a microsystem of lung where a micro layer of hydrogel was included, produced through the combination of collagen type I and matrigel, which allowed the growth of smooth muscle cells and their interaction with bronchial epithelial cells. This device made possible the study of chronic lung diseases (HUMAYUN *et al.*, 2018).

As previously reported examples, new human disease models on a chip can leverage research and development with greater accuracy and effectiveness of new therapeutic targets and new drugs. These relatively simple systems can be sufficient for screening the toxicity of drugs administered by air, which demonstrates the advantage of applying OOAC models in current trials.

5.3 Heart-on-a-chip

The heart is one of the fundamental organs in the human body, which performs the function of dynamically pumping blood to the entire body. It is responsible for transporting oxygen and nutrients to all other organs and collecting their cellular waste through the systemic vascular system, leading to the body's homeostasis. Despite its impact on the whole organism, the heart does not have the capacity to regenerate damaged heart tissues like other organs, which prevents the spontaneous recovery of the myocardium in case of hypoxia (KANKALA *et al.*, 2017; ZHANG *et al.*, 2016). In order to overcome this challenge, the search for biomimetic cardiac substitutes became the focus of several researches. There has been a noteworthy progress in the development of new functional cardiac tissues, which has been explicitly dedicated to significant recapitulation of the anatomical and physiological attributes of the heart.

There are currently several heart-on-a-chip systems that have been created to mimic the physical, mechanical and biological functions of the living heart, which are not achieved with the use of conventional 2D culture systems. A microfluidic device has been developed to cultivate mouse cardiomyocytes in a flexible structure coated with fibronectin, with the objective of forming anisotropic muscle tissue. The thin muscle layer demonstrated a chronotropic effect in response to increasing concentrations of epinephrine, which is in line with the expected dose-response curve. This platform was improved by adapting a fluid flow control system and introducing platinum electrodes. These were used to collect accurate

information related to the contractile response of the tissue to a drug with agonistic action on the adrenergic receptors β (isoproterenol) (AGARWALA *et al.*, 2013; AUNG *et al.*, 2016). This high-performance heart-on-a-chip device has proven to be extremely useful for evaluating cardiac drugs, as it allows the collection of a quantity of relevant data for the continued development of the drug and opens the possibility of integration in a multi-organs platform.

Microfluidic technology can be combined with stem cell engineering and thus result in the production of platforms with patient derived cardiac tissue. In this line of thought, another microfluidic system with a perfunded microchannel emerged, from which it was possible to collect iPSC-derived cardiac tissue. The cardiomyocytes clusters exhibited sarcomeres and cardiac protein markers, besides the similarity with *in vivo* cardiac function (KENSAH *et al.*, 2013). A non-invasive recording method was incorporated in this device, using a common microscope. Finally, the 3D cardiac tissue produced was incubated with several drugs separately and its response was evaluated. It was concluded that the response observed was similar to that achieved in living human heart tissue.

AUNG and collaborators developed a different approach using microfluidics for the production of hydrogels containing cardiomyocytes in order to evaluate the functionality of the developed cardiac tissue. The heart cells were cultivated in a perfused hydrogel layer and the contractile stress of the heart tissue was monitored in situ in real time (AUNG *et al.*, 2016). This platform has successfully characterized the contractile stresses generated by encapsulated cardiac cells in real time. It was possible to demonstrate its potential to respond to exogenous molecules (epinephrine) and the use of human - iPSCs leverages the potential of this system to improve existing cardiac disease systems.

In another study, a microphysiological cardiac system was elaborated with the purpose of recapitulating a minimalist human cardiac micro tissue in a central microchannel of the microfluidic device. This biomimetic platform allows the self-organization of human iPSC-derived cardiomyocytes in 3D structures similar to cardiac organoids, which have demonstrated the ability to develop spontaneously beats. To these microtissues were administered multiple drugs in various concentrations, and more consistent results were obtained with the reference data of the tissue scale compared to the values at cellular level (MATHUR *et al.*, 2015).

5.4 Multi-Organs-on-a-Chip

The remarkable advances in single organ-on-chip technology demonstrate the importance of integration and consequent interaction between different types of cells inserted in the same functional unit when simulating a tissue or study organ. The more complex microphysiological systems resembling an organ correspond to the representation of tissue-tissue interface and microchambers connected to microchannels, which prompt to the development of multiple organs in a single platform.

