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Breaking through the barrier: Modelling and exploiting the physical microenvironment to enhance drug transport and efficacy

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ABSTRACT

Pharmaceutical compounds are the main pillar in the treatment of various illnesses. To administer these drugs in the therapeutic setting, multiple routes of administration have been defined, including ingestion, inhalation, and injection. After administration, drugs need to find their way to the intended target for high effectiveness, and this penetration is greatly dependent on obstacles the drugs encounter along their path. Key hurdles include the physical barriers that are present within the body and knowledge of those is indispensable for progress in the development of drugs with increased therapeutic efficacy. In this review, we examine several important physical barriers, such as the blood-brain barrier, the gut-mucosal barrier, and the extracellular matrix barrier, and evaluate their influence on drug transport and efficacy. We explore various *in vitro* model systems that aid in understanding how parameters within the barrier model affect drug transfer and therapeutic effect. We conclude that physical barriers in the body restrict the quantity of drugs that can pass through, mainly as a consequence of the barrier architecture. In addition, the specific physical properties of the tissue can trigger intracellular changes, altering cell behavior in response to drugs. Though the barriers negatively influence drug distribution, physical stimulation of the surrounding environment may also be exploited as a mechanism to control drug release. This drug delivery approach is explored in this review as a potential alternative to the conventional ways of delivering therapeutics.

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Contents

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1. Introduction

Drugs can be introduced into living organisms in several ways, including intravenous administration (where the drug is directly injected into the bloodstream), oral, topical, sublingual, rectal, intramuscular, or subcutaneous routes. Each of these routes can be beneficial for a specific application, and the administration route is chosen based on the targeted tissue, necessary treatment, and patient-specific factors. The administered drugs need to overcome the body's various external and internal biological barriers, such as the skin or blood-brain barrier (BBB). Crossing some of the barriers can be directly circumvented upon injection, where the drug immediately enters the cardiovascular system, which runs through the entire organism, connecting its most vital organs [1]. Therefore, intravenous injection is an especially efficient drug delivery method, as the drug can be transported along the vessels with blood flow. However, the injected drugs get diluted by the blood flow, and, as a result, the amount of drug uptake in the targeted organ may be relatively low. Although the uptake through the gastrointestinal tract (GI) or skin is circumvented with this approach, the presence of trained healthcare professionals is often necessary, and, in some patients, resistance towards injections is developed. Additionally, circumventing the biological barriers is not always possible. For instance, the BBB is characterized by limited transport between the lumen of the vessels and interstitial space due to the formation of tight junctions in between endothelial cells hampering the permeability of therapeutics. The permeability is also severely limited because of efflux transporters found on the luminal side of endothelial cells, such as P-glycoprotein, multidrug resistance proteins, and breast cancer resistance protein [2]. Another characteristic of the BBB is its exposure to mechanical forces such as shear stresses and stretches (wall strains) in the capillaries caused by the blood flow. Such forces can have an influence on drug administration and should be taken into account while considering drug delivery approaches aimed at the BBB [3]. Orally administered drugs face the additional challenge of passing the GI. The GI has a function of a barrier, with physiologically relevant characteristics including peristaltic motion, further affecting the drug transport. Besides the peristaltic motion, the mucus layer also affects oral drug absorption and it can be considered as one of the major barriers in the intestine. The mucus properties vary across the different parts of the GI and therefore, it has diverse effects on drug transport.

The fraction of the drug that will pass through the endothelial or epithelial barrier will be transported throughout the body, where it may face additional physical restraints. The extracellular matrix (ECM), secreted by cells, serves as structural support for the cells and regulates their various functions. The ECM is present in all tissues and can significantly vary in composition and properties, depending on the tissue type and its state, e.g. diseases such as cancer can substantially change the mechanical properties of ECM, influencing drug penetration. Finally, once the drug crosses the barrier to the diseased zone, it can enter cells in several ways, depending not only on the cell type but also on the drug formulation. The drug can permeate the cell membrane by passive diffusion or by binding itself to the receptors present on the membrane. It can also be incorporated via endocytosis, a process in which the cell itself engulfs external particles. This mechanism is most common among drugs with nanometric sizes [4] and has been extensively reviewed elsewhere [5].

The different ways of drug delivery can be tested in *in vivo* models. However, these offer limited flexibility to study individual parameters. The possibility to independently tune and alternate various parameters and elements of the biomimetic physical tissue microenvironment acknowledges the vast potential of *in vitro* approaches to study and predict drug efficacy and transport through barriers. Additionally, the interest in *in vitro* models as alternative methods for testing drug adequacy is growing due to the desire to reduce animal usage. Yet, typically employed *in vitro* models have limited complexity. As such, they need to incorporate additional parameters to fully replicate the native barriers in the human body. For example, the most commonly used *in vitro* models for the GI and BBB are static Transwell models in two-dimensional (2D) culture formats. These models use inserts with porous membranes that can be mounted onto a well plate and serve as permeable support for growing cells either in mono- or co-culture. Even though multiple cell types can be combined in this system, the static models lack full physiological relevance as mechanical stimuli, including flow and peristaltic motion, are typically not included. Such mechanical stimuli are crucial for cell proliferation and differentiation, showing the need for more advanced barrier models than the traditional static ones.

Knowing different biological barriers which need to be overcome by drugs before reaching the designated tissue is necessary to design their most optimal formulation and choose the right dose required for therapeutic effects. Without this knowledge, the drug could undergo inactivation before reaching its target. It could also get activated in a tissue that is not targeted or reach the target zone in insufficient quantities for successful treatment. Therefore, in this review, we provide a broad perspective on the main barriers in the human body - that should be considered in drug development and drug delivery studies. For in-depth characteristics of drug transport through each of the discussed barriers, the reader is referred to these specific reviews [6–11]. As the drug formulation and dosage, before reaching clinical trials, could be tested and monitored in detail in *in vitro* platforms, we also present here different approaches to recapitulate physical microenvironments and their role in drug uptake. Finally, we discuss how drug efficacy can be modulated via physical stimulation for each of the main barriers discussed.

2. Tissue characteristics as natural barriers for drug transfer

Natural barriers are characterized by a dynamic and complex environment, which requires thorough understanding to successfully design appropriate drugs and models for uptake studies. In the human body there is a multitude of natural barriers, which should be considered while developing therapeutics and new drug enhancement strategies. Therefore, in the following sections, we will discuss, one by one, the endothelial barrier, epithelial barrier, and the physical barrier of bulk tissue along with the ECM.

2.1. Endothelial barrier

Intravenous administration is one of the most common drug delivery routes. Consequently, the first barrier most drugs need
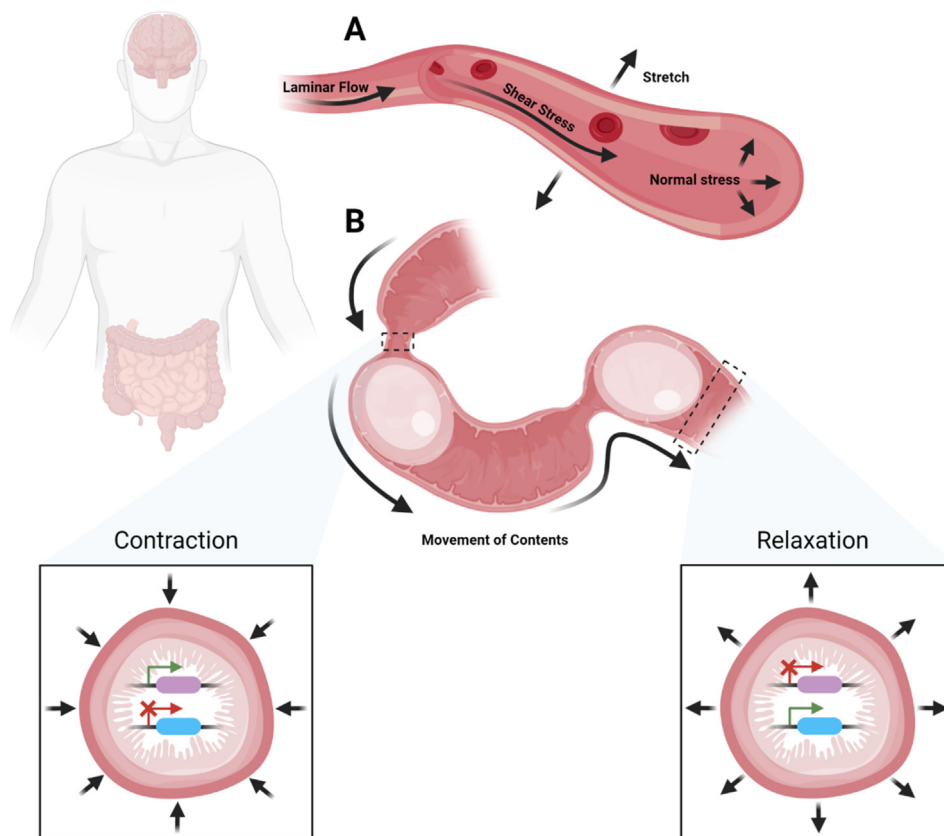
Fig. 1. Mechanical forces in tissues: A) Blood vessel; B) Intestine. The dynamic environment affects gene expression, cell differentiation, and signaling pathways, therefore it is considered crucial for establishing relevant models (set of different processes specific for contraction (purple) and relaxation (blue) shown schematically in the close-ups).

to pass is connected to the cardiovascular system, notably the vascular endothelium, which is an interface separating the blood from tissues. Solutes and cells can pass through this barrier into the extravascular space in a regulated manner, to maintain homeostasis [12]. The blood vessels are lined with endothelial cells, blocking the transport of molecules bigger than 66 kDa [13]. Typically, the transport through the endothelial layer occurs via the transcellular and paracellular routes.

2.1.1. Blood flow and vessels

Blood flow is an inseparable part of the cardiovascular system and therefore, of blood vessels. In straight parts of blood vessels, the movement of blood is laminar, which means that the motion of blood could be represented as a set of parallel flow lines, showing a gradual decrease in velocity from the center of the vessel to its wall [14]. In the direction parallel to the blood flow, shear stress is generated [15]. It can be characterized as the tangential drag force exerted by the blood moving through the luminal side of the vessel [16]. The velocity profiles of laminar blood flow through the vessels show that the layer of blood which is in the closest proximity to the vessel's inner wall is relatively stationary in comparison with the innermost layer [17,18]. The velocity of blood along with its viscosity are factors directly determining the shear stress value to which the vessel's endothelium is exposed [19]. The indirect factor playing a role in shear stress' magnitude is the vessel diameter [17]. Moreover, it has been shown by Zarins *et al.* that the arteries' diameter is regulated by the flow of blood and, more precisely, by the homeostatic adaptation to keep the shear stress at an optimal level. The lumen's diameter increases together with the increase of flow and shear stress [20,21]. There-

fore, the diameter of a blood vessel can be used as an indicator of the expected values of shear stress. Laminar flow is often utilized in *in vitro* models, even though it is a simplified way of mimicking blood movement through the vessels. Blood flowing in the vessel generates forces acting in more than one direction. Blood pressure, as force-directed perpendicularly to the inner (luminal) part of the vessel, generates normal stress and cyclic circumferential stretch [22]. In the direction perpendicular to normal stress and shear stress, the endothelial cells are subjected to stretching (See Fig. 1**A**). Additionally, under physiological conditions in the human body, the blood flow is pulsatile and not laminar. Oscillatory stress results from disturbances in the flow of blood, and it is mainly observed in large arteries' curves and bifurcations as well as in stenotic arteries [23].

Various *in vivo* experiments reflecting different organs have captured physiological values of shear stress. Lipowsky and colleagues investigated the microvasculature of cat mesentery and found the shear stress inside the blood vessels to vary from 29.0 dyn/cm^2 in venules to 47.1 dyn/cm^2 in arterioles [24]. In the studies of coronary arteries, Krams *et al.* showed that the values of shear stress in the endothelium ranged from around 20 to 80 dyn/cm^2, depending on the inflow velocities used (from 10 to 30 cm/s) [25]. Cucullo *et al.* focused on the brain vascular system *in vivo* and found the value of shear stress in capillaries to fall between 5 and 23 dyn/cm^2 and in venules to oscillate around 3 dyn/cm^2 [26]. In turn, Santa-Maria *et al.* indicated that the shear stress of 1.6 dyn/cm^2 is an accurate *in vitro* representation of the native brain postcapillary venule conditions [27]. As the numbers differ depending on the vessel's geometry and curvature, it is imperative that *in vitro* models recapitulate conditions relevant for a specific microenvironment.
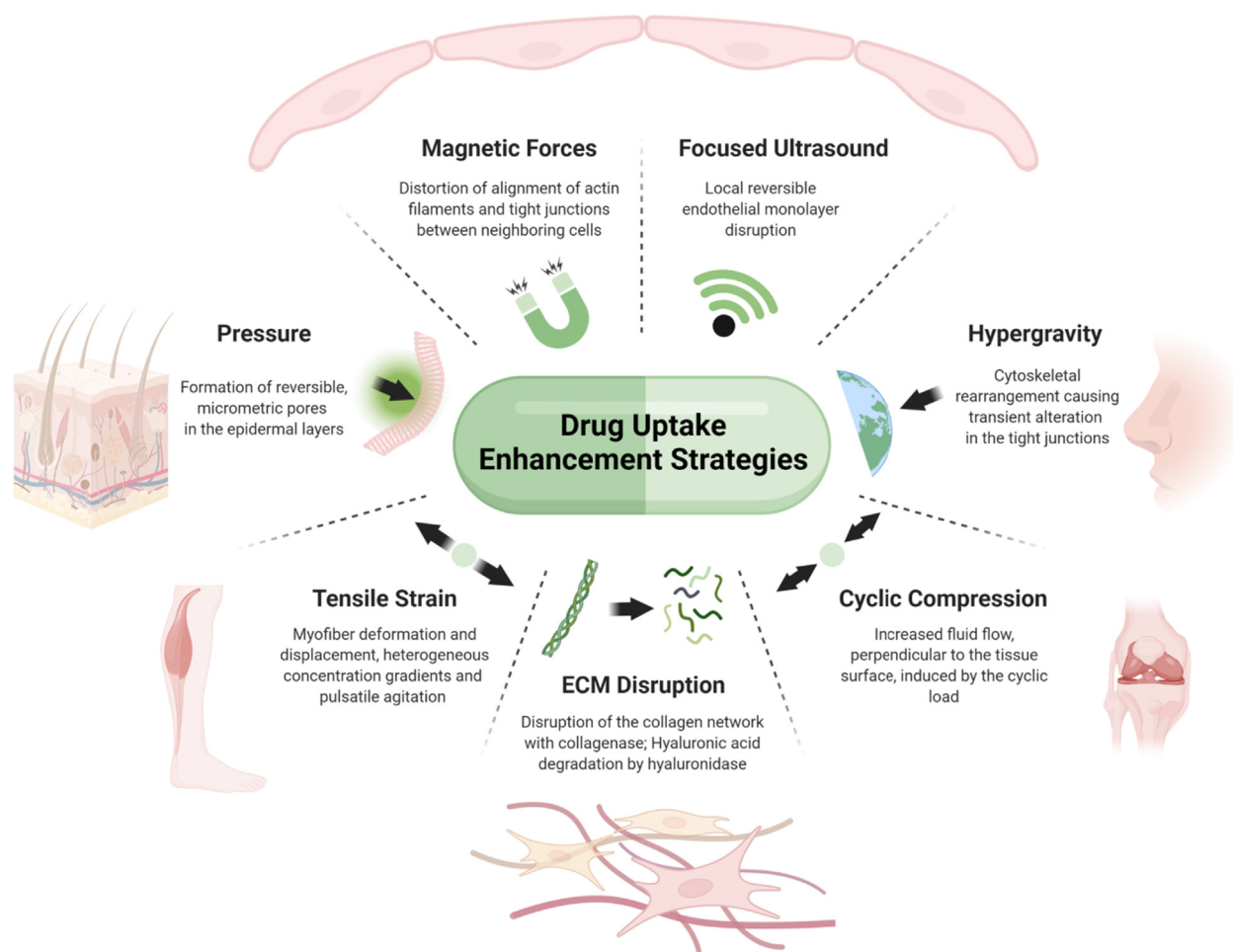
Fig. 2. Graphical representation of drug uptake enhancement strategies, based on physical stimulation and enzymatic ECM disruption, for various tissues.