The organs and tissues of the human body are integrated in a highly dynamic microenvironment interconnected with continuous exchange of mechanical and biochemical signals. Note that an isolated organ does not have the ability to demonstrate all of its characteristics when isolated, due to lack of communication with the other constituents of the human system. An action directed at a specific tissue or organ, such as the administration of a drug, may have implications for other organs. Thus, standard OOAC models, which simulate functional units of individual organs, are unable to predict side effects outside the target-organ. In an attempt to overcome this limitation, integrated OOAC have been developed, which combine different tissues representative of organs on a single platform. These systems are identified as multi-organs-on-a-chip (multi-OOAC) and provide a physiologically similar dynamic microenvironment where it is possible to recapitulate human organ-organ interactions. Different compartments are used where several cell types are kept in a long-term culture system, integrated in a unique platform.

During the last decade, several multi-organs-on-a-chip platforms have been developed with enormous potential for application in disease modeling, as well as in the discovery of new drugs and drug toxicity screening. These systems allow improving knowledge in the process of absorption, distribution, metabolism and excretion (ADME) of a drug, offering a means of identifying unexpected effects on secondary organs, as well as a tool to evaluate the transport of a drug throughout the human body.

To design a multi-OOAC platform, it is necessary to take into consideration some critical aspects in order to simulate the physiologically relevant organ as faithfully as possible. These includes the size of the organs and their proportionality with the organ *in vivo*, the average volume ratio (ratio between the organ size scale and the vascular flow *in vivo*) and the interactions between different organs. WIKSWO and co-workers addressed relevant aspects to establish the scale in a multi-organs system, which served as design guidance in the development of a universal culture medium (WIKSWO *et al.*, 2013).

The multi-OOAC functional systems are explored and developed for multiple purposes. In order to study the cross effects of tissues, they were cultivated in microdevice slices of intestine and liver isolated from the same rat (ABACI and SHULER, 2015). These slices remained functional under specific flow conditions, demonstrating the potential of this platform for the evaluation of interactions between organs. WAGNER and collaborators cultivated human primary hepatocytes in a microfluidic device simultaneously with skin biopsy for 28 days (WAGNER *et al.*, 2013). This work demonstrated that human primary tissues could maintain their functions *in vitro* under dynamic culture conditions. Other work suggests that the combined tissues become more sensitive and responsive to a given molecule when compared to their single tissue culture, attributing this change to the tissue-tissue interactions experienced in multi-organs platforms. It is well established that most orally administered drugs are absorbed and metabolized in the liver or small intestine and excreted by the kidneys. However, it is impossible to simulate this *in vitro* process using conventional cell culture models.

As example of a device containing three organs, there is a chip that was developed based on a 3D hydrogel culture, where three cell lines were included in order to evaluate the cytotoxicity of anti-cancer drugs influenced by their metabolism (SUNG and SHULER, 2009). In this microsystem, liver cells, myeloblasts and cancer cells, representing the liver, bone marrow and cancerous tissue, were cultivated in separate microchambers. Tegafur, an oral pro-drug that after its metabolization originates the 5-fluoruracil, mostly used in breast cancer, was introduced in the system to evaluate its cytotoxicity in this three cell lines. This platform allowed the reproduction of Tegafur metabolism in 5-fluoruracil at the hepatic level and consequent cellular death as a result of the action of the toxic metabolite, contrary to what was observed in conventional multi-well plates that did not show the same results. The working group of ESCH *et al.*, (2014) designed a micro-scale device including liver, grastrointestinal tract and other tissues in order to simulate the oral absorption of nanoparticles. In this system, it became evident the capacity of the nanoparticles to cross the gastrointestinal compartment and subsequent interaction with the liver cells, eventually causing damage in the latter (ESCH *et al.*, 2014).

The potential of multi-OOAC was increased with the design of a microphysiological system of four organs, containing the liver, intestine, kidney and skin, which maintained their functions for 28 days (MASCHMEYER *et al.*, 2015). Considering the proportionality of the size of the tissues in relation to the living organs, the representative tissues of the skin and intestine present a size 100,000 times smaller than the size of their equivalent organs *in vivo*.

To simulate liver function in the system, researchers used the equivalent of ten hepatic lobes to form the 3D liver microtissue. Kidney function was achieved by building the proximal tubular barrier formed from the human proximal tubular cell line RPTEC/TERT1. In this system, a peristaltic micropump was integrated, which allowed the pulsed medium flow and the connection of the four organs through microchannels. A second circuit ensured the drainage of the fluid excreted by the renal barrier. In the conception of this platform, the physiological fluid-tissue relationship was considered, as it proved to be an important factor for the accurate simulation of drug metabolism in these systems. Additionally, this microsystem exhibited a robust homeostasis capacity and remarkable functionality of the four tissues, presenting itself as a promising platform for the study of ADME *in vitro*. Another type of system including four organs was developed to represent the liver, kidney, lung and fat tissue (ZHANG *et al.*, 2009). In this platform, the effect of transforming growth factor beta 1 (TGF-β1) was evaluated. In this way, specifically controlling the TGF-β1 may have different actions on different types of cell populations, which demonstrates the compartmental isolation between different types of cells, similar to what is verified *in vivo*. This method can be used to simultaneously cultivate several cell types and control their cell functions individually.

Finally, a common culture medium that is capable of overcome the needs of all cell types present in the system still remains a critical factor in the development of multi-organ-on-a-chip.

6. Organ-on-a-chip pharmaceutical applications

The Food and Drug Administration (FDA), the regulatory authority responsible for marketing approval of new drugs in the USA, has shown a declared interest in the development and regulatory approval of new platforms with pharmaceutical application, which demonstrate a beneficial impact on the various stages of drug development, including screening of drugs, evaluation of efficacy and toxicity and analysis of pharmacokinetic (PK) and pharmacodynamic (PD) parameters, thus allowing responses with greater accuracy and speed (SKARDAL *et al.*, 2016). The most promising models that have attracted investors' attention are the organs-on-a-chip and a more complete version of the multi-organs-on-a-chip, also including the development of models of barrier systems, such as the blood-brain barrier (study of the ability of the drug to reach the brain tissue through the bloodstream) and the placenta (study of teratogenic effects on the fetus). In addition, these systems can provide relevant information to allow a standardized comparison of accurate biological

response, facilitating the development of disease models in the emerging area of personalized medicine in order to decrease inconsistency of therapeutic response from patient to patient (TEJAVIBULYA and SIA, 2016).

6.1 Disease Modeling

The simulation of diseases on a chip is one of the main applications of the organ-on-a-chip technology, having the ability to represent various pathological situations, ranging from genetic and infectious diseases to cancers and degenerative diseases. They are useful to understand the etiology of diseases, develop diagnostic strategies and effective therapies (SKARDAL *et al.*, 2016).

6.1.1 Inflammatory Diseases

Several diseases are closely associated with inflammatory processes that can trigger a set of severe symptoms and/or worsen the condition of the disease. Pneumonia is an infection in the alveolar sacs of the lung and adjacent tissues, and is considered one of the most common lung diseases in the world, affecting mostly young children and the elders. It is a complex pathology characterized by an acute onset and difficult to control. For the complete understanding of the mechanism of this challenging disease is crucial to establish *in vitro* models that are able to reproduce the existing pathophysiology *in vivo* and enable the screening of efficient drugs.

The lung platform that mimicked lung respiration, previously mentioned in this work, demonstrated the ability to simulate the endothelium-epithelium lung interface under fluid flow and mechanical stress conditions, resembling the lung functional unit. Inflammatory models based on this system were induced through the administration of the tumor necrosis factor (TNF-α), cytokine with pro-inflammatory action, and bacteria that promote the increase of surface protein expression, Intercellular Adhesion Molecule 1 (ICAM-1), in endothelial cells, activating the recruitment of human neutrophils (TAVANA *et al.*, 2011). The same working group also succeeded in developing a model of IL-2 induced pulmonary edema (HUH *et al.*, 2010).

A robust *in vitro* model of lung airways on chip was developed to study chronic obstructive pulmonary disease - COPD and evaluate its response to various pharmacological anti-inflammatory therapies. Healthy microvascularized lung endothelial cells and epithelial lung cells from patients were used. This system recreated many properties of the structures and functions of human lung bronchioles and maintained them for several weeks *in vitro*, which is

extremely advantageous in the study of chronic diseases. Using this model, it was possible to discover new biomarkers of the exacerbation of this disease (BENAM *et al.*, 2016).

6.1.2 Brain diseases

Neurodegenerative diseases are characterized by a progressive degenerative process culminating in very debilitating conditions. They can affect people of all ages, worsening over time, and come from progressive degradation and/or death of neurons. This decrease in active cells of the nervous system can compromise some body movements (ataxia) and the functioning of the brain, giving rise to dementia. Its cause still remains unknown.