2.1.1.1. Enhanced uptake strategies. Vascular endothelium poses a challenge for larger molecules and therapeutics, such as nanoparticles, which may not be able to cross it unless the barrier is disrupted. Therefore, some advances in enhanced drug delivery strategies have been made. Various strategies will be introduced and discussed throughout the manuscript (See Fig. 2).

Qiu *et al.* demonstrated that drug transport through the endothelial layer could be enhanced using magnetic forces [28]. The authors used both an *in vitro* model based on microfluidic channels and an *in vivo* mouse model. They showed that by applying a controlled magnetic field in the presence of magnetic nanoparticles, based on iron oxide-poly(ethylene glycol) (PEG), cellular uptake of nanoparticles could be observed, together with the distortion of alignment of actin filaments and tight junctions between neighboring cells. As a result, the paracellular pathway was activated, and the local delivery of drugs circulating in the neighboring bloodstream was increased. The ability to penetrate the endothelial layer by a 150 kDa anti-collagen molecule in the *in vitro* model and an indocyanine green fluorophore in the *in vivo* model was shown. The induced changes were temporal, and after removal of magnetic force, the original endothelial cell junctions and actin arrangement could be restored. Improving transport through the endothelial barrier is very promising for a broad application in drug delivery. It allows control over intravenous paths and circulatory systems in various diseases.

2.1.2. Blood-brain barrier (BBB)

One of the most prominent, yet very specific, examples of the blood-to-tissue barriers is the BBB, an interface between the bloodstream and the brain tissue. The BBB is the main obstacle that drugs intended to treat diseased brain tissue need to overcome. Its properties hinder conventional drug therapies, e.g. targeting brain tumors, leading to the low survival rate of patients with glioblastoma, a malignant type of brain cancer [29]. Therefore, it is essential to investigate the BBB environment *in vitro* to have a detailed understanding of the mechanisms hindering the drug uptake and potentially enhancing it. In recent years a multitude of various *in vitro* BBB models were proposed, taking into account the dynamic, multicellular environment of native BBB.

The BBB is a selective interface between the blood vessels composed of a monolayer of endothelial cells and a part of the central nervous system mostly made up of astrocytes, pericytes, and neurons. The permeability of the BBB is highly restricted and the transport mechanisms are limited to transmembrane and transcellular passive diffusion (in the case of lipophilic molecules with molecular weight lower than 400 Da), active transport (either in a form of carrier-mediated transporters or as active efflux transporters, which can carry larger or hydrophilic molecules as well as macromolecular complexes), endocytosis, and the extracellular pathways [6,30,31]. As a result, substances such as hydrophilic molecules can only pass through the BBB in case of its disruption or via
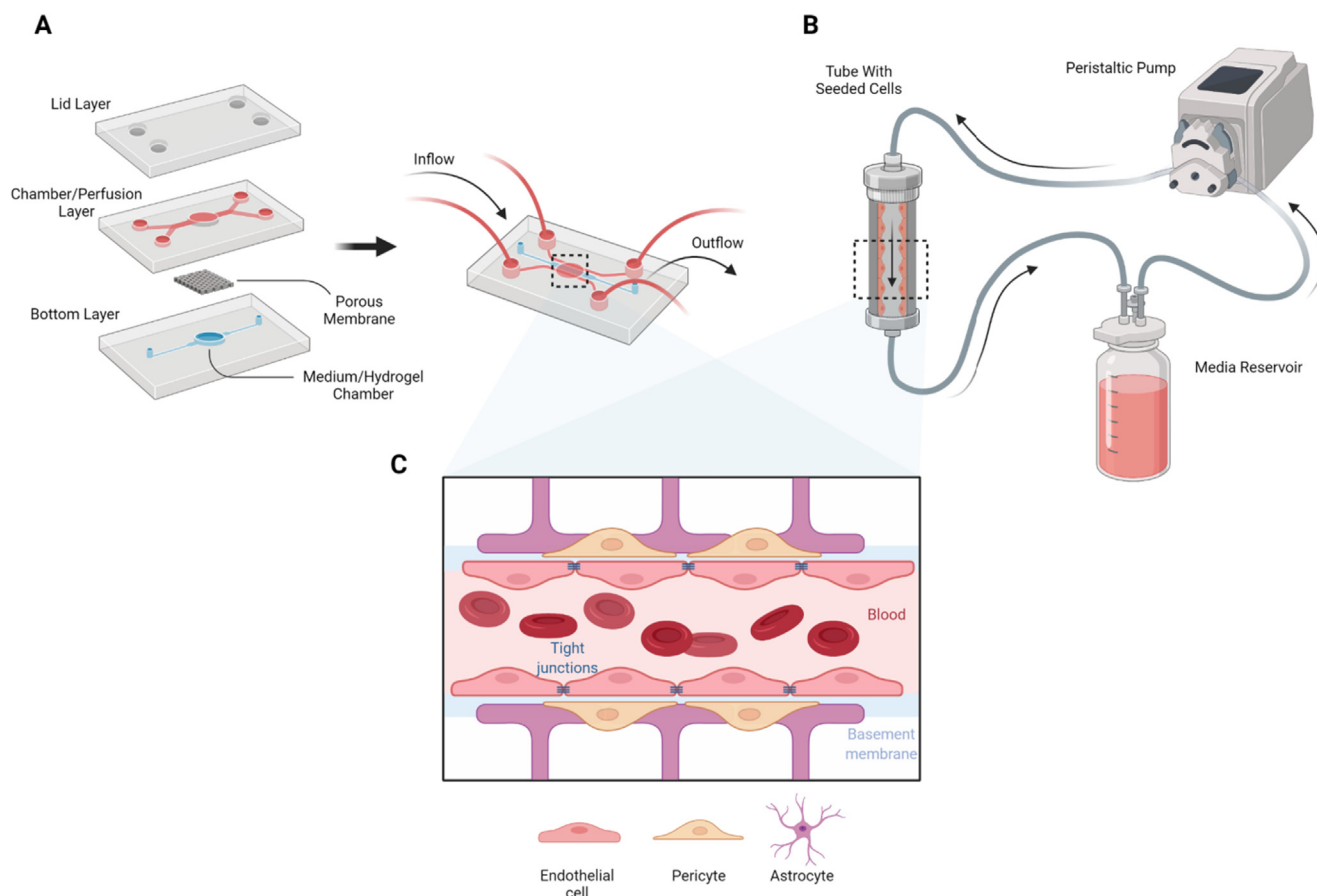
Fig. 3. Schematic representations of dynamic *in vitro* blood-brain barrier models with the introduced flow. A) Microfluidic setup with a medium/hydrogel reservoir and porous membrane serving as the basement membrane on which the cells are grown. The medium flow, and thus shear stress, is introduced to the system via tubing connected to the chip. B) A dynamic model consisting of a tube inside which endothelial cells are grown, a peristaltic pump providing medium flow, and a medium reservoir, all interconnected with tubing. C) A close-up of the *in vivo* BBB environment, which the models strive to recapitulate.

mentioned specific receptor-mediated transporters. In between the blood and the brain part of the barrier lies the basement membrane, which primarily consists of collagen type IV and laminin, along with nidogen and heparan sulfate proteoglycans [32]. Tight junctions (also known as zonula occludens) are crucial components of the BBB, protecting the brain from the uptake of non-selective substances such as pathogens and therapeutics. Tight junctions are complex protein structures, mainly formed by claudins and occludins. Their formation inhibits substrates from passing through the areas in between endothelial cells, leaving only the transcellular route as a way to access the brain [33]. This zonula occludens formation makes the BBB endothelium unique among the capillary blood vessels, as it hampers the ability of drugs to cross and move from the bloodstream to the brain (cancer) tissue.

The brain microenvironment is subjected to flow and shear stress. These, in turn, influence drug transport efficacy. The flow in venous vessels present in the brain is pulsatile. Endothelial cells on the luminal side of blood vessels are constantly subjected and affected by all of the aforementioned forces (laminar/pulsatile flow, shear stress, normal stress, cyclic circumferential stretch, and oscillatory stress). Additionally, the drug's transport, when the flow is not laminar, is influenced by its relative residence time on the vessel's endothelium, dependent on the time-averaged wall shear stress and oscillatory shear index [34]. Therefore, it is crucial to study how blood flow inside the brain vasculature impacts the transport of drugs through the layer of endothelial cells.

As the tightness of the BBB is one of its most crucial characteristics, the most frequently used method for the quantitative mea-

surement of barrier permeability in microfluidic devices is transendothelial electrical resistance (TEER) investigation. TEER measurement is a common way of investigating the integrity of a cell monolayer, and thus its paracellular permeability. It is based on the assessment of either ohmic resistance or impedance using a wide frequency range. The values obtained by these measurements reflect cell barrier integrity and are usually used to evaluate the model before drugs are introduced to it for penetration studies [35]. However, a study conducted by Costa *et al.* revealed that, although TEER is a convenient and widely used method of assessing the integrity of the investigated BBB model, the resistance values may change due to a variety of physical parameters and cells used. They showed that in comparison with human primary brain endothelial cells, the frequently used human cortical microvessels endothelial cells/D3 (hCMEC/D3) cell line shows a significant decrease in claudin-5, occludin, and JAM2 expression, which consequently leads to lowered TEER values for hCMEC/D3 cells [36].

2.1.2.1. Modelling of the blood-brain barrier and uptake studies. Various dynamic *in vitro* models have been developed to recapitulate the native BBB (See Fig. 3). Garcia-Polite *et al.* utilized a system based on a silicone tube coated with fibronectin to create an engineered vasculature model [37]. Human brain microvascular endothelial cells (hBMEC) were seeded inside the tube, after which the construct was connected to a perfusion bioreactor and a peristaltic pump. A range of shear stress values was applied to the system to examine how changes in the flow, and consequently shear stress, affected the formation of tight junctions between the cells.

For this investigation, the authors used markers for zonula occludens-1 (ZO-1) and claudin-5. ZO-1 is the main cytoskeletal organizer for endothelial cells, as it controls F-actin distribution and governs the recruitment of proteins responsible for tight junctions development. Therefore, it regulates barrier formation, which is imperative in terms of selective permeability of the BBB and thus drug penetration into the brain. Experiments on mice have shown that claudin-5 inhibits the passage through the BBB of molecules smaller than 800 Da. The EZ-Link^{TM} Sulfo-NHS-Biotin, with a molecular weight of 443 Da, and Hoechst stain, with a molecular weight of 562 Da, were used as model substrates [38]. They also showed that high shear stress values of 40 dyn/cm^2, as well as pulsatile blood flow, result in a decrease in the expression of tight junction markers, namely ZO-1 and claudin-5, to the values noted for static controls at 0 dyn/cm^2. Upon pulsatile flow being introduced and maintained for 96 h, translocation of ZO-1 from in between the endothelial cells towards the direction of the nucleus and cytoplasm was observed. This situation is atypical, indicating inhibited formation of tight junctions. hBMEC showed restricted ZO-1 expression when grown under static conditions. The authors also presented that hBMEC cultured under physiologic shear stress (10–20 dyn/cm^2) exhibit upregulated markers associated with tight junctions. Compared to non-shear conditions, ZO-1 was 1.7-fold higher and claudin-5 more than 2-fold. Interestingly, no astrocytes were involved in the model used. Instead, the authors applied a human astrocyte-conditioned medium, which enhanced the development of tight junctions.

Another essential factor for BBB permeability, in addition to claudin-5 and ZO-1, is P-glycoprotein (P-gp), a drug transporter found in the plasma membrane. P-gp was shown to limit penetration through the BBB of various hydrophobic molecules with atomic masses larger than 400 Da, exporting them from the endothelial cells back to the bloodstream. This was shown via studies using cells from knockout mice and P-gp blocking agents [39]. P-gp expression was also studied by the authors in an *in vitro* model system. They found that under capillary-like values of shear stress at 10–20 dyn/cm^2, P-gp is upregulated by 1.5- to 1.6-fold. However, its activity decreased under high shear stress, namely 40 dyn/cm^2, and pulsatile flow. Interestingly, hampered permeability induced by 40 dyn/cm^2 can be reversed by applying 10 dyn/cm^2, as indicated by recovered upregulation of tight junction markers. Overall, these studies show that the type and value of the medium flow, and consequently the induced shear stress, play an essential role in tight junction formation.

Elbakary *et al.* designed another BBB *in vitro* perfusion system intended to study cytotoxicity along with the permeation of drugs. It consisted of three chambers connected to a reservoir bottle and a peristaltic pump, forming a complete circuit. The system enabled co-culture and drug perfusion studies, but compared to the one by Garcia-Polite *et al.*, it lacks the curvature of a blood vessel [37,40]. Endothelial cells were grown both on the coverslips and on inserts. The flow rate of media and oxygen could be adjusted using the peristaltic pump, and the rates investigated had values of 275 μL/min (low rate) and 550 μL/min (high rate). In this system, the coverslip's location is an essential factor, as the shear stress decreases 200–300-fold when the coverslip approaches the bottom of the chamber [41]. Elbakary *et al.* measured TEER in both static and dynamic conditions. The TEER was enhanced and maintained longer when the fluid flow was applied to the system. Consequently, a reduction in its flux across endothelium could be seen when mitoxantrone was introduced. Mitoxantrone (444 Da) is used to assess barrier function, as this drug cannot penetrate the BBB unless the barrier is disrupted. Elbakary *et al.* investigated how flow at 550 μL/min influences mitoxantrone permeability through the endothelial monolayer. In the model, there was a larger flux of mitoxantrone from the basolateral (blood) to the apical

(brain) side than in the opposite direction. The ratio between apparent permeability coefficients for both directions (efflux ratio) was 1.6-fold greater for static and 3.6-fold greater for flow conditions. It suggests that the efflux process is active, and the difference in ratios may be related to the formation of a tighter barrier in dynamic conditions. The flow application can positively influence the formation of an impenetrable barrier, as shown by the increase in TEER values and the difference in efflux ratios.

Santa-Maria *et al.* built a BBB lab-on-a-chip system made of polydimethylsiloxane (PDMS) compartments connected by channels. A porous membrane with a pore size of 0.45 μm was placed inside the device. Gold electrodes connected to glass slides were placed over the top and bottom PDMS parts for TEER measurements. Cell culture medium was supplied to the system by a peristaltic pump connected to the chip via silicone tubing [27,42]. The authors investigated the BBB microfluidic system under two shear stress values, namely 0.4 dyn/cm^2 and 1.6 dyn/cm^2. The latter was chosen to represent conditions present in brain postcapillary venules. They investigated human brain-like endothelial cells (BLECs) and observed a downregulation in claudin-1, −3, and −7, while expression levels of tight proteins associated with tight junctions (occludin, JAMs, ESAM, MARVELDs) were unaltered. claudin-5 investigated in co-culture with pericytes under flow showed a decrease in expression, as opposed to its expression in co-culture with astrocytes. This effect might suggest that the addition of pericytes is not essential for tight barrier formation. In turn, genes for adherens junctions remained unaltered or upregulated due to introduction of flow. The addition of shear stress at 0.4 and 1.6 dyn/cm^2 elevated the TEER values significantly by 18%. Along with an increase in resistance, the decrease in paracellular permeability for the model compounds Lucifer yellow (molecular weight: 457 Da) by 78% and Evans blue labeled albumin (molecular weight: 67.5 kDa) by 93% was observed. A comparison was made with the static control, showing that the endothelial cells formed a tighter barrier. Santa-Maria *et al.* also reported the gene expression of ATP-binding cassette transporter (ABC transporters or efflux transporters) to be at the same level in the static and dynamic model. The efflux transporters prevent molecules, which manage to diffuse into the endothelial cells, from penetrating the brain by redirecting them into the bloodstream [43,44]. The solute carrier transporters, which take part in the transport of various substrates, such as amino acids, glucose, and organic ions, across the BBB [45], showed either upregulated or unchanged expression levels under dynamic conditions. On the other hand, the authors observed that the endothelium was more negatively charged in the dynamic conditions and had more lectin binding sites, specific to endothelial glycocalyx [46]. This was the desired effect, as the abluminal side of both the endothelial monolayer and basement membrane is highly negatively charged *in vivo*, serving as an additional protective mechanism to prevent positively charged substrates from crossing the BBB [47]. This lab-on-a-chip approach showed that not only the choice of endothelial cells but also pericytes and/or astrocytes could influence the formation of tight junctions. An increase in TEER values under flow was observed; however, the expression of significant tight junction proteins and ABC transporters was unaltered or even downregulated. It might be connected to the choice of BLECs and indicate that other endothelial cell types would perform better in dynamic conditions.