It is estimated that 153000 Portuguese people, 9.9 million European people and 35.6 million people around the world will suffer from some form of dementia. These diseases have a great impact on the patient's family, social and professional life, and can cause a total incapacity to exercise any type of daily activity. However, one of their main problems is their late detection, which reduces treatment options and their effectiveness. The development of *in vitro* models that characterize these pathologies, such as Alzheimer's Disease (AD), will be a useful resource to better understand their etiology, investigate the mechanisms of brain diseases, elaborate efficient treatment strategies and better control the symptoms by minimizing side effects.

A group of researchers presented a microfluidic device capable of creating 3D neurospheroids that best simulate the brain microenvironment *in vivo*. Its development in microwell matrices with interstitial flow demonstrated a better formation of neuronal networks when compared to static culture models, allowing a longer viable culture time. To this system, the peptide β-amyloid (main actor in the AD) was added to evaluate its potential as a model of brain disease *in vitro*, under conditions of interstitial flow. This study evidenced the neurotoxic effects of peptide β-amyloid that caused a decline in the viability of neuronal cells and the destruction of neuronal networks by inducing synaptic dysfunction. Thus, this platform demonstrates a great potential for the investigation of strategies to address the disease and treatment, aiming at the application of tests with drugs (PARK *et al.*, 2015).

6.1.3 Cancer

Cancer corresponds to a family of diseases and it is estimated that 90% of cases are related to the environment and unhealthy habits, with only 10 to 15% of the probability of developing cancer from genetic information coming from the parents. In other words, these pathologies are mostly an acquired or behavioural disease. The development of tumors presupposes abnormal cell division and the development of the ability to invade other

tissues. Cancer cells have the ability to spread throughout the body through the circulatory and lymphatic systems, giving rise to metastases. The development of *in vitro* models capable of studying the biological behaviour of cancer cells, their ability to migrate to other organs and evaluate the effect of anti-cancer drugs is crucial for the advancement of medicine in this area.

Several working groups have developed chip cancer models capable of simulating tumor microenvironments, which exhibit complex cell-cell and matrix-cell interactions, cytokine gradients and biophysical signs (CHOI *et al.*, 2015; KUO *et al.*, 2014). As an example, Xu and co-workers developed a microfluidic device with the purpose of mimicking the invasion of the glioblastoma in the ECM under different concentrations of oxygen. This research demonstrated the role of hypoxia and the epithelial-mesenchymal transition (EMT) process in glioblastoma. Hypoxia promotes the proliferation of cancer cells, while the expression of proteins associated with EMT, is involved in the cell migration stimulation (XU *et al.*, 2015). Another microdevice was developed by LI and collaborators to assess the response of lung adenocarcinoma cells to different soluble factors and to ECM proteins (LI *et al.*, 2013). Applying also the microfluidic technology, several studies were directed to the research of cancer cell extravasation and to the investigation of the molecular mechanisms of the action of immune cells in cancer cells (BUSINARO *et al.*, 2013).

6.2 Drug screening

The research and development of a new molecule or new chemical entity (NCE) requires a long and arduous time of research and demonstration of its unequivocal benefit. Despite the success in the evolution of various molecules for clinical application, it is surprising how many drugs have been removed by the regulatory authorities (e.g. FDA and EMA) after undergoing clinical trials and have been marketed for years. Most of them were antidiabetic and anti-inflammatory drugs that caused severe toxic effects on liver and heart. Thus, more and more importance is given to how the various organs of the human body will respond to the administration of a new molecule (SKARDAL *et al.*, 2016). One relevant issue relies on the inadequacy of conventional drug screening methods. OOAC may facilitate the efficient screening of new pharmaceutical compounds and help to more clearly identify an effect or cause that may trigger the cancellation of the clinical trial or the withdrawal of the drug from the market at a later date. In this way, when designing an OOAC platform for drug screening it is imperative to include a tissue representative of the liver, as it is the organ most exposed to xenobiotics and plays a vital role in the biotransformation of molecules (KNOWLTON and TASOGLU, 2016; PROT *et al.*, 2011). The metabolic activation is a critical step in the

system of screening of new molecules, because it can originate active metabolites that will have great impact on the effective analysis of efficacy and other pharmacological parameters. On the other hand, it also allows estimating the effective concentration that exerts the intended therapeutic effect with minimal or non-existent side effects. After the selection of the molecule, these systems can be applied in the evaluation of the release profile of several formulations, and contribute to the choice of the formulation with the best therapeutic profile. In this context, OOAC may be used to generate accurate results for the continuous development of drugs, sparing time and resources (ZHANG *et al.*, 2017).

6.3 Drug testing - efficacy and toxicity evaluation

A major challenge in the development of new drugs is their low efficacy and unexpected toxicity in clinical trials, resulting in the absence of the intended therapeutic effect. The approaches employed in the pre-clinical stages of the study do not have the ability to accurately predict the efficacy and possible toxicity of the drug *in vivo* before it reaches the clinical phase, being considered one of the reasons for the unexpected results. In order to overcome the limitations of current methods, organ-on-a-chip emerge as a promising tool capable of modelling human physiological and pathological functional units of living organs. These allow reconstructing and modulating the main etiologies, mechanisms and relevant clinical responses with various levels of biological complexity, managing to predict unexpected toxicity outside the target. The latter becomes more evident in the analysis of multi-organs systems (SEIDI *et al.*, 2011). A heart-on-a-chip composed of multiple thin layers of mouse cardiomyocytes, described in the previous section, was applied to study the ionotropic effect of the agonist β-adrenergic isoproterenol, enabling to obtain identical results to those previously obtained with rats (AGARWALA *et al.*, 2013). In another study, a 3D model of EMT was described with the objective of testing the efficacy of a drug (AREF *et al.*, 2013). This model, which simulated the progression of cancer, demonstrated the cancer dispersion induced by EMT. Twelve drugs were perfused through the vascular microchannels, which included approved drugs, but also molecules with potential effect at an early stage of discovery, in order to evaluate the potential of this project as a drug screening platform. The ability to inhibit EMT was analyzed through direct visualization of cancer spheroids. The results showed that the effective concentrations were higher when compared to 2D culture systems to obtain the same effect. In turn, these were closer to the range of effective drug concentrations determined in clinical trials. Other cancer models and multi-organs of cervical cancer also demonstrate the difference between the effective

concentration values recorded in organ platforms and conventional 2D culture systems (TATOSIAN and SHULER, 2009).

XU *et al.*, (2016) and his group developed a 3D model of the blood-brain barrier (BBB) that simulates the complex multicellular architecture, functions and mechanical properties of BBB *in vivo* (XU *et al.*, 2016). The system included essential components, such as primary microvascular brain endothelial cells, astrocytes and ECM that organized themselves to form a rigorous BBB structure. This model was used to understand the interaction between BBB and exogenous cancer cells during metastases in the brain. The efficient delivery of drugs to the brain is still a challenge today, ascribed to the selective permeability of BBB to different external molecules. The BBB model described in this work demonstrated robust and realistic experimental results regarding the response to various drugs directed to brain tumors, becoming a useful tool for simultaneous screening of several drugs, which is not possible in other existing BBB models, nor in animal studies.

Unexpected adverse effects to drugs are another common cause that results in costly withdrawal of drugs from the market or failure of clinical trials. Animal trials are sometimes unable to reveal important toxic effects in humans, which can trigger late rejection of drug candidates due to biological discrepancy and specific toxicity pathways in animals, or genetic history. Several OOAC have been used to evaluate liver toxicity and analyze the mechanism. In a microfluidic device, human hepatocytes were cultivated with the aim of monitoring the metabolic response to flutamide (anti-cancer drug) and the hepatotoxic action of flutamide and its active metabolite, hydroxiflutamide. This study proved the toxic response of this drug and its metabolite and described which metabolic pathways were activated. Furthermore, this liver model can be used as a platform for the identification of potential hepatotoxicity biomarkers. The drug metabolites generated from the biotransformation process in the liver can trigger toxic side effects in other organs, as exemplified in the liver and kidney platform on a chip. This study mimicked the systemic interaction between the two organs and recapitulated the nephrotoxic response of the ifosphamide metabolite produced by hepatocytes. These platforms significantly increase the detection of toxicological effects and drug efficacy, with a reduction in cost, time, protocol requirements (sample size), and ethical and legal issues associated with animal and human trials (SNOUBER *et al.*, 2013).