Cucullo *et al.* utilized a dynamic *in vitro* BBB model (DIV-BBB), composed of 50 polypropylene porous fibers, hollow inside to facilitate nutrient and gas exchange in the interface between the luminal and the abluminal compartment. A pump provided pulsatile flow to the system [48,49]. hBMEC co-cultured with human astrocytes under both static and dynamic conditions were investigated. The authors observed that exposure to shear stress at 6.2 dyn/cm^2 upregulated expression of occludin, claudin-5, N-cadherin, and VE-

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cadherin (vascular endothelial cadherin). VE-cadherin is especially important in relation to the permeability of the BBB, as it is characteristic of endothelial cells and plays a role in their adhesion, cell-cell communication, and tight junction formation [50]. However, it was shown that once the vasculature is well-formed, VE-cadherin is no longer needed to sustain the barrier's integrity [51]. N-cadherin ensures the integrity of cell-cell adhesion, and it can replace VE-cadherin when it is lacking in between endothelial cells [52,53]. Furthermore, Cucullo *et al.* reported that capillary-like shear stress increased the RNA levels of members of CYP1, -2, and -3 families, namely, CYP1A1, CYP2B6, CYP2C8, CYP2J2, CYP3A4, and CYP3A5 of cultured hBMEC. CYP enzymes play a key role in drug metabolism, so their upregulation might indicate neuroprotective system formation. Some of them, like CYP1B1, have been found in the human BBB *in vivo* [54], though expression varies from one individual to another. Interestingly, the presence of CYP3A4, which was upregulated in the *in vitro* study of Cucullo *et al.*, was not found in the BBB *in vivo* [47]. This observation indicates that although those enzymes seem desirable in terms of drug metabolism, their appearance in the model system might indicate that the actual *in vivo* conditions were not reproduced. Cucullo *et al.* found that in dynamic conditions with a shear stress of 6.2 dyn/cm^2, TEER values of the co-culture model increased seven-fold compared to the static control (700 Ω/cm^2 vs. 100 Ω/cm^2). Permeability studies using D-glucose, Dilantin, L-asparaginase, Morphine, D-aspartic acid, Sucrose, and Mannitol as model compounds have shown that the permeability values change under flow. In general, the values for static conditions were a few orders of magnitude higher than those observed in flow models (one order of magnitude for D-glucose and L-asparaginase, two for Sucrose, and three orders for Morphine and D-aspartic acid). The values obtained with flow closely resembled the values detected in *in vivo* models. The studies performed by Cucullo *et al.* corroborated findings by Garcia-Polite *et al.* [37], Elbakary *et al.* [40], and Santa-Maria *et al.* [27], with TEER values increasing under flow conditions, in comparison with static conditions. The values might differ, as they depend on various factors, but the trend is uniform. Permeability for different drugs also decreased when dynamic conditions were applied.

Another microfluidic chip design was reported by Partyka *et al.*, who constructed a device with a reservoir filled with a hydrogel obtained by mixing collagen type I with Matrigel and hyaluronic acid with embedded astrocytes [55]. The model investigated tight junction formation under low shear stress values (0.5 dyn/cm^2) and cyclic strain. Tight junctions were formed under shear stress conditions when cultured for 96 h, as shown by ZO-1 staining, which indicated an intact barrier, as opposed to the static control in which gaps along the endothelium could be seen. The authors also measured the TEER after exposure to shear stress. They observed that the resistance increased significantly after three days of a dynamic culture compared to the static control and in relation to the initial TEER values. Apart from the tight endothelial monolayer formation, another explanation for the increase in TEER levels could be additional impedance stemming from the hydrogel used in the system. Partyka *et al.* used 4 kDa fluorescein isothiocyanate-labeled (FITC) dextran as a model compound to investigate how pulsatile flow, and thus pulsatile strain occurring in the vessels, impacted endothelial permeability. The use of FITC-dextrans is a common practice when evaluating perivascular transport in the microfluidic channels. Dextran is one of the reagents frequently used to examine BBB tightness, as it can controllably vary in molecular weight, which is one of the factors determining drug permeability [56]. The authors concluded that pulsatile flow induced retrograde transport of dextran along the endothelium. The permeability coefficient values were much lower for shear stress conditions (approximately 0.8–1.6 $\cdot$ 10^{-6} cm/s) than in static channels (approximately 10 $\cdot$ 10^{-6} cm/s). The lowest

permeability to FITC-dextran was observed in the model with both endothelial cells and astrocytes subjected to shear stress. When subjected to cyclic stretch, this model showed two-fold higher values. Based on the permeability studies performed with 4 kDa FITC-dextran, the authors found that barrier development is dependent primarily on mechanical stimuli rather than direct contact with astrocyte endfeet. This result has been confirmed by Kubotera *et al.,* who removed the astrocyte endfeet covering rat capillaries to determine the influence on the integrity of the BBB. The authors did not observe relevant changes in permeability and, therefore, concluded that the astrocytes are not imperative for BBB fabrication [57]. The permeability coefficient measured with FITC-dextran for endothelial cells in flow conditions cultured without astrocytes was higher than those in co-culture. Furthermore, similarly high values for the permeability coefficient were obtained for co-cultures undergoing cyclic stretch, where radial strain values oscillated between 0 and 7.8 ± 0.4%. Another observation by Partyka *et al.* was that a tight BBB environment could be successfully reproduced without adding pericytes, which raises the question of how important those cells are for BBB models recapitulating barrier function. Astrocytes and pericytes are frequently used in BBB models because they are present in the human organism in close proximity to the endothelial monolayer, forming together the BBB. However, it has been shown in more than one model that they do not contribute directly to tight junction formation, and therefore they do not influence the permeability [37,57]. An interesting finding of the study conducted by the authors is the positive effect of pulsatile flow application on dextran transport, and that barrier development is facilitated better without the addition of astrocytes, which was suggested earlier by Santa-Maria *et al.* [27]. However, Partyka *et al.* emphasize that mechanical stimuli, such as shear stress and cyclic stretch, are imperative to stimulate the formation of a non-permeable BBB environment *in vitro*. This trend connected to shear stress has also been observed by Cucullo *et al.* [48,49] though investigated using different drugs.

Another approach was implemented by Wang *et al.* They developed a microfluidic system, encompassing an insert with a co-culture of endothelial cells and astrocytes grown on the opposite sides of a porous membrane (See Fig. 4). The researchers refer to their system as BBBoC (BBB-on-a-chip) [58]. The BBBoC consisted of a lid layer, a neuronal chamber for cell culture and medium reservoirs, and a layer with medium perfusion channels. The neuronal chamber was located centrally in between two reservoirs filled with medium. To circumvent the wall shear stress resulting from the reciprocating flow, a "step chamber" has been introduced inside the neuronal chamber. The insert was put over the delivering channel running underneath. The BBBoC was produced via stereolithography. In this system, the flow was introduced using a rocking platform, generating oscillatory shear stress due to the reciprocating flow. This BBBoC model used gravity-based medium flow to mimic the blood's residence in brain tissues. TEER values obtained by the step chamber introduction were roughly one order of magnitude higher than for other systems mentioned above (See Table 1). The authors also investigated the permeability of the gravity-based device with minimized shear stress using FITC-dextrans. They observed that FITC-dextran permeability decreased by approximately 90% for 70 kDa compared to 4 kDa FITC-dextran. These results corroborate the research by Matter and Balda. They showed that smaller molecules could diffuse across endothelium via both transcytosis and paracellular diffusion. However, if tight junctions are well-formed, larger molecules will be excluded from the paracellular transport route [59]. Wang *et al.* investigated the permeability of their BBB microfluidic model using drugs varying in their perfusion mechanisms. They utilized doxorubicin, which does not penetrate the BBB, as it is transported back to the bloodstream via active efflux transporters [60], cimetidine, which can
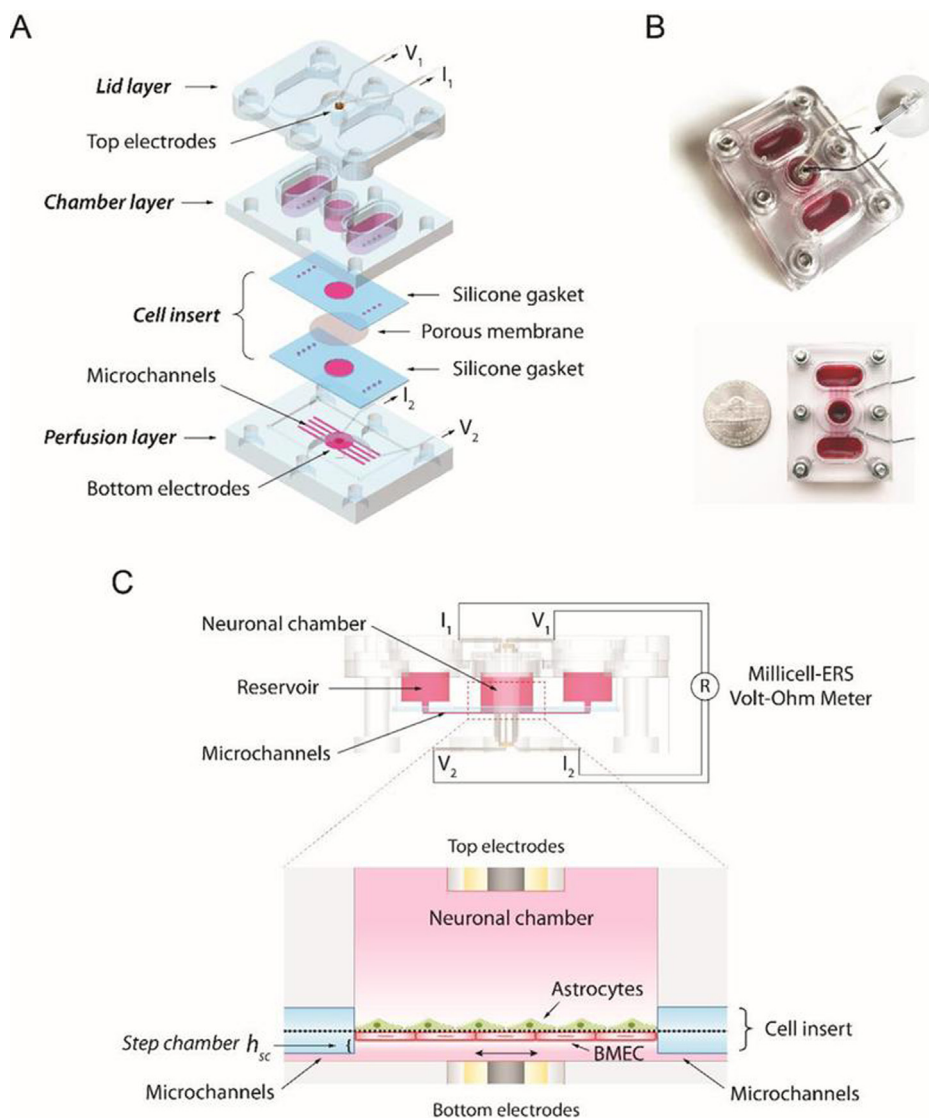
Fig. 4. BBB-on-a-chip presented by Wang et al. A) Schematic representation of the layers from which the device is made. B) Overview of the assembled chip. C) Side view with a close-up on the neuronal chamber and the cells grown on both sides of the porous membrane. Figure reprinted from Wang *et al.* [58], with permission from Wiley.

pass through the BBB with the use of organic cation transporters or efflux transporters [58], and caffeine, which penetrates the *in vivo* BBB rapidly either via carrier-mediated transport or via simple diffusion [61]. The permeability values of the model of Wang *et al.* correlate with those obtained in *in vivo* studies on drug penetration across the BBB [62,63]. Indeed, the highest value was obtained for caffeine ($4.85 \pm 1.84 \cdot 10^{-4}$ cm/s), intermediate for cimetidine ($1.11 \pm 0.09 \cdot 10^{-6}$ cm/s) and the lowest for doxorubicin ($1.54 \pm 0.66 \cdot 10^{-7}$ cm/s). In their setup, the authors chose to minimize shear stress on the endothelial cells and refrain from using pumps for medium flow introduction. They observed a tight endothelial barrier, as investigated via TEER measurements. The TEER values were higher for the system of Wang *et al.* [58] than for other microfluidic systems mentioned beforehand, which implemented pump-based medium flow, and therefore unidirectional laminar shear stress [27,37,40,48,49,55]. The findings of Wang *et al.* [58] do not indicate any negative impact of astrocytes on barrier formation, as it was in cases of Santa-Maria *et al.* [27] and Partyka *et al.* [55].

An overview of the dynamic set-ups used for BBB fabrication is presented in Table 1.

Different endothelial cell types were used for the models presented in this review. As such, the models cannot be directly compared with one another, as mentioned by Wang *et al.*, who stated that hBMECs have proven to respond differently to shear stress than other cell types commonly used in microfluidic devices. All the above examples confirm the indisputable value of flow in *in vitro* BBB models. Flow enables a constant supply of nutrients and oxygen with simultaneous waste removal. It increases the resemblance to the human organism by preventing phenomena like gravitational settling and aggregation of nano-sized drugs used in the systems. Lack of flow is one of the hurdles in static *in vitro* systems, which are less representative of the human body's physiological environment, where no settling can be observed [64]. Dynamic models show enhanced tight junction formation and lower permeability. Yet, the variety of flow rates used in the systems, and therefore the variety of shear stress values, pose the question of which values are needed to have a truly representative model. Further studies of dynamic models incorporating investigation of shear stress conditions are needed to develop a better understanding of the cellular mechanisms hampering drug transport across the BBB.

Table 1
The overview of dynamic *in vitro* systems with the introduced flow.

System	Flow Perfusion Bioreactor [37]	Kirkstall Quasi Vivo (QV600) [40]	Lab-on-a-chip [27]	DIV-BBB [48,49]	BBB-on-a-chip [55]	BBB-on-a-chip [58]
Type of used endothelial cells	hBMEC	PBMEC	BLEC	hBMEC	hCMEC/D3	BMECs derived from human iPSCs
Co-culture	No	No	Yes	Yes	Yes	Yes
Flow type	Steady/pulsatile	Laminar	Laminar	Pulsatile	Steady/pulsatile	Reciprocating
Shear stress (dyn/cm^2)	0–40	0–$110 \cdot 10^{-6}$	0.4 and 1.6	4 and 6.2	0.7	0.014–0.023
TEER ($\Omega \cdot$ cm^2)	Not measurable	448.1 ± 11.3	361.8 ± 166.3 – 425.5 ± 188.8	700	150–1200	2000–4000
Immunofluorescence staining (tight junctions detection)	ZO-1, claudin-5	ZO-1	ZO-1, claudin-5, β-catenin, P-glycoprotein (P-gp), glucose transporter-1 (GLUT-1), ICAM-1, VCAM-1	Analysis via gene array (ZO-1, claudin 3 and 5, cadherins, catenin α2, and β1, and actin α2)	ZO-1, GFAP	ZO-1, claudin-5
Permeability assay	–	Mitoxantrone transport assay	Lucifer yellow, Evans blue labeled albumin	D-glucose, Dilantin, L-asparaginase, Morphine, D-aspartic acid, Sucrose, Mannitol	FITC-dextrans	FITC-dextrans, caffeine, cimetidine, doxorubicin
Additional tests	Western blot	MTT cellular viability assay	RNA sequencing analysis, zeta potential measurements, cell surface glycocalyx staining	Glucose consumption, lactate production, RNA extraction, and gene analysis, endothelial cells protein separation, 2D gel electrophoresis	Micro-particle image velocimetry, copGFP transfection	–
Outcome on drug transfer	–	The ratio between apparent permeability coefficients for both directions (efflux ratio) was 1.6-fold greater for static and 3.6-fold greater for flow conditions	Along with an increase in resistance, the decrease in paracellular permeability for Lucifer yellow (by 78%) and Evans blue labeled albumin (by 93%) was observed	The values for static conditions were a few orders of magnitude higher than those observed in flow models. The values obtained with the use of flow resemble closely the values detected in *in vivo* models	The permeability coefficient values are much lower for shear stress conditions than in static channels. The lowest permeability to FITC-dextran was observed in the model with both endothelial cells and astrocytes subjected to shear stress. When subjected to cyclic stretch, this model showed two-fold higher values	FITC-dextran permeability decreased by approximately 90% for 70 kDa in relation to 4 kDA. The highest permeability value was obtained for caffeine ($4.85 ± 1.84 \times 10^{-4}$ cm/s), intermediate for cimetidine ($1.11 ± 0.09 \times 10^{-6}$) and the lowest for doxorubicin ($1.54 ± 0.66 \times 10^{-7}$ cm/s)

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Overall, in vitro models employed to study the BBB are an extremely valuable tool enabling research on a molecular level. They provide an opportunity to build the BBB environment in a controlled way, where small alterations in just one of the components, e.g. absence and presence of flow, the value of flow, absence, and presence of astrocytes and pericytes, can be monitored. With this approach, complementary to *in vivo* tests that explore systemic effects, more light can be shed on the significance of each of the monitored components.