6.4 Personalized medicine

It is widely recognized that the segment of personalized medicine is growing and it has an impact on clinical practice due to the unique variations of the human genome. This type of

practice aims to respond to the therapeutic needs of the individual patient or a specific group of patients based on their genetic and phenotypic characteristics, exhibiting similar clinical presentations, with the objective of significantly improving clinical treatment and minimizing adverse effects. In the last decade, there has been an evident progress in precision medicine, much due to advances in the areas of genetics, proteomics, informatics, epigenetics, DNA sequencing, metabolomics, among others (JAMESON and LONGO, 2015). Pharmacogenomics has brought the opportunity to reformulate the medical prescription based on the patient's genome, which will have a great impact on the choice of the drug with maximum effectiveness and in appropriate doses, preventing unexpected adverse effects.

Most of the treatments followed are chosen based on the overall success rate of a given drug, for a disease, in the general population, rather than on the response expected from the particular patient. Thus, an unmet clinical need arises in which, to make the decision of which medicine is more adequate, to achieve the greatest therapeutic effect on the patient and the least adverse effect, becomes a process of trial and error. In this context, the concept of personalized medicine has been evolving, using the genetic profile of the tooth to identify possible treatment targets (TRAN *et al.*, 2015). With the significant advances and advantageous properties of OOAC, the creation of customized models would allow predicting the effectiveness of a treatment using the patient's own cells. Thus, these systems could be applied in the more challenging areas of medicine, such as oncology, in predicting the progression of a cancer and the response to a therapeutic protocol (GERAILI *et al.*, 2018). BENAM and collaborators demonstrated how it would be possible to turn organs-on-a-chip into efficient models with patient specific cells and their adequate analysis (BENAM *et al.*, 2016).

MUSAH1 and colleagues have developed a model of human renal glomeruli *in vitro* from podocytes derived from iPSCs. This system was used to demonstrate the mechanism of renal disease and evaluate new drugs. It should be noted that human iPSCs showed a great capacity for self-renewal and differentiation into other cell types (MUSAH1 *et al.*, 2017). In another work, it was produced a cardiac organoid through the cultivation of iPSC-derived cardiomyocytes in a standardized gelatin layer. This model was applied in drug screening by monitoring physical and chemical parameters in real time (ZHANG *et al.*, 2017).

By combining OOAC production with cells collected from the patient and the evaluation of his or her genetic profile, it is possible to predict the progression of the disease, an efficient screening of drugs and their response to therapy. The development of multi-OOAC systems

with patient specific cells, appears as a relevant tool in the evaluation and screening of cancer progression and metastasis development.

Despite the advantageous attributes of this technology, the production of models on a chip with specific patient cells presents some limitations, in particular the inability of cell regeneration. This limitation can be overcome by using human iPSCs that have the ability to differentiate. However, some previous works have indicated that the development of cells with high levels of heterogeneity, present predominantly immature phenotypes, which limits their applicability, being necessary the implementation of a specific pre-defined method, which originates the desired mature cell lines. From the beginning, it is advantageous to customize drug screening, while all organs are monitored to assess possible adverse effects. This technology will efficiently transform patient care with an improved therapeutic result (TEJAVIBULYA and SIA, 2016).

7. Current status of use and approval of organ-on-a-chip systems

7.1 Standardization and validation

The examples of systems presented throughout this monograph generally differ in one or more parameters of the model. For example: origin and type of cells, mechanical properties of the material, biochemical stimuli, chip design. Although it is beneficial for the evolution of this technology, the increasing diversity of ways in which it is developed causes a delay in the adoption of a standardized industrial model. The new functionalities that arise in each platform require standardized procedures of Good Manufacturing Practices - GMP and Good Laboratory Practices - GLP enabling the comparison of data between the various studies and entities, in order to obtain approval for the commercialization of these systems and enhancing the adherence of regulatory entities that will have to analyze data from these platforms. The validation of the devices and functionalities of the tissues produced *in vitro* must be robust, reliable and reproducible. However, the process of standardization and validation of the device is complex, because there is no standard validation method or test that has the ability to adapt to all tissues that are produced *in vitro* through this technology.

In the inspection of a project that intends to model and quantify the transport through barrier tissues, it is important to evaluate the integrity of the junctions that compose that barrier. Thus, a possible strategy for the evaluation of the viability of the junctions could be the quantification of the protein of expression of that same junction (SHAH *et al.*, 2016). However, this measurement would not be very useful, neither adequate in the study of a

non-barrier tissue model. This example demonstrates the need to validate specificities and functionalities of the created tissue applied to the experimental model designed for a particular use.