2.1.2.2. Enhanced uptake strategies. To increase or control the drug uptake through BBB, several approaches can be employed. Ultrasonic stimulation is a physical factor studied for its ability to increase drug efficiency, e.g. in cancer treatment [65–68] and transport through the endothelial layer or the BBB, as reviewed elsewhere [69]. The application of ultrasound is based on thermal or mechanical effects. For example, injection of microbubbles can further increase drug penetration through the endothelial layer and, as such, stimulate drug uptake due to the increased permeability of the vasculature and cell membranes. The bubbles can contract and expand under the ultrasonic wave and subsequently collapse. This leads to the formation of small fluid jets that can impact surrounding cells [68]. The focused ultrasound (FUS) combined with microbubbles enhanced drug delivery through the blood-tumor barrier. The ultrasound and microbubble destruction causes high mechanical forces, directly disturbing the vascular endothelial cell monolayer or leading to inertial cavitation that releases drugs from liposomes [67]. When using FUS, the temperature can be locally increased, leading to tissue ablation and, consequently, increasing the blood circulation and drug transport in the surroundings [68]. Ultrasound was shown to increase drug penetration through the BBB due to the joined effect of tight junction disturbance, induction of transcytosis, and induction of pores in the vascular endothelium [69]. In another study, Arvanitis *et al.* [65] analyzed the effect of FUS using an orthotopic mouse HER2-positive xenograft to model breast cancer metastasis to the brain. Based on quantitative analysis of fluorescence imaging, the authors observed increased drug penetration for two tested molecules, i.e. the low–molecular-weight chemotherapeutic agent doxorubicin (580 Da) and the antibody-drug conjugate ado-trastuzumab emtansine (T-DM1; 66.5 kDa). The increase in extravasation of the drug was seven-fold and two-fold, for doxorubicin and T-DM1, respectively, and in penetration depth was greater than 100 μm vs. < 20 μm for doxorubicin, and 42 ± 7 μm vs. 12 ± 4 μm for T-DM1, when compared to the control samples without FUS. Based on the experimental results and modeling, the authors concluded that FUS enhanced interstitial convective transport due to the increased hydraulic conductivity. For small molecules (doxorubicin), the transmembrane transport, drug uptake, and binding to the nucleus in the endothelial cells were also increased. Additionally, poorly perfused vessels with negative transvascular pressure, lying near highly perfused vessels with positive transvascular pressure, attracted the drug and increased transport into poorly perfused tumor areas. In another study, Ho *et al.* used ultrasound at 2 MHz and lipid-shell microbubbles of 1.12 μm in size [66]. The antivascular effect and doxorubicin penetration were observed in the *in vitro* and *in vivo* mouse model. The higher the acoustic pressure used, the bigger the vessels that could be disrupted, the higher the distance of drug penetration, and the greater the tumor growth inhibition was. Tardoski *et al.* used low-intensity ultrasound, at 2.9 and 1.3 MHz, with maximal pressure of 0.29 MPa [67]. The authors aimed at avoiding cavitation effects to eliminate the risk of tissue damage. They observed the increased bisphosphonate uptake in the form of zoledronic acid (270 Da) into MCF-7 human breast cancer cells via cadherin-mediated endocytosis. Typically, the zoledronic acid could not pass

the membrane by diffusion due to its negative charges. A further increase in zoledronic acid uptake was observed when additionally mild hyperthermia (approximately 43–44 °C) was induced via continuous ultrasound delivery. The effects were assigned to the induced mechanical stresses, which did not affect cell membrane integrity or cell growth rate, but induced endocytosis. The thermal effects led to increased blood flow and increased endothelial permeability. Accumulation of small drugs in the heated area was also reported [68].

Mechanical stresses were also tested to increase drug transport through the BBB [55]. The authors studied how the vessel wall stretch influences transport through BBB, using a microfluidic approach integrated with a 3D hydrogel cell culture model. Controlled shear stress (ca. 0.7 dyn/cm^2) and cyclic strain, driven by fluid flow, were applied. It was observed that both the shear stress and cyclic stretch decreased the transport of 4 kDa dextran through the vessel walls. It was also shown that the cyclic stretch facilitates retrograde convective transport along the basement membrane. As the stretch-induced transport is understood to contribute to waste removal from the brain tissue, these findings can help to explain pathological states, caused e.g. by vessel wall stiffening, leading to the altered strains in the vasculature.

Overall, physical stimulation of tissues offers an attractive approach in improving drug efficiency and targeted drug delivery, reducing dose-limited side effects of chemical modulations, and eliminating increased drug resistance. It can be tuned to obtain local, minimally invasive, and transient effects. Thus the approach is very promising to enhance the delivery of therapeutics into complex and challenging environments such as tumors or brain tissue (See Fig. 2). However, several issues remain to be addressed, including well-controlled stimulation in different tissues and at different depths and locations. Elimination of other accompanying effects, such as tissue heating or alteration of the signal by complex anatomical structures, needs to be resolved as well.

2.2. Epithelial barrier

Besides the endothelial barrier, a key barrier that needs to be overcome for drug delivery is the epithelial barrier which defines the interface between separate compartments. The epithelium is the primary constituent of the intestinal barrier which on the apical side forms a continuous border restricting the passage of molecules [70]. Even though the interstitial epithelial barrier plays a key role in drug transport, the epithelial barrier is also important for skin and nasal drug delivery. Several recent reviews covered the latter topics, to which we refer readers for more in-depth information [71–74].

2.2.1. Gastrointestinal barrier

One of the most common ways of drug administration is via the oral route, where the drug first needs to pass the GI tract to reach systemic circulation [75]. When drugs are administered orally, a key barrier that needs to be overcome is the mucus layer present in the small intestine. This layer acts as a physical barrier for drug transport [76]. The major structural components of the mucosal barrier are the mucins, which are highly glycosylated proteins interacting with each other and forming a mesh-like structure [77], which can regulate drug penetration and diffusion. The small intestine is composed of one layer of mucus situated in near proximity of the epithelial cell affecting the absorption of molecules, whereas the colon has an inner and outer mucus layer. The mucosal layer that is close to the epithelial cells is more stationary with no flow, suggesting a longer retention time of drugs. The second, loosely adhesive mucus layer, which is facing the luminal content, is characterized by more flow and a shorter drug retention time.

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Modeling the human gut physiology *in vitro* is a challenging task where multiple strategies have been applied to date to construct the most physiologically relevant model. The mucosal barrier has been recreated in several ways *in vitro,* including using mucus-secreting cell lines and applying mucus on inserts [76]. However, these models lack the dynamic nature of physiological conditions. Direct comparison between the classical Transwell and dynamic *in vitro* tissue models is limited to only a few studies, which have compared the transport of compounds such as caffeine and atenolol [78], paracetamol [79], verapamil, and ergotamine [80]. These studies show that the two models lead to different results. Specifically, caffeine showed higher transport in the dynamic model, whereas ergotamine showed reduced transport. Furthermore, the dynamic conditions offer multiple other advantages, such as effects on cell differentiation. A recent study by Kulthong *et al.* investigated the differences in gene expression in Caco-2 cells cultured under static and dynamic conditions [81]. More than five thousand genes were differentially expressed when comparing the static and dynamic conditions, both up- and down-regulated. Specifically, fluid flow caused upregulation of specific metabolizing enzymes such as cytochrome P450 (CYP) A1A, essential for drug metabolism in the small intestine. However, the overall cellular metabolism was decreased based on the downregulation of various individual genes, including enzymes responsible for drug metabolism. In general, differences in static and dynamic models suggest the importance of external stimulation, with dynamic models more closely mimicking *in vivo* environments.

Microfluidic models can be exploited to incorporate dynamic elements, identify drug transport, test new therapies, and even conduct drug safety testing. The establishment of physiologically relevant dynamic GI models allows *in vitro* screening of drug molecules for their ability to cross the intestinal barrier. Current models mainly rely on the previously mentioned static Transwell models, which fail to entirely recapitulate the complex gut morphology [82]. The human gut microenvironment largely depends on the mechanical stress applied by the peristaltic motion which relaxes and contracts the tissues to move the gut contents through the GI tract [83] (See Fig. 1**B**).

Moreover, the peristaltic motions shape the human intestinal microenvironment affecting drug transport and absorption. Therefore, mimicking together the peristalsis and the mechanical stretching of the endothelial and epithelial layers is crucial for developing physiological relevant GI models [84]. Additionally, in the *in vitro* models, compounds often fail to cross the mucin layer due to the limited residence time on the epithelial surface [85]. Ideally, to test the drug permeability across the mucus layer, the models need to recapitulate the human mucus composition as close to the physiological state as possible, including the mechanically moving parts. The conventional static models fail to incorporate the flow and peristaltic motion, essential for mimicking human gut physiology. Organ-on-a-chip platforms offer the ability to recapitulate the gut in a three-dimensional (3D) design which may establish a more physiologically relevant model [86]. Specifically, most gut-on-a-chip models are based on two microchannels separated by a porous membrane. Commonly, Caco-2 cells are seeded into the microchannels under static conditions to improve adherence, and after that, a dynamic microenvironment can be established by having peristaltic pumps creating media flow [87]. Various studies have investigated different intestinal models by considering multiple factors influencing drug transport. Here, we will discuss these models in more detail, focusing on how peristaltic movement affects drug transport.

2.2.1.1. Microfluidic gut-on-a-chip models and drug transfer studies. One of the first dynamic GI models, including intestinal

peristalsis-like motions, was the gut-on-a-chip model developed by Kim *et al.* in 2012 [82]. The proposed chip device comprised two layers of closely opposed microfluidic channels separated by an ECM coated porous membrane lined with human Caco-2 intestinal epithelial cells. To mimic fluid flow culture, the medium was perfused through both channels. The established fluid flow was at a relatively low rate of 30 μL/h. The peristalsis-like motion was achieved by applying cyclic suction regulated by a vacuum manifold to vacuum chambers on both sides of the channels to stretch and relax the ECM porous membrane. Upon the applied flow, the Caco-2 cells underwent phenotypic modifications. Specifically, the cells spontaneously organized into folds that appeared to exhibit the morphology of intestinal villi. This spontaneous intestinal villi formation is potentially related to mimicking the physiological mechanical microenvironment with fluid flow and peristaltic motions. The established gut-on-a-chip model recapitulated the dynamic features of the human intestine, which play a crucial role in mimicking the physiological intestinal transport affecting the cell differentiation to more physiologically relevant cell types. More recent work from the same research group developed an even more advanced gut-on-a-chip model where besides peristalsis, inflammatory events were recreated *in vitro* [88]. These events could serve as a base for studying how drug transport is affected by pathophysiology. The villi in the model were able to show immunological properties and drug-metabolic activity by expressing cytochrome P450 enzymes. Moreover, the gut-on-a-chip model developed by Kim *et al.* holds the potential to be used as an *in vitro* tool for drug transport testing as well as for drug development. Additionally, Beaurivage *et al.* created a gut-on-a-chip model, using the OrganoPlate, to mimic inflammatory bowel disease *in vitro.* They tested the validity of this model for drug discovery [89]. The OrganoPlate was composed of 40 microfluidic chips, each containing three microfluidic channels where an ECM precursor was incorporated into the central channel, and Caco-2 cells were seeded in the top channel. The OrganoPlate device was able to perform drug screening in a high-throughput manner. Specifically, they exposed the cells to different concentrations of an anti-inflammatory compound, TPCA-1, which caused reduced basal cytokine secretion. Treating the cells with 1.25 μM TPCA-1 retained barrier integrity and did not affect the viability of the cells. However, a higher concentration of TPCA-1 caused cell death, affecting the barrier function. Therefore, this model can be used to assess the effect of various compounds on barrier integrity. Recently, Tan *et al.* also used Caco-2 cells to create a multichamber microfluidic intestinal barrier model with peristalsis-like movements, which can be used for drug transport studies. The cultured Caco-2 cells in their model were able to differentiate and polarize upon the presence of a relatively high continuous flow rate (65 μL/min) [90]. Interestingly, they used an alternative to PDMS, namely a thiol-ene based novel membrane system. This material has a low affinity for the absorption of molecules compared to PDMS, which is notorious for absorbing small hydrophobic molecules, making its use challenging for drug transport studies [91]. Specifically, the permeability of the thiol-ene microfluidic device was investigated by applying mannitol, dextran, and insulin as model compounds. The permeability of all three compounds was higher in the microfluidic model. The Caco-2 cells used in the model can show discrepancies in the expression of enzymes and transporters, but also the differences in tight junction formation compared to the human intestine [92]. Furthermore, the most commonly used Caco-2 cell lines for intestinal models do not produce the major mucin types, such as MUC2, which is crucial for the establishment of the mucus layer [93]. Therefore, the Caco-2 cells need to be co-cultured with other mucin-producing cells to represent the mucus layer closely. When it comes to microfluidic models, to mimic the physiological drug

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permeability, it is essential, besides including peristaltic motions, to include the mucus layer. To study the impact of the mucus barrier on drug diffusion, a comparison between mucus-containing and non-containing models needs to be performed.

Besides the Caco-2 cell models, Lee *et al.* designed a stomach-on-a-chip using human-induced pluripotent stem cells (hiPSCs) differentiated into gastric organoids, where luminal flow and peristaltic movement was observed [94]. The luminal flow was established by using biomimetic periodic pumping. The device was manufactured by 3D printing, followed by PDMS molding in which hiPSC gastric organoids were integrated. The peristaltic movement was confirmed by visualizing FITC-dextran as a model compound, where each contraction corresponded to the peristaltic pump speed. This stomach-on-a-chip model holds potential for personalized drug screening with models composed of patient-derived iPSCs. However, future studies are needed to establish the ability to investigate drug transport in this model. Besides using iPSCs in stomach-on-a-chip, Workman *et al.* have differentiated iPSCs to human intestinal organoids to establish an intestine-on-a-chip model [95]. Their model was composed of a porous PDMS membrane and incorporated continuous flow at a rate of 30 μL/h. The continuous flow exposure resulted in the formation of polarized intestinal folds containing enterocytes, Paneth cells, goblet cells, and endocrine cells. Specifically, the presence of different cell types created a more physiologically relevant microenvironment. Overall, based on cell differentiation and, therefore, the presence of a more complex mixture of cell types, these studies show that the use of iPSCs can mimic the physiological conditions more realistically when compared to the Caco-2 cell models. Additionally, using iPSCs can potentially be used to test drug transport in specific disease conditions where for example, the peristalsis is affected and therefore affects drug transport. Kasendra *et al.* developed a duodenum intestine chip for preclinical drug assessment [96]. In their model, intestinal organoid cultures isolated from healthy individuals were seeded on one side of ECM-coated PDMS membranes, while on the other side primary human intestinal microvascular endothelial cells (HIMECs) were used. The cells were exposed to cyclic mechanical strain to create physiologically relevant forces mimicking peristaltic motion at 60 μL/h. The application of flow accelerated the formation of densely packed apical microvilli and stimulated the maturation of the epithelium polarization. To test the intestinal barrier function and permeability of the model, the authors investigated the permeability of dextran. The low permeability coefficient of FITC-dextran indicates that their model successfully forms a functional barrier. The results show that the proposed duodenum intestine-chip model can mimic *in vivo* results and aid preclinical drug assessment. The authors compared the mRNA expression of relevant cell types, including enterocytes and goblet cells of the intestinal chip to RNA values directly isolated from human intestinal tissue. Furthermore, they observed that the expression of P-gp in their model had a relatively smal increase (~1.5–2.6 fold) compared to human native tissue. Even though their model showed intestinal permeability, future studies need to determine the ability of this model to assess the permeability of different drugs. Interestingly, besides drug transport studies, the platform allowed the investigation of CYP450 induction which is a promising way to investigate drug metabolism *in vitro*. For instance, Pocock *et al.* recently established a microfluidic intestine-on-a-chip model for evaluating the transport of the cancer pro-drug SN38, which has very low bioavailability [78]. The same research group designed several lipophilic pro-drugs of SN38, which were used to evaluate their model for transepithelial transport of drugs. The intestine-on-a-chip model was composed of four membranes, three of which were PDMS and one polycarbonate. On the apical side of the model, Caco-2 cells were cultured, and fluid shear stress was applied. To test the drug transport, SN38

and the prodrug formulations were applied to the apical side of the model. At 30 min intervals samples were collected from the basal side, and the drug concentration was measured by using ultra-fast liquid chromatography. Besides the SN38 prodrugs, they also used model compounds, such as caffeine and atenolol to test the system's permeability, which revealed that the coefficients obtained were in approximate comparison to physiological conditions. Overall, besides the model compounds, the SN38 prodrugs permeability coefficients could be determined, suggesting that this model mimics key characteristics of the intestinal epithelium, including the dynamic conditions. To study the mucus layer *in vitro*, Elberskirch *et al.* developed a microfluidic mucus-chip system composed of a porous silicon nitride membrane, an upper channel with a porcine intestinal mucus layer, and a fluidic channel connected to a peristaltic pump [97]. This mucus-chip system can evaluate the permeability of compounds via the mucus barrier, which is often neglected in the traditional Transwell systems. As the mucus composition varies in different physiological conditions, the mucus-chip system allows multiple adjustments, such as adapting the mucus layer thickness based on the mimicked *in vivo* situation. The system was validated by measuring the diffusion of the relatively small molecules caffeine (194.2 Da) and diclofenac sodium (318.1 Da), as well as a large molecule FITC-dextran (70,000 Da) under fluidic conditions for three hours with and without the mucus layer. The results showed that the test compounds crossed the mucus barrier with different permeability based on their size, which is considered a major permeability factor across the mucus barrier. Using a mucus-chip system allows testing the drug mucus barrier permeability. All of the above-discussed models vary in their components and manufacturing process, making it challenging to determine the most appropriate characteristics of an ideal GI *in vitro* model. However, the importance of implementing peristaltic motion was observed in all of them. Overall, mimicking the key characteristics of the GI, such as peristalsis *in vitro*, has significant potential to improve the current set-ups for *in vitro* models used for drug transport studies.

2.2.2. Enhanced uptake strategies

The intestinal thiol-ene microfluidic model of Tan *et al.* investigated the transport of drugs with low absorption rates including insulin [90]. To enhance the uptake they applied a permeability enhancer namely tetradecyl-maltoside. When the permeability enhancer was introduced, the permeability increased in both static and microfluidic models across the Caco-2 monolayer. The permeability enhancer was beneficial for insulin due to its low oral bioavailability. The model allowed permeability studies of three different compounds combined with permeability enhancers, which might serve as a relevant model for drug transport and delivery studies. Another potential way of enhancing oral drug delivery is by using liposomes that can successfully cross the mucus barrier [98]. Li *et al.* used Pluronic F127 (PF127) polymer modifier for the composition of the liposomes. The usage of PF127 improved the stability of the liposomes. Additionally, they observed that their liposomes could diffuse through the rat intestinal mucus barrier. Taking into account their results, the usage of PF127-modified liposomes seems like a promising way of improving the drug delivery via the mucus barrier. Besides developing strategies for increased drug uptake upon oral administration, enhancement strategies are popular for other ways of administration. Specifically, nasal and topical drug delivery are very attractive routes for drug administration due to the direct accessibility, high available absorption area, low risk of infection, and possibility of painless and patient-friendly drug application. Typically, these tissues reveal limited permeability, especially for larger drugs. The opportunity to increase the uptake by physical, mechanical or electrical simulation, not requiring any additional chemicals, can be

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considered safe and, therefore, very interesting from the application point of view (See Fig. 2).

Increased drug delivery under load was observed for the nasal mucosa. Kwon *et al.* applied centrifugation hypergravity (10 × increased gravity force) on a human nasal epithelial cell line and primary human nasal epithelial cells cultured in the plate [99]. Hypergravity caused a decrease in the TEER. As a result, the cell barrier was weakened, and the passage of polar drugs (fluorescently labeled 4 kDa dextran and sodium salt) was increased by 16–19%. The uptake mechanism was via the paracellular route - the gaps between the cells. A slight decrease in cell proliferation (ca. 7%), changes in the cytoskeleton organization, and upregulation of the expression of the junctions-related genes were observed. The authors concluded that hypergravity leads to cytoskeletal rearrangement, causing transient alteration in the tight junctions and consequently increasing drug permeability.

Lio *et al.* proposed another approach to decrease the tight junction and cell barrier in the skin tissue [100]. The authors used neodymium magnets to pinch the skin and induce temporal pressure (0.14–0.4 MPa). They showed that a one-minute treatment with 0.28 MPa pressure led to the formation of reversible, approximately 3 μm-size pores in the epidermal layers, allowing the transport of different drugs (nanoparticles up to 500 nm, macromolecules up to 20 kDa) in a distance of 430 μm from the skin surface. After such optimized treatment, the skin barrier was not compromised, as observed by lack of epidermis thickening or inflammation. More prolonged application of pressure of over five minutes and higher pressure values further increased the drug uptake. However, this induced adverse effects on the skin. This approach successfully delivered insulin via the topical route to diabetic mice. The authors suggested that the transport of drugs happens through both the paracellular and transcellular routes. A different approach used photomechanical pulses in the form of pressure waves to increase the transport of drug molecules through the skin [101,102]. A single, 23-ns mechanical pulse generated by a laser allowed for a transient increase in permeability of the stratum corneum, the most outer layer of the skin, and delivery of fluorescently labeled 40 kDa dextran and 20 nm latex particles, to the depth of ca. 50 μm into viable dermis and epidermis [101].

3. Load-bearing tissues and enhancement strategies

From a drug delivery perspective, load-bearing tissues are of great interest, as drug efficacy may depend on tissue mechanics and physical parameters. The dependence between mechanical load and drug diffusion was established by analyzing drug uptake in load-bearing tissues, such as cartilage, tendon, or muscle. DiDomenico and Bonassar studied the diffusion of solutes with different charges within articular cartilage in *ex vivo* experiments [103]. The authors used the fluorescently labeled 150 kDa-size antibodies and traced their penetration into cartilage. The highest solute concentration was observed 200–300 μm in depth from the cartilage surface. At these distances, the relative content of ECM (e.g. collagen) in the tissue is lower, and the pore sizes are larger when compared to the superficial tissue. As a result, the contribution of diffusion increased, specifically 1) for positively charged drugs, which can be explained by the electrostatic interactions with the net negative charges of the tissue matrix [104], and 2) under compressive loading applied at the frequency of 1 Hz (1.5–3 fold), scaling up with increased cyclic amplitude [103]. The latter result was explained by the fluid flow perpendicular to the tissue surface, induced by the cyclic load. In other studies, the dynamic compression was shown to increase the effectiveness of insulin-like growth factors in articular cartilage explants, as measured by an increase in the protein and proteoglycan synthesis, also due to increased fluid flow

[105]. Interestingly, Lima *et al.* have shown that the dynamic mechanical loading can outstandingly improve mechanical and biochemical properties of the engineered cartilage tissue when provided after discontinued supplementation of transforming growth factor- β3 (TGF β3) [106]. The authors claim that providing mechanical stimulation and the addition of growth factors sequentially, yields optimal results. In another study, Huang and Gu used a numerical approach to test the effect of tension-compression nonlinearity on drug transport under dynamic loading [107]. They observed that both, the nonlinearity of tension-compression and dynamic compression without nonlinearity, enhanced the transport of larger molecules (2–4 nm size) in the cartilage due to induced convection. This effect was diminished for smaller molecules.

The specific function of muscle, having the ability to contract with given force and frequency, influences drug transport and efficacy in a complex manner, going beyond passive diffusion [108–110]. Several studies have investigated the uptake of drugs or model compounds connected to the metabolic activity of contracting muscle [111–113]. The studies of Wu and Edelman went beyond this paradigm, testing the combined effect of tissue dynamics and architecture on the local drug distribution in the muscle [108]. They used custom-made devices to apply controlled tensile strain, in the physiological range of 0–20%, on isolated rat soleus muscle immersed in a model compound solution of 20 kDa FITC-dextran. Drug uptake and distribution were analyzed at the planar and curved muscle surface. The tissue geometry and type of load both showed a significant effect on the local uptake. The drug penetration was higher at the curved surfaces than planar surfaces, and it increased under cyclic strains compared to static load by approximately 1.6-fold. The higher surface area explained these observations in contact with the drug at the curvatures. Additionally, the drug penetration from tissue margins encountered the transport from the planar surfaces, increasing the local drug concentration. The static loading contributed to the drug uptake by decreasing the volume of the stretched muscle and the intramuscular gradient of pressure. The dynamic loading caused further increase by two mechanisms. Firstly, the cyclic contractions led to higher time-average intramuscular porosity due to myofiber deformation and displacement, and to heterogeneous concentration gradients accelerating diffusion. Secondly, the dynamic motion led to a pulsatile agitation increasing the drug dispersion and spread. The differences in local muscle architecture at the curved surfaces led to even greater porosity differences and higher drug dispersion in the dynamic mode, causing synergistic effects of dynamic motion and curvature in the drug uptake.

In a follow-up study, the authors systematically tested the influence of static and cyclic strain on drug transport at the planar surface of the cartilage [109]. They observed that the drug penetration increased by 1.45 fold at 10% strain and was further intensified by cyclic loading and isometric twitch contractions with a maximum of 0.5 Hz (1.52 fold increase). Histological analysis revealed that mechanical loading influenced porosity, fiber area, and fiber density, thereby dictating the efficiency of drug transport under load. The starched fibers were thinned in the static load, and the resulting porosity increased, enhancing drug transport. In the dynamic mode, the effectiveness of the transport followed the trend from static load but was also influenced by the loading rate. In a contraction experiment, where a twitching force was applied, the authors suggested a specific mechanism of myofibers' behavior. Swelling of fibers may appear in the stretched state, leading to the displacement of the cytoplasm into the direction of the middle of the fibers, causing the lower porosity and drug transport hindrance. After relaxation, fibers are thin, and porosity increases. The switch between the swollen and relaxed state leads to the dispersive effect and the improved drug transport. The maximum drug

penetration at optimal frequency is connected to the increased temporal steric hindrance and interstitial pressures and dispersion effects. The optimal frequency is expected to be related to the specific optimal muscle length (maximal force generation) defined by the peak muscle performance, namely 110% of the nominal muscle length in the performed studies. It was shown that swelling or shrinkage of the fibers could be dictated by the strength of stimulation, where higher strength leads to shrinkage, and moderate strength leads to swelling [113]. The authors also studied the dependency of drug size on their efficacy. Small molecules revealed better diffusion, explained by the steric hindrance connected to pore size. In dynamic load, the dispersion formation increased transport beyond the molecular diffusion. The drug concentration further influenced the drug transport due to the combined effects of architecture-driven diffusion and muscle-motion-related dispersion. Modeling and numerical approaches were used to validate the architectural and dispersive effects on drug delivery.

Overall, mechanical loading influences both the load-bearing tissues' architecture and functional properties, such as fluid distribution, interstitial pressure, and force transmittance, determining the drug transport [109] (See Fig. 2). Mechanical activity should be considered when assessing drug efficacy in such microenvironments and could be used to control the drug delivery. The support of computational modeling of multifactorial systems will allow better understanding and more accurate predictions of drug uptake in load-bearing tissues.

4. Tissue matrix and its effect on drug delivery

Zooming in from the macroscopic to the microscopic properties of tissues, a key component is the cells themselves. Cells in the tissue that are residing close to the vasculature may limit drug transport, by active sequestration of the administered drug. In addition to the mode of action of the specific drug, the cellular density of tissue is also an important determinant. Amongst others, this became evident in studies on doxorubicin distribution in tumor tissue, where doxorubicin was found to strongly interact with the nuclei of the cells close the vasculature [114].

In addition to cells, our tissues also contain ECM. It is increasingly recognized that ECM is not passive and static, playing only a structural role. Instead, it is a highly dynamic and complex component, serving a deeper purpose in cell signaling. In the context of drug penetration, the role of the ECM is two-fold. Firstly, the ECM may pose a physical, restrictive barrier for drugs to be effectively distributed evenly throughout the tissue. Secondly, depending on its characteristics, through the interaction with cells, the ECM may trigger alterations in cell behavior that can subsequently affect the response of cells to the drug. We will discuss both effects in the following sections.

4.1. The ECM as a physical barrier for drug transport

The barrier function of the ECM for the transport of drugs can be a consequence of various factors. First of all, the composition of the ECM plays an important role. ECM components themselves were found previously to sequester drugs, as was shown in a study by Chang *et al.* for the interaction between cisplatin and collagen [115].

Secondly, the transport of drugs through the extravascular space makes use of a combination of diffusion and convection, processes that can be affected by the ECM. Diffusion is a mechanism that is thought to be used mainly by small molecules and is driven by gradients in concentration. Convection, on the other hand, is the preferred mechanism of larger molecules and is driven by gradi-

ents in pressure. The extent to which these processes can take place depends on the properties of the drug itself, but also on the properties of the matrix. If the ECM is very dense in a certain tissue, it may prove to act as a physical barrier for the transport of drugs administered. Ghajar *et al.* studied the transport of FITC-conjugated molecular mass markers by diffusion through fibrin gels of different densities within microfluidic channels. Their results showed that increased fibrin concentration led to reduced transport, but that also the molecular weight of the marker influenced this [116]. Studies on tissue-mimics in an *in vitro* setting showed that the increased presence of ECM components reduced transport of a subset of test molecules, including the anticancer drug paclitaxel [117]. In this study, elastin was identified as a key ECM constituent affecting transport. Furthermore, the penetration of therapeutics was suggested to be reduced in tumors containing large amounts of collagen compared to tumors with low collagen content [118,119]. This is likely due to reduced rates of transport in the denser ECM network and the raised interstitial fluid pressure as a consequence of the dense ECM [120]. Disruption of the collagen network with collagenase was shown to increase the diffusion of IgG in xenografted tumor tissue in mice [119,121]. Hyaluronic acid, a glycosaminoglycan, through which other ECM components can be crosslinked, is another key component of the ECM. As such, hyaluronic acid contributes to the mechanical properties of the ECM. Hyaluronic acid can be enzymatically broken down by hyaluronidase. Delivery of gemcitabine and liposomal doxorubicin was found to be improved after treatment with PEGylated hyaluronidase, indicating a potential barrier function of hyaluronic acid in the ECM [122,123].

In addition, the barrier effect exerted by the ECM will heavily depend on the properties of the drugs used. Size, shape, charge, lipophilicity are all major determinants of transport through tissue. Classically, our therapeutics have largely relied on small molecules, but with the arrival of antibody-based therapeutics and nano-sized drug delivery systems, amongst others, the diversity of drug formulations has increased. Each of these formulations will have its own characteristics when it comes to tissue penetration. As such, nanoparticle size and surface charge were found to dictate uptake in tumor spheroid models. Albanese *et al.* showed that in a tumor spheroid-on-a-chip model, the accumulation of nanoparticles was dependent on their size. Larger nanoparticles (greater than 100 nm in size) were taken up to a lesser extent than smaller particles (40 nm) [124]. This was corroborated by Tchoryk *et al.*, who further showed that also surface charge of the nanoparticles used affects their uptake [125].

The disease state of tissues is another factor that needs to be taken into consideration when exploring novel therapeutics with increased tissue penetration. The ECM is highly likely to change as a result of pathological remodeling, for example during fibrosis and cancer. These alterations may affect various characteristics of the ECM, for example, the accumulation of ECM (the density), its composition (e.g. electrostatic properties), and organization (alignment and orientation of fibers), which in turn can affect drug transport. When designing novel drugs, the potentially pathological ECM should be taken into consideration, as excess ECM deposition can inhibit drug transport, whereas reduced or disrupted ECM can accelerate transport.