Organ-on-a-chip, which can be integrated in a body-on-a-chip, entails as main objective to reduce or completely replace the use of animal models through the qualification achieved by the FDA - Drug Development Tools (DDTs) validation. The validation process of relevant physiological systems *in vitro* must follow the criteria presented by the DDT guideline (REBELO *et al.* 2016). The current DDT program is voluntary and qualifies the platform as "fit-for-purpose". Compared to the validation of an animal model, when it is approved as qualified in a specific context of use as DDT, it can be used by the pharmaceutical industry during the time of product development and its results are considered reliable by the regulatory authorities, without further data being required to prove the quality of the study. As such, OOAC that meet the requirements for DDT should provide a reliable and quality approach that encourages their use in the discovery of new molecules and further studies.

7.2 Evolution of financing and investment in organs-on-chip systems

With the development of the first organ-on-a-chip prototypes and the prohibition of the use of experimental animal models in cosmetic development studies in Europe, according to the guidelines of the Organisation for Economic Co-operation and Development (OECD) (3 R's principle: replacement, reduction and refinement), there was a stimulus in the European Union for the development of several projects to this end. In particular, a project lasting 36 months was funded with 1.4 million euros to support the development and innovation of alternative devices for drug testing (KANAMORI *et al.*, 2017). In the USA, the evolution of microphysiological systems technology has been boosted by investments exceeding $140 million from the National Institutes of Health (NIH), the Food and Drug Administration, and the Defense Advanced Research Projects Agency (DARPA) (SUTHERLAND *et al.*, 2013). The Japan Agency for Medical Research and Development (AMED) has initiated a five-year program with a budget of ¥500 million (~$4.5 M) per year, which aims to develop microphysiological systems based on a chip that will be applied in the drug discovery process, recognizing the potential of this technology (KANAMORI *et al.*, 2017).

It is necessary to focus on the development for the optimization of similarity, productivity, reproducibility, robustness and validation of OOAC in order to promote the access and use of this technology by academic researchers and the pharmaceutical sector. Great efforts are being made to transform OOAC into industry relevant models, but the full transition of

these devices requires the demonstration of utility and cost per chip (ZHANG and RADISIC, 2017).

7.3 Marketing and regulation

In order to standardize and promote the use of this technology, the National Center for Advancing Translational Sciences (NCATS) was created in 2017. It financed the development of the Tissue Chip Testing Centers, whose function is to independently evaluate the robustness and reliability of various microphysiological systems. The pharmaceutical industry in collaboration with the FDA promoted the validation of several results obtained with organs-on-a-chip according to the evolution of published projects. These records were compiled in a centralized and standardized database of microphysiological systems (MPS-Db) that allows researchers to access public data on this technology (LOW and TAGLE, 2017; SAKOLISH *et al.*, 2018).

7.4 Industrial transition and clinical applications

Several device options for biomedical applications such as pharmacological and toxicological studies, drug discovery and development are beginning to emerge. Simultaneously with this advance, other clinical and industrial applications occur, such as patient-on-a-chip (POAC), which includes the screening of drugs and the evaluation of their potential effect on the patient, with the objective of selecting a targeted therapy that involves less risk for the patient. Finally, collaborations have been initiated between hospitals and laboratories to develop clinical programs for cancer-on-a-chip patients (CPOAC) with the objective of developing personalized models of patient-specific diseases, using iPSCs obtained from them. Accordingly, specific cancer biomarkers will be screened for evaluation of the metastatic properties of the tumor (FERREIRA *et al.*, 2016; WARKIANI *et al.*, 2016). Unfortunately, their clinical application is still very limited, despite recent progress in the development of these platforms. It is necessary to include in these systems advanced properties compatible with the intended analyses in this type of pathologies, such as the collection of high-definition target images, biosensors with sensitive and specific detection levels. CPOAC-based devices present numerous economic barriers that limit their use and incorporation in the pharmaceutical, biotechnological and clinical markets, in addition to the technical and biological challenges related to the interconnection of different tissues. Some articles have compiled the challenges and requirements for the application of these systems in drug development and mechanism evaluation studies, which include important approaches by researchers, clinicians, industry and regulators (WATSON *et al.*, 2017).

Summing-up, the vast scientific, clinical and regulatory investment and development that has been observed in the field of organ-on-a-chip technology demonstrates the interest and high expectations of the scientific community for its application in research.