Overall, the ECM is an important physical barrier that may hinder the penetration of drugs into tissue. To date, several approaches have been suggested to help overcome this. These have mainly focused on controlling ECM in cancer, a key strategy being disrupting ECM structure in tumor tissue (see Fig. 2). For more extensive information on this topic, we would like to refer to previously published work [126,127].

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4.2. Effects of ECM stiffness on drug efficacy in cells

In addition to the ECM itself being a physical barrier to drug penetration, the response cells launch upon drug exposure may be influenced by the mechanical properties of the ECM. The different tissues in our bodies are characterized by differences in their mechanical properties. In this respect, various mechanical properties can be recognized, such as stiffness and elasticity, also depending on the method used for characterization. For example, bone is the stiffest tissue we know, with elastic moduli in the GPa range, whereas brain and lung tissue are amongst the softest, with elastic and shear moduli in the low kPa range [128]. A key component contributing to these properties is the ECM. The composition of the ECM can vary greatly between different tissues, and also disease can lead to alterations in the properties of the ECM. As such, the disease state can severely influence the mechanical properties of tissues. Fibrosis and cancer are examples in which tissue rigidity increases primarily through alterations in the ECM. The stiffness of the ECM found in tumors was ∼4–10 kPa higher than the stiffness of the healthy tissues [129]. The mechanics and associated characteristics of the ECM influence the behavior of the cells residing in a given environment. The cells inhabiting a tissue will respond to physical and biochemical cues in the ECM, which can affect their overall response to therapeutics. To date, various studies have explored how the mechanical properties of the cellular environment can influence the therapy response, making use of various in vitro ECM models and mimics. Below we will discuss examples thereof and highlight the strategies taken to study this phenomenon.

Schrader et al. investigated the effects of matrix stiffness on two-dimensional-cultured hepatocellular carcinoma cancer cells [130]. The authors used polyacrylamide gels of variable stiffness coated with cell adhesive materials. Huh7 and HepG2 cells were found to have higher proliferation rates when grown on the substrate with the higher elastic modulus (12 kPa) compared to the lower elastic modulus (1 kPa). Treatment with the chemotherapeutics cisplatin and 5-fluorouracil revealed that the cells grown on the 12 kPa substrates were more sensitive than those grown on 1 kPa substrates. The stiffness of the substrate was further shown to regulate intracellular signaling events, which were found to be responsible for differences in cell behavior. Qin and coworkers used polyacrylamide gels, with Young's moduli that were classified as low (10 kPa), intermediate (38 kPa), and high (57 kPa), thereby mimicking the rigidity of normal breast tissue, and benign and malignant breast tumors, respectively [131]. The substrates of intermediate stiffness were found to reduce cell death upon treatment with doxorubicin in MDA-MB-231 breast cancer cells. Drug uptake was shown to be decreased in cells cultured on these substrates, and as such, increased resistance. Exploration of the mechanisms involved revealed that the Integrin-Linked Kinase (ILK) was induced and in turn mediated the activation of Yes-Associated Protein (YAP) and P-gp. Lin et al. examined how matrix mechanics affect the response to the HER2-targeted kinase inhibitor lapatinib in HER2-amplified breast cancer cells [132]. The authors relied on polyacrylamide gels and tissue culture plastic coated with collagen-I to create soft and stiff environments. HCC1569 breast cancer cells showed enhanced sensitivity to lapatinib on the soft substrates compared to the stiff substrates. BT549 cells, which do not carry a HER2 amplification, were not affected by lapatinib or changes in rigidity. Subsequent analysis of the mechanisms involved also led these authors to the YAP/TAZ pathway. Knockdown of these components using small interfering RNA abolished the modulus-dependent lapatinib resistance. In their 2019 study, Medina et al. used fibronectin-functionalized polyacrylamide gels with increasing elastic modulus to investigate the effect of substrate stiffness on therapy sensitivity in a panel of breast cancer

cells [133]. They also used a two-dimensional configuration and analyzed the efficacy of several different cancer drugs. Overall, the authors reported that cancer cells cultured on substrates that mimicked the stiffness of tumor tissue were more resistant to therapeutics than those cultured on the stiffer substrates. Another factor analyzed in this study is the origin of the cells used. The authors made a comparison between well-established breast cancer cell lines, acclimatized to growing on stiff cell culture plastics and primary breast cancer cells, directly obtained from an in vivo mouse model. They reported that the primary cells predicted the in vivo response more accurately than the cell lines could.

Shin et al. relied on a hydrogel system based on RGD-conjugated alginate to control the matrix mechanics and ligand density [134]. A set of myeloid leukemia subtypes carrying different genetic mutations were used and cultured in a three-dimensional configuration. Both stiffness and ligand density were shown to control the proliferation rates in a distinct way for the cell model. Next, the authors studied cell responses to a panel of therapeutics. As such, they distinguished three classes of drugs: drugs that cells are resisting regardless of matrix stiffness, drugs that act in a matrix stiffness-dependent manner, and drugs that cells are sensitive to regardless of matrix mechanics. However, this classification turned out to depend on the cell type studied. Overall, the authors observed differential chemosensitivity that not only depended on matrix stiffness but also on the leukemia cell type examined. Nguyen and colleagues studied the effects of stiffening of the ECM on resistance to the Raf kinase inhibitor sorafenib [135]. They used a high-throughput biomaterial platform based on PEG-phosphorylcholine (PEG-PC) hydrogels coated with basement membrane-like ECM or collagen-rich ECM. This system had tunable stiffness, reported to be between 6 and 400 kPa. In the cell types studied, the liver carcinoma cell line HEP3B and the breast carcinoma cell lines BT-549, MDA-MB-231, and SkBr3, a significant increase in the IC-50 value of sorafenib was found upon increasing stiffness of the cell culture substrate. This matrix-mediated drug resistance did not depend on the activity of ROCK but was mediated by JNK. As such, adding a JNK inhibitor to sorafenib treatment led to the cells regaining sensitivity. Tokuda et al. explored the effect of matrix elasticity on drug response in melanoma cells, using tunable and chemically defined polyethylene glycol (PEG) hydrogels [136]. The Young's modulus of the gels ranged from 0.6 to 13.1 kPa, to mimic the elasticity of non-cancerous and cancerous tissue. The PEG chains were functionalized with CGRGDS peptide and crosslinked with a matrix-metalloproteinase (MMP)-degradable crosslinker. WM35 cells (early-stage melanoma) and A375 cells (metastatic stage) were exposed to vemurafenib (PLX4032), a drug in clinical use for the treatment of metastatic melanoma, while cultured 2D on the PEG gels. As measured by ATP content, analysis of the resulting metabolic activity revealed no significant differences between substrates of different elasticity. Assessment of apoptosis by measuring caspase 3 activity showed an increase in WM35 cells upon drug treatment that was strongest on the softest substrates. On the other hand, the A375 cells showed no difference in response on the different substrates. As such, in this particular experimental set-up, the effects of substrate elasticity observed appear to depend heavily on the cell types that are being studied. Recently, Deng et al. reported that the stiffness of the extracellular environment contributes to therapy resistance by inducing a more efficient DNA repair [137]. They cultured human embryonic kidney-293 (HEK293) cells on fibronectin-coated acrylamide hydrogels, of which the stiffness varied from 0.5 kPa to 30 kPa. Treatment with various genotoxic agents, including radiotherapy, cisplatin, and etoposide, showed that the cells grown on the substrates with the lower stiffness were more sensitive compared to cells on higher stiffness substrates. Investigations into the underlying
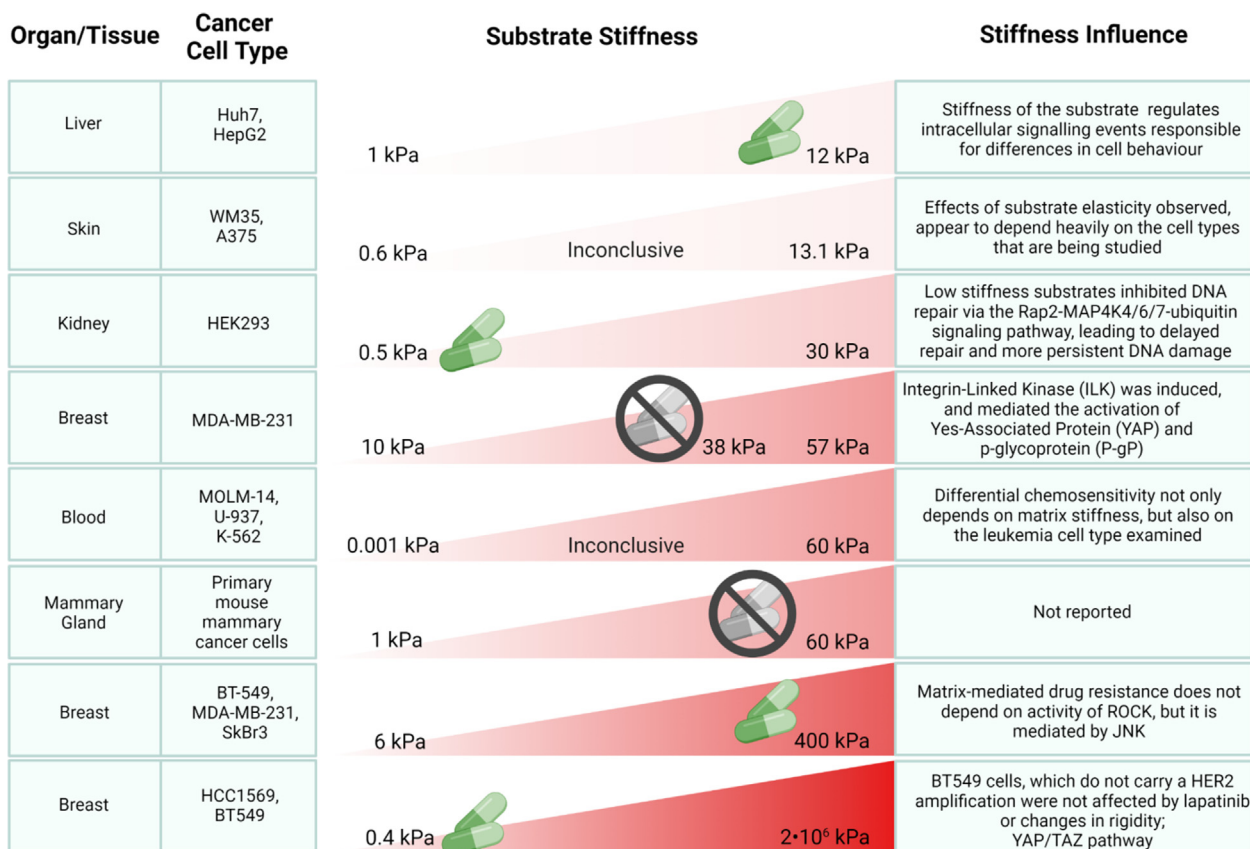
Organ/Tissue	Cancer Cell Type	Substrate Stiffness		Stiffness Influence
Liver	Huh7, HepG2	1 kPa	12 kPa	Stiffness of the substrate regulates intracellular signalling events responsible for differences in cell behaviour
Skin	WM35, A375	0.6 kPa / Inconclusive	13.1 kPa	Effects of substrate elasticity observed, appear to depend heavily on the cell types that are being studied
Kidney	HEK293	0.5 kPa	30 kPa	Low stiffness substrates inhibited DNA repair via the Rap2-MAP4K4/6/7-ubiquitin signaling pathway, leading to delayed repair and more persistent DNA damage
Breast	MDA-MB-231	10 kPa	38 kPa 57 kPa	Integrin-Linked Kinase (ILK) was induced, and mediated the activation of Yes-Associated Protein (YAP) and p-glycoprotein (P-gP)
Blood	MOLM-14, U-937, K-562	0.001 kPa / Inconclusive	60 kPa	Differential chemosensitivity not only depends on matrix stiffness, but also on the leukemia cell type examined
Mammary Gland	Primary mouse mammary cancer cells	1 kPa	60 kPa	Not reported
Breast	BT-549, MDA-MB-231, SkBr3	6 kPa	400 kPa	Matrix-mediated drug resistance does not depend on activity of ROCK, but it is mediated by JNK
Breast	HCC1569, BT549	0.4 kPa	$2 \cdot 10^6$ kPa	BT549 cells, which do not carry a HER2 amplification were not affected by lapatinib or changes in rigidity; YAP/TAZ pathway

Fig. 5. Schematic representation of various cancer cells' response to both substrate stiffness and drug treatment. Green symbols were put next to stiffness values which have proven to increase drug uptake by the cells. Grey symbols represent inhibited drug uptake due to the stiffness value of the substrate used for the cell culture.

mechanisms revealed that the low stiffness substrates inhibited DNA repair via the Rap2-MAP4K4/6/7-ubiquitin signaling pathway, leading to delayed repair and more persistent DNA damage.

Collectively, these studies show that the mechanical microenvironment impacts the cellular response to therapeutics (See Fig. 5). However, the direction in which matrix mechanics affect drug response, i.e. resistance, sensitivity, or no change, depends heavily on the environmental mechanics used. The range of stiffness used in the studies discussed above varies between the different reports. Furthermore, the drug(s) that are being studied may have a different dependence on matrix mechanics, and also, the cell models being used are influenced differently. Cells of various origins may launch diverse intracellular pathways in response to a change in matrix mechanics. This may lead to differential sensitivity, even when the same drug is applied in identical mechanical conditions.

5. Future perspectives

As discussed in this review, multiple different physical and biological barriers in the body, dependent on the chosen administration way, affect the drug transport and efficacy. Overcoming these barriers for effective drug delivery is highly challenging in the native physical microenvironment. However, strategies for crossing these barriers are continuously being improved and (re-) invented. An essential pillar in this context is formed by relevant *in vitro* models, with which the effects of the various physical barriers on drug efficacy, and ways to cross these barriers, can be studied in detail. In recent years, the complexity of such models that can be exploited for drug transport studies has significantly increased, including the use of organ-on-chip systems.

In vitro BBB models should ideally implement flow to achieve close physiologic resemblance. The flow induces shear and affects the cell fate and interactions, including the formation of crucial components of the BBB, such as tight junctions. Additionally, involving co-cultures in the model was shown to increase the expression of proteins associated with tight junctions, revealing more *in vivo*-like characteristics. Orally administered drugs additionally need to cross the intestinal barrier to reach the systemic circulation. In the GI tract, they experience peristaltic motions and fluid flow, affecting the transport across the membrane. Therefore, gut epithelial models that introduce both flow and peristaltic motion may be required to predict the drug efficacy successfully. Besides the peristalsis-like movements, the thickness of the mucus barrier can affect the amount of drug crossing the intestinal barrier and the efficacy of the drug. In addition, the characteristics of the ECM, such as stiffness and elasticity, can significantly affect drug penetration across the tissue and dictate intracellular signaling events. Therefore, mechanical and structural properties should not be neglected. Finally, the influence of external stimulation, such as electrical or mechanical, may further tune and influence drug transport and efficacy. Overall, based on recent studies, multiple aspects of the physical microenvironment should be considered together to understand the drug transport inside the body thoroughly.

With the advance of the available *in vitro* models, the opportunity to test new strategies of effective drug delivery in well-controlled and adjustable environments is also becoming increasingly accessible. The increasing knowledge on the physical barriers in the body that drive sub-optimal drug delivery offers the potential to explore less conventional approaches to aid drug transport across these barriers. Those approaches can include the use of

mechanical forces already present in the tissue or exerted on it to disrupt the barrier in a reversible way, which enhances drug uptake (See Fig. 2 for a summary of enhancement strategies). Another futuristic example is an orally administered capsule which has been designed in such a way that it can perform gastric injections once it reaches gastric submucosa. The capsule formulation includes a multi-spring actuation system, which is responsible for making the injection [138].

Based on the newest scientific reports, we envision that further progress in the field of drug delivery is connected with the development of new high adequacy *in vitro* testing platforms of high complexity, including organ-on-chips. These systems will allow optimization of conventional routes of drug delivery through the barriers in the body and develop novel approaches based on external stimulation. By better understanding how to model the biological barriers, we foresee that non-invasive drug delivery will be highly advanced. The novel non-invasive drug delivery will be a more patient-friendly alternative as well as a game-changer for developing countries where currently there are drug administration challenges due to the lack of medical facilities. It may also contribute to further improvement in the accuracy of drug delivery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

References

[1] T. Kerz, G. Paret, H. Herff, Routes of drug administration, in: Card. Arrest Sci. Pract. Resusc. Med., Elsevier, 2007, pp. 614–638. 10.1017/CBO9780511544828.035.

[2] Y. Li, Q. Wu, C. Li, L. Liu, K. Du, J. Shen, Y. Wu, X. Zhao, M. Zhao, L. Bao, J. Gao, R. F. Keep, J. Xiang, Role of Human Breast Cancer Related Protein versus P-Glycoprotein as an Efflux Transporter for Benzylpenicillin: Potential Importance at the Blood-Brain Barrier, PLoS ONE 11 (2016), https://doi.org/10.1371/JOURNAL.PONE.0157576 e0157576.

[3] J.M.J.R. Carr, P.N. Ainslie, Shearing the brain 129 (2020) 599–602. 10.1152/JAPPLPHYSIOL.00658.2020.

[4] T. Tree-Udom, J. Seemork, K. Shigyou, T. Hamada, N. Sangphech, T. Palaga, N. Insin, P. Pan-In, S. Wanichwecharungruang, Shape Effect on Particle-Lipid Bilayer Membrane Association, Cellular Uptake, and Cytotoxicity, ACS Appl. Mater. Interfaces. 7 (2015) 23993–24000, https://doi.org/10.1021/ACSAMI.5B06781.

[5] Chapter 6. Absorption/Transport Mechanisms, Handb. Basic Pharmacokinet. … Incl. Clin. Appl. Seventh Ed. (2009). 10.21019/9781582121260.CH6.

[6] W.M. Pardridge, Drug transport across the blood-brain barrier, J. Cereb. Blood Flow Metab. 32 (2012) 1959–1972, https://doi.org/10.1038/jcbfm.2012.126.

[7] C.M. Bellettato, M. Scarpa, Possible strategies to cross the blood-brain barrier 11 Medical and Health Sciences 1109 Neurosciences, Ital. J. Pediatr. 44 (2018) 1DUNNY, https://doi.org/10.1186/S13052-018-0563-0/FIGURES/1.

[8] D. Dahlgren, H. Lennernäs, Intestinal Permeability and Drug Absorption: Predictive Experimental, Computational and In Vivo Approaches, Pharm. 2019, Vol. 11, Page 411. 11 (2019) 411. 10.3390/PHARMACEUTICS11080411.

[9] J. Müller, M. Keiser, M. Drozdzik, S. Oswald, Expression, regulation and function of intestinal drug transporters: An update, Biol. Chem. 398 (2017) 175–192, https://doi.org/10.1515/HSZ-2016-0259.

[10] R. Nandigama, B. Upcin, B.H. Aktas, S. Ergün, E. Henke, Restriction of drug transport by the tumor environment, Histochem. Cell Biol. 150 (2018) 631–648, https://doi.org/10.1007/S00418-018-1744-Z.

[11] J.L.S. Au, B.Z. Yeung, M.G. Wientjes, Z. Lu, M.G. Wientjes, Delivery of cancer therapeutics to extracellular and intracellular targets: Determinants, barriers, challenges and opportunities, Adv. Drug Deliv. Rev. 97 (2016) 280–301, https://doi.org/10.1016/J.ADDR.2015.12.002.

[12] N. Wettschureck, B. Strilic, S. Offermanns, Passing the Vascular Barrier: Endothelial Signaling Processes Controlling Extravasation, Physiol. Rev. 99 (2019) 1467–1525, https://doi.org/10.1152/PHYSREV.00037.2018.

[13] Y. Komarova, A.B. Malik, Regulation of endothelial permeability via paracellular and transcellular transport pathways, Annu. Rev. Physiol. 72 (2010) 463–493, https://doi.org/10.1146/ANNUREV-PHYSIOL-021909-135833.

[14] J.D. Pollock, I. Murray, S. Bordes, A.N. Makaryus, Physiology, Cardiovascular Hemodynamics, StatPearls. (2021). https://www.ncbi.nlm.nih.gov/books/NBK470310/ (accessed February 3, 2022).

[15] S. Chien, Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell, Am. J. Physiol. - Hear. Circ. Physiol. 292 (2007) 1209–1224, https://doi.org/10.1152/AJPHEART.01047.2006.

[16] K.S. Cunningham, A.I. Gotlieb, The role of shear stress in the pathogenesis of atherosclerosis, Lab. Investig. 85 (2005) 9–23, https://doi.org/10.1038/labinvest.3700215.

[17] J.J. Paszkowiak, A. Dardik, Arterial wall shear stress: observations from the bench to the bedside, Vasc. Endovascular Surg. 37 (2003) 47–57, https://doi.org/10.1177/153857440303700107.

[18] B. Zhang, J. Gu, M. Qian, L. Niu, H. Zhou, D. Ghista, Correlation between quantitative analysis of wall shear stress and intima-media thickness in atherosclerosis development in carotid arteries, Biomed. Eng. Online. 16 (2017) 1–17, https://doi.org/10.1186/S12938-017-0425-9.

[19] E.A.V. Jones, F. Le Noble, A. Eichmann, What determines blood vessel structure? Genetic prespecification vs. hemodynamics, Physiology. 21 (2006) 388–395, https://doi.org/10.1152/PHYSIOL.00020.2006.

[20] C.K. Zarins, M.A. Zatina, D.P. Giddens, D.N. Ku, S. Glagov, Shear stress regulation of artery lumen diameter in experimental atherogenesis, J. Vasc. Surg. 5 (1987) 413–420, https://doi.org/10.1016/0741-5214(87)90048-6.

[21] E. Roux, P. Bougaran, P. Dufourcq, T. Couffinhal, Fluid Shear Stress Sensing by the Endothelial Layer, Front. Physiol. 11 (2020) 861, https://doi.org/10.3389/FPHYS.2020.00861.

[22] E.E. Van Haaften, T.B. Wissing, M.C.M. Rutten, J.A. Bulsink, K. Gashi, M.A.J. Van Kelle, A.I.P.M. Smits, C.V.C. Bouten, N.A. Kurniawan, Decoupling the Effect of Shear Stress and Stretch on Tissue Growth and Remodeling in a Vascular Graft, Tissue Eng. - Part C Methods 24 (2018) 418–429, https://doi.org/10.1089/ten.tec.2018.0104.

[23] D.C. Fernandes, T.L.S. Araujo, F.R.M. Laurindo, L.Y. Tanaka, Hemodynamic Forces in the Endothelium: From Mechanotransduction to Implications on Development of Atherosclerosis, Endothel. Cardiovasc. Dis. Vasc. Biol. Clin. Syndr., Elsevier (2018) 85–95, https://doi.org/10.1016/B978-0-12-812348-5.00007-6.

[24] H.H. Lipowsky, S. Kovalcheck, B.W. Zweifach, The distribution of blood rheological parameters in the microvasculature of cat mesentery, Circ. Res. 43 (1978) 738–749, https://doi.org/10.1161/01.RES.43.5.738.

[25] R. Krams, J.J. Wentzel, J.A.F. Oomen, R. Vinke, J.C.H. Schuurbiers, P.J. De Feyter, P.W. Serruys, C.J. Slager, Evaluation of Endothelial Shear Stress and 3D Geometry as Factors Determining the Development of Atherosclerosis and Remodeling in Human Coronary Arteries in Vivo, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 2061–2065, https://doi.org/10.1161/01.ATV.17.10.2061.

[26] L. Cucullo, M. Hossain, W. Tierney, D. Janigro, A new dynamic in vitro modular capillaries-venules modular system: Cerebrovascular physiology in a box, BMC Neurosci. 14 (2013) 1–12, https://doi.org/10.1186/1471-2202-14-18.

[27] A.R. Santa-Maria, F.R. Walter, R. Figueiredo, A. Kincses, J.P. Vigh, M. Heymans, M. Culot, P. Winter, F. Gosselet, A. Dér, M.A. Deli, Flow induces barrier and glycocalyx-related genes and negative surface charge in a lab-on-a-chip human blood-brain barrier model, J. Cereb. Blood Flow Metab. 41 (2021) 2201–2215, https://doi.org/10.1177/0271678X21992368.

[28] Y. Qiu, S. Tong, L. Zhang, Y. Sakurai, D.R. Myers, L. Hong, W.A. Lam, G. Bao, Magnetic forces enable controlled drug delivery by disrupting endothelial cell-cell junctions, Nat. Commun. 2017 81. 8 (2017) 1–10. 10.1038/ncomms15594.

[29] M.T.C. Poon, C.L.M. Sudlow, J.D. Figueroa, P.M. Brennan, Longer-term (≥2 years) survival in patients with glioblastoma in population-based studies pre- and post-2005: a systematic review and meta-analysis, Sci. Reports 2020 101. 10 (2020) 1–10. 10.1038/s41598-020-68011-4.

[30] W.A. Banks, Characteristics of compounds that cross the blood-brain barrier, BMC Neurol. 9 (2009) 1–5, https://doi.org/10.1186/1471-2377-9-S1-S3.

[31] C.W. Fong, Permeability of the Blood-Brain Barrier: Molecular Mechanism of Transport of Drugs and Physiologically Important Compounds, J. Membr. Biol. 248 (2015) 651–669, https://doi.org/10.1007/S00232-015-9778-9.

[32] M.S. Thomsen, L.J. Routhe, T. Moos, The vascular basement membrane in the healthy and pathological brain, J. Cereb. Blood Flow Metab. 37 (2017) 3300–3317, https://doi.org/10.1177/0271678X17722436.

[33] S. Ohtsuki, T. Terasaki, Contribution of Carrier-Mediated Transport Systems to the Blood–Brain Barrier as a Supporting and Protecting Interface for the Brain; Importance for CNS Drug Discovery and Development, Pharm. Res. 2007 249. 24 (2007) 1745–1758. 10.1095-007-9374-5.

[34] H.A. Himburg, D.M. Grzybowski, A.L. Hazel, J.A. LaMack, X.M. Li, M.H. Friedman, Spatial comparison between wall shear stress measures and

porcine arterial endothelial permeability, Am. J. Physiol. - Hear. Circ. Physiol. 286 (2004) 1916–1922, https://doi.org/10.1152/AJPHEART.00897.2003.

[35] B. Srinivasan, A.R. Kolli, M.B. Esch, H.E. Abaci, M.L. Shuler, J.J. Hickman, TEER Measurement Techniques for In Vitro Barrier Model Systems, J. Lab. Autom. 20 (2015) 107–126, https://doi.org/10.1177/2211068214561025.

[36] A. Fabulas-Da Costa, C. Landry, L. Dehouck, E. Sevin, M.-P. Dehouck, R. Cecchelli, M. Culot, Blood–Brain Barrier In Vitro Models and Their Applications in Toxicology, (2014) 147–166. 10.1007/978-1-4939-0521-8_7.

[37] F. Garcia-Polite, J. Martorell, P. Del Rey-Puech, P. Melgar-Lesmes, C.C. O'Brien, J. Roquer, A. Ois, A. Principe, E.R. Edelman, M. Balcells, Pulsatility and high shear stress deteriorate barrier phenotype in brain microvascular endothelium, J. Cereb. Blood Flow Metab. 37 (2017) 2614–2625, https://doi.org/10.1177/0271678X16672482.

[38] T. Nitta, M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, S. Tsukita, Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice, J. Cell Biol. 161 (2003) 653–660, https://doi.org/10.1083/jcb.200302070.

[39] A.H. Schinkel, P-Glycoprotein, a gatekeeper in the blood-brain barrier, Adv. Drug Deliv. Rev. 36 (1999) 179–194, https://doi.org/10.1016/S0169-409X(98)00085-4.

[40] B. Elbakary, R.K.S. Badhan, A dynamic perfusion based blood-brain barrier model for cytotoxicity testing and drug permeation, Sci. Reports 2020 101. 10 (2020) 1–12. 10.1038/s41598-020-60689-w.

[41] P. Miranda-Azpiazu, S. Panagiotou, G. Jose, S. Saha, A novel dynamic multicellular co-culture system for studying individual blood-brain barrier cell types in brain diseases and cytotoxicity testing, Sci. Rep. 8 (2018) 1–10, https://doi.org/10.1038/s41598-018-26480-8.

[42] F.R. Walter, S. Valkai, A. Kincses, A. Petneházi, T. Czeller, S. Veszelka, P. Ormos, M.A. Deli, A. Dér, A versatile lab-on-a-chip tool for modeling biological barriers, Sens. Actuators B Chem. 222 (2016) 1209–1219, https://doi.org/10.1016/J.SNB.2015.07.110.

[43] W. Löscher, H. Potschka, Blood-brain barrier active efflux transporters: ATP-binding cassette gene family, NeuroRx. 2 (2005) 86–98, https://doi.org/10.1602/neurorx.2.1.86.

[44] D.S. Miller, Regulation of ABC transporters at the blood–brain barrier, Clin. Pharmacol. Ther. 97 (2015) 395–403, https://doi.org/10.1002/CPT.64.

[45] K.A. Nałęcz, Solute Carriers in the Blood–Brain Barier: Safety in Abundance, Neurochem. Res. 2016 423. 42 (2016) 795–809. 10.1007/S11064-016-2030-X.

[46] S. Beyer, A. Blocki, M.C.Y. Cheung, Z.H.Y. Wan, B. Mehrjou, R.D. Kamm, Lectin Staining of Microvascular Glycocalyx in Microfluidic Cancer Cell Extravasation Assays, Life 2021, Vol. 11, Page 179. 11 (2021) 179. 10.3390/LIFE11030179.

[47] S. Dauchy, F. Dutheil, R.J. Weaver, F. Chassoux, C. Daumas-Duport, P.O. Couraud, J.M. Scherrmann, I. De Waziers, X. Declèves, ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood–brain barrier, J. Neurochem. 107 (2008) 1518–1528, https://doi.org/10.1111/J.1471-4159.2008.05720.X.

[48] L. Cucullo, M. Hossain, E. Rapp, T. Manders, N. Marchi, D. Janigro, Development of a humanized in vitro blood-brain barrier model to screen for brain penetration of antiepileptic drugs, Epilepsia. 48 (2007) 505–516, https://doi.org/10.1111/j.1528-1167.2006.00960.x.

[49] L. Cucullo, N. Marchi, M. Hossain, D. Janigro, A dynamic in vitro BBB model for the study of immune cell trafficking into the central nervous system, J. Cereb. Blood Flow Metab. 31 (2011) 767–777, https://doi.org/10.1038/jcbfm.2010.162.

[50] O. F, G. C, F. A, C. M, G. A, S. S, R. G, M. L, K. GY, F. D, K. V, P. D, B. P, M. D, G.L. M, D. E, Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo, Nat. Commun. 3 (2012). 10.1038/NCOMMS2199.

[51] C.N. Duong, D. Vestweber, Mechanisms Ensuring Endothelial Junction Integrity Beyond VE-Cadherin, Front. Physiol. (2020) 519, https://doi.org/10.3389/FPHYS.2020.00519.

[52] Y. Miyamoto, F. Sakane, K. Hashimoto, N-cadherin-based adherens junction regulates the maintenance, proliferation, and differentiation of neural progenitor cells during development 9 (2015) 183–192. 10.1080/19336918.2015.1005466.

[53] C. Giampietro, A. Taddei, M. Corada, G.M. Sarra-Ferraris, M. Alcalay, U. Cavallaro, F. Orsenigo, M.G. Lampugnani, E. Dejana, Overlapping and divergent signaling pathways of N-cadherin and VE-cadherin in endothelial cells, Blood 119 (2012) 2159–2170, https://doi.org/10.1182/BLOOD-2011-09-381012.

[54] C.R.M. Rieder, R.B. Parsons, N.J.S. Fitch, A.C. Williams, D.B. Ramsden, Human brain cytochrome P450 1B1: immunohistochemical localization in human temporal lobe and induction by dimethylbenz(a)anthracene in astrocytoma cell line (MOG-G-CCM), Neurosci. Lett. 278 (2000) 177–180, https://doi.org/10.1016/S0304-3940(99)00932-5.