8. Conclusions and outlook

The organ-on-a-chip systems are able to establish a strict control of the architecture and space of tissues and organs, along with the transport of physiological substances and the biochemical and biophysical signalling present in the human body. This approach offers unique advantages in the study of intercellular communications, tissue microenvironment, disease modelling and pharmacological testing, in addition to existing methods. Thus, OOAC arises as a new strategy that will complement traditional cell culture and animal models by providing a biomimetic tissue niche. Also, the design of the organ-on-a-chip is compatible with the integration of various methods of analysis and biosensors, which enables the real-time recording of physiological and pathological information, and extends its usefulness for biomedical applications.

Although the emerging technology of OOAC has revealed important information about the cellular behaviour and relevant functions of organs, much research still has to be done to complete the simulation of the functions of the organs and thus achieve the body-on-a-chip model with extended application for disease studies and drug discovery. These challenges include the correct choice of material, which influences the mode of production and the design of the platform.

PDMS has been the most used material in these constructions due to its flexibility, transparency, air permeability and biocompatibility. However, this material can absorb small hydrophobic molecules, which can lead to an underestimation of the toxicity and efficacy of the drug, limiting its practical utility in the discovery of drugs and evaluation of pharmacological parameters. To improve the ability of OOAC in order to provide more comprehensive information on cellular behaviour, metabolism and drug response, it is necessary to adapt or refine the monitoring systems. Analysis systems already associated with OOAC technology, such as polymerase chain reaction (PCR), confocal microscopy and mass spectrometry, require better integration in the cellular device for complete real-time analysis, so as to improve these platforms and making them more inclusive for obtaining biological information.

The main limitation for the construction of multi-organs-on-a-chip analogues of the human body is the source of cells. Human primary cells are ideal resources for modelling human tissues. To this end, iPSCs have been investigated in order to enhance the mimicking of organs on chip. Patient-specific iPSCs provide a promising cellular source to recapitulate the mechanism of disease on a chip, enabling its use for the study of customized drugs. To evaluate organ-organ interactions on multi-OOAC, it is necessary to improve the common culture medium, as different cell types require different nutritional components, although they are cultivated on the same platform. To overcome this challenge, human plasma or serum may be a desirable substitute as a supplement to the culture medium for the various organ-specific cell lines. Thus, a new biomimetic microsystem with improved materials and a cell source adapted for the construction of organs-on-a-chip will be achieved.

Although the development of organs-on-a-chip is still at an early stage and many parameters need to be adjusted yet, its potential to recapture diseases and predict the human response under physiological and pathological conditions is enormous. This technology is expected to evolve towards more realistic, accessible, practical and robust human models for end use, by combining stem cell biology and engineering in biological trials. Finally, the success of this OOAC will depend on the collaboration of academic researchers, industry and regulators to achieve the commercialization of a standardized and recognized platform.

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10. Appendix

Table 1. Comparison between 2D and 3D tissue platforms in terms of biological complexity, manufacturing and outputs (adapted from LIU *et al.*, 2018)

	Conventional 2D systems	**3D systems**		
		Engineered tissues (cells + scaffolds)	**Organoid**	**Organ-on-a-chip**
Production method and timing	Differentiated, grown on rigid flat surfaces as monolayer; fast	Fabricated with scaffold and casting mold; slow	Embedded in matrigel to self-organize; slow	Seeded in engineered chambers with perfusion; fast
Maturation	Immature	Improved but still lacking	Improved but still lacking	Improved but still lacking
Cell morphology and type	Usually monotype, not resembling physiological conditions	Size and shape similar to *in vivo*	Size and shape similar to *in vivo*	Depends on platform design
ECM	Limited composition	Similar to *in vivo*	Similar to *in vivo*	Depends on platform design
Tissue architecture	Absent	Simple	Complex, similar to organ developmental stages	Complexity depends on platform design
Diffusion of signal factors and nutrients	Short distances (through cell membranes)	Concentration gradients may exist (may be affected by ECM properties)	Inner cells may die or lack maturity due to ineffective transport to interior	Precisely controlled temporal and spatial gradients

Perfusion	No	No	No	Yes
Variability; reproducibility; controllability; difficulty of use	Low; high; high; easy	High; low; low; difficult	High; low; very low; easy	High; sometimes low; very high; difficult
Characterization and analyses	Limited, but easy cell retrieval	Tissue function analyses possible, but cell retrieval and phenotypic analyses can be hard.	Tissue function analyses possible, but cell retrieval and phenotypic analyses can be hard.	Real-time tissue/organ function analyses possible, with easy cell retrieval