[55] P.P. Partyka, G.A. Godsey, J.R. Galie, M.C. Kosciuk, N.K. Acharya, R.G. Nagele, P.A. Galie, Mechanical stress regulates transport in a compliant 3D model of the blood-brain barrier, Biomaterials 115 (2017) 30–39, https://doi.org/10.1016/J.BIOMATERIALS.2016.11.012.

[56] Y. Xu, Q. He, M. Wang, X. Wang, F. Gong, L. Bai, J. Zhang, W. Wang, Quantifying blood-brain-barrier leakage using a combination of evans blue and high molecular weight FITC-Dextran, J. Neurosci. Methods. 325 (2019), https://doi.org/10.1016/J.JNEUMETH.2019.108349.

[57] H. Kubotera, H. Ikeshima-Kataoka, Y. Hatashita, A.L.A. Mascaro, F.S. Pavone, T. Inoue, Astrocytic endfeet re-cover blood vessels after removal by laser ablation, Sci. Reports 2019 91. 9 (2019) 1–10. 10.1038/s41598-018-37419-4.

[58] Y.I. Wang, H.E. Abaci, M.L. Shuler, Microfluidic blood–brain barrier model provides in vivo-like barrier properties for drug permeability screening, Biotechnol. Bioeng. 114 (2017) 184–194, https://doi.org/10.1002/bit.26045.

[59] K. Matter, M.S. Balda, Functional analysis of tight junctions, Methods 30 (2003) 228–234, https://doi.org/10.1016/S1046-2023(03)00029-X.

[60] T. Ohnishi, I. Tamai, K. Sakanaka, A. Sakata, T. Yamashima, J. Yamashita, A. Tsuji, In vivo and in vitro evidence for ATP-dependency of P-glycoprotein-mediated efflux of doxorubicin at the blood-brain barrier, Biochem. Pharmacol. 49 (1995) 1541–1544, https://doi.org/10.1016/0006-2952(95)00082-B.

[61] A.L. McCall, W.R. Millington, R.J. Wurtman, Blood-brain barrier transport of caffeine: Dose-related restriction of adenine transport, Life Sci. 31 (1982) 2709–2715, https://doi.org/10.1016/0024-3205(82)90715-9.

[62] L. Shi, M. Zeng, Y. Sun, B.M. Fu, Quantification of blood-brain barrier solute permeability and brain transport by multiphoton microscopy, J. Biomech. Eng. 136 (2014), https://doi.org/10.1115/1.4025892.

[63] A. Avdeef, Absorption and Drug Development, (2012) 742. https://www.wiley.com/en-us/Absorption+and+Drug+Development%3A+Solubility%2C+Permeability%2C+and+Charge+State%2C+2nd+Edition-p-9781118057452 (accessed December 12, 2021).

[64] S.K. Mahto, T.H. Yoon, S.W. Rhee, A new perspective on in vitro assessment method for evaluating quantum dot toxicity by using microfluidics technology, Biomicrofluidics. 4 (2010), https://doi.org/10.1063/1.3486610.

[65] C.D. Arvanitis, V. Askoxylakis, Y. Guo, M. Datta, J. Kloepper, G.B. Ferraro, M.O. Bernabeu, D. Fukumura, N. McDannold, R.K. Jain, Mechanisms of enhanced drug delivery in brain metastases with focused ultrasound-induced blood–tumor barrier disruption, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E8717–E8726, https://doi.org/10.1073/PNAS.1807105115.

[66] Y.J. Ho, T.C. Wang, C.H. Fan, C.K. Yeh, Spatially Uniform Tumor Treatment and Drug Penetration by Regulating Ultrasound with Microbubbles, ACS Appl. Mater. Interfaces. 10 (2018) 17784–17791, https://doi.org/10.1021/ACSAMI.8B05508.

[67] S. Tardoski, E. Gineyts, J. Ngo, A. Kocot, P. Clézardin, D. Melodelima, Low-Intensity Ultrasound Promotes Clathrin-Dependent Endocytosis for Drug Penetration into Tumor Cells, Ultrasound Med. Biol. 41 (2015) 2740–2754, https://doi.org/10.1016/J.ULTRASMEDBIO.2015.06.006.

[68] C.Y. Lai, B.Z. Fite, K.W. Ferrara, Ultrasonic enhancement of drug penetration in solid tumors, Front. Oncol. 3 AUG (2013) 204, https://doi.org/10.3389/FONC.2013.00204.

[69] K.F. Timbie, B.P. Mead, R.J. Price, Drug and gene delivery across the blood-brain barrier with focused ultrasound, J. Control. Release. 219 (2015) 61–75, https://doi.org/10.1016/J.JCONREL.2015.08.059.

[70] M.A. Odenwald, J.R. Turner, The intestinal epithelial barrier: a therapeutic target? Nat. Rev. Gastroenterol. Hepatol. 2016 141. 14 (2016) 9–21. 10.1038/nrgastro.2016.169.

[71] L.A. Keller, O. Merkel, A. Popp, Intranasal drug delivery: opportunities and toxicologic challenges during drug development, Drug Deliv. Transl. Res. 2021 (2021) 1–23, https://doi.org/10.1007/S13346-020-00891-5.

[72] J. Xu, J. Tao, J. Wang, Design and Application in Delivery System of Intranasal Antidepressants, Front. Bioeng. Biotechnol. 8 (2020) 1450, https://doi.org/10.3389/FBIOE.2020.626882.

[73] A.Z. Alkilani, M.T.C. McCrudden, R.F. Donnelly, Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the Stratum Corneum, Pharm. 2015, Vol. 7, Pages 438-470. 7 (2015) 438–470. 10.3390/PHARMACEUTICS7040438.

[74] D.K. Mishra, V. Pandey, R. Maheshwari, P. Ghode, R.K. Tekade, Cutaneous and Transdermal Drug Delivery: Techniques and Delivery Systems, Basic Fundam. Drug Deliv. (2019) 595–650, https://doi.org/10.1016/B978-0-12-817909-3.00015-7.

[75] G.J. Mahler, M.B. Esch, R.P. Glahn, M.L. Shuler, Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity, Biotechnol. Bioeng. 104 (2009) 193–205, https://doi.org/10.1002/BIT.22366.

[76] M. Boegh, H.M. Nielsen, Mucus as a Barrier to Drug Delivery – Understanding and Mimicking the Barrier Properties, Basic Clin. Pharmacol. Toxicol. 116 (2015) 179–186, https://doi.org/10.1111/bcpt.12342.

[77] J. Dekker, W.M.O. Van Beurden-Lamers, A. Oprins, G.J. Strous, Isolation and structural analysis of rat gastric mucus glycoprotein suggests a homogeneous protein backbone, Biochem. J. 260 (1989) 717, https://doi.org/10.1042/BJ2600717.

[78] K. Pocock, L. Delon, V. Bala, S. Rao, C. Priest, C. Prestidge, B. Thierry, Intestine-on-A-Chip Microfluidic Model for Efficient in Vitro Screening of Oral Chemotherapeutic Uptake, ACS Biomater. Sci. Eng. 3 (2017) 951–959, https://doi.org/10.1021/ACSBIOMATERIALS.7B00023.

[79] T.M. Marin, N. de Carvalho Indolfo, S.A. Rocco, F.L. Basei, M. de Carvalho, K. de Almeida Gonçalves, E. Pagani, Acetaminophen absorption and metabolism in an intestine/liver microphysiological system, Chem. Biol. Interact. 299 (2019) 59–76. 10.1016/J.CBI.2018.11.010.

[80] M.J.C. Santbergen, M. van der Zande, A. Gerssen, H. Bouwmeester, M.W.F. Nielen, Dynamic in vitro intestinal barrier model coupled to chip-based liquid chromatography mass spectrometry for oral bioavailability studies, Anal. Bioanal. Chem. 412 (2020) 1111–1122, https://doi.org/10.1007/S00216-019-02336-6.

[81] K. Kulthong, G.J.E.J. Hooiveld, L. Duivenvoorde, I. Miro Estruch, V. Marin, M. van der Zande, H. Bouwmeester, Transcriptome comparisons of in vitro intestinal epithelia grown under static and microfluidic gut-on-chip conditions with in vivo human epithelia, Sci. Reports 2021 111. 11 (2021) 1–13. 10.1038/s41598-021-82853-6.

[82] H.J. Kim, D. Huh, G. Hamilton, D.E. Ingber, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, Lab Chip. 12 (2012) 2165–2174, https://doi.org/10.1039/C2LC40074J.

[83] General Principles of Gastrointestinal Function — Motility, Nervous Control, and Blood Circulation, Pocket Companion to Guyt. Hall Textb. Med. Physiol. (2015) 477–483. 10.1016/B978-1-4160-5451-1.00062-1.

[84] J.B. Dixon, S. Raghunathan, M.A. Swartz, A tissue-engineered model of the intestinal lacteal for evaluating lipid transport by lymphatics, Biotechnol. Bioeng. 103 (2009) 1224–1235, https://doi.org/10.1002/BIT.22337.

[85] P. Viswanathan, Y. Muralidaran, G. Ragavan, Challenges in oral drug delivery: a nano-based strategy to overcome, Nanostructures Oral Med. (2017) 173–201, https://doi.org/10.1016/B978-0-323-47720-8.00008-0.

[86] K. Ronaldson-Bouchard, G. Vunjak-Novakovic, Organs-on-a-Chip: A Fast Track for Engineered Human Tissues in Drug Development, Cell Stem Cell 22 (2018) 310–324, https://doi.org/10.1016/J.STEM.2018.02.011.

[87] A. Bein, W. Shin, S. Jalili-Firoozinezhad, M.H. Park, A. Sontheimer-Phelps, A. Tovaglieri, A. Chalkiadaki, H.J. Kim, D.E. Ingber, Microfluidic Organ-on-a-Chip Models of Human Intestine, Cell. Mol. Gastroenterol. Hepatol. 5 (2018) 659–668, https://doi.org/10.1016/J.JCMGH.2017.12.010.

[88] H.J. Kim, H. Li, J.J. Collins, D.E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, Proc. Natl. Acad. Sci. USA 113 (2016) E7–E15, https://doi.org/10.1073/PNAS.1522193112.

[89] C. Beaurivage, E. Naumovska, Y.X. Chang, E.D. Elstak, A. Nicolas, H. Wouters, G. van Moolenbroek, H.L. Lanz, S.J. Trietsch, J. Joore, P. Vulto, R.A.J. Janssen, K. S. Erdmann, J. Stallen, D. Kurek, Development of a Gut-on-a-Chip Model for High Throughput Disease Modeling and Drug Discovery, Int. J. Mol. Sci. 2019, Vol. 20, Page 5661. 20 (2019) 5661. 10.3390/IJMS20225661.

[90] H.Y. Tan, S. Trier, U.L. Rahbek, M. Dufva, J.P. Kutter, T.L. Andresen, A multi-chamber microfluidic intestinal barrier model using Caco-2 cells for drug transport studies, PLoS ONE 13 (2018), https://doi.org/10.1371/JOURNAL.PONE.0197101 e0197101.

[91] K.Y. Chumbimuni-Torres, R.E. Coronado, A.M. Mfuh, C. Castro-Guerrero, M.F. Silva, G.R. Negrete, R. Bizios, C.D. Garcia, Adsorption of proteins to thin-films of PDMS and its effect on the adhesion of human epithelial cells, RSC Adv. 1 (2011) 706–714, https://doi.org/10.1039/C1RA00198A/.

[92] H. Sun, E.C.Y. Chow, S. Liu, Y. Du, K.S. Pang, The Caco-2 cell monolayer: usefulness and limitations 4 (2008) 395–411. 10.1517/17425255.4.4.395.

[93] S. Parry, F.G. Hanisch, S.H. Leir, M. Sutton-Smith, H.R. Morris, A. Dell, A. Harris, N-Glycosylation of the MUC1 mucin in epithelial cells and secretions, Glycobiology 16 (2006) 623–634, https://doi.org/10.1093/GLYCOB/CWJ110.

[94] K.K. Lee, H.A. McCauley, T.R. Broda, M.J. Kofron, J.M. Wells, C.I. Hong, Human stomach-on-a-chip with luminal flow and peristaltic-like motility, Lab Chip. 18 (2018) 3079–3085, https://doi.org/10.1039/C8LC00910D.

[95] M.J. Workman, J.P. Gleeson, E.J. Troisi, H.Q. Estrada, S.J. Kerns, C.D. Hinojosa, G.A. Hamilton, S.R. Targan, C.N. Svendsen, R.J. Barrett, Enhanced Utilization of Induced Pluripotent Stem Cell-Derived Human Intestinal Organoids Using Microengineered Chips, Cell. Mol. Gastroenterol. Hepatol. 5 (2017) 669–677. e2, https://doi.org/10.1016/J.JCMGH.2017.12.008.

[96] M. Kasendra, R. Luc, J. Yin, D.V. Manatakis, G. Kulkarni, C. Lucchesi, J. Sliz, A. Apostolou, L. Sunuwar, J. Obrigewitch, K.J. Jang, G.A. Hamilton, M. Donowitz, K. Karalis, Duodenum Intestine-Chip for preclinical drug assessment in a human relevant model, Elife. 9 (2020), https://doi.org/10.7554/ELIFE.50135.

[97] L. Elberskirch, T. Knoll, A. Moosmann, N. Wilhelm, H. von Briesen, S. Wagner, A novel microfluidic mucus-chip for studying the permeation of compounds over the mucus barrier, J. Drug Deliv. Sci. Technol. 54 (2019), https://doi.org/10.1016/J.JDDST.2019.101248.

[98] X. Li, D. Chen, C. Le, C. Zhu, Y. Gan, L. Hovgaard, M. Yang, Novel mucus-penetrating liposomes as a potential oral drug delivery system: preparation, in vitro characterization, and enhanced cellular uptake, Int. J. Nanomedicine. 6 (2011) 3151–3162, https://doi.org/10.2147/IJN.S25741.

[99] D. Kim, Y.H. Kim, S. Kwon, Enhanced nasal drug delivery efficiency by increasing mechanical loading using hypergravity, Sci. Reports 2017 81. 8 (2018) 1–8. 10.1038/s41598-017-18561-x.

[100] D.C.S. Lio, D.C.S. Lio, D.C.S. Lio, R.N. Chia, M.S.Y. Kwek, M.S.Y. Kwek, C. Wiraja, L.E. Madden, L.E. Madden, H. Chang, S.M.A. Khadir, X. Wang, X. Wang, X. Wang, X. Wang, D.L. Becker, D.L. Becker, C. Xu, C. Xu, C. Xu, C. Xu, Temporal pressure enhanced topical drug delivery through micropore formation, Sci. Adv. 6 (2020), https://doi.org/10.1126/SCIADV.AAZ6919.

[101] S. Lee, D.J. McAuliffe, T.J. Flotte, N. Kollias, A.G. Doukas, Photomechanical transcutaneous delivery of macromolecules, J. Invest. Dermatol. 111 (1998) 925–929, https://doi.org/10.1046/J.1523-1747.1998.00415.X.

[102] A.G. Doukas, N. Kollias, Transdermal drug delivery with a pressure wave, Adv. Drug Deliv. Rev. 56 (2004) 559–579, https://doi.org/10.1016/J.ADDR.2003.10.031.

[103] C.D. DiDomenico, L.J. Bonassar, The Effect of Charge and Mechanical Loading on Antibody Diffusion Through the Articular Surface of Cartilage, J. Biomech. Eng. 141 (2019), https://doi.org/10.1115/1.4041768/424335.

[104] A.G. Bajpayee, A.J. Grodzinsky, Cartilage-targeting drug delivery: can electrostatic interactions help?, Nat. Rev. Rheumatol. 2017 133. 13 (2017) 183–193. 10.1038/nrrheum.2016.210.

[105] L.J. Bonassar, A.J. Grodzinsky, E.H. Frank, S.G. Davila, N.R. Bhaktav, S.B. Trippel, The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I, J. Orthop. Res. 19 (2001) 11–17, https://doi.org/10.1016/S0736-0266(00)00004-8.

[106] E.G. Lima, L. Bian, K.W. Ng, R.L. Mauck, B.A. Byers, R.S. Tuan, G.A. Ateshian, C.T. Hung, The beneficial effect of delayed compressive loading on tissue-engineered cartilage constructs cultured with TGF-beta3, Osteoarthr. Cartil. 15 (2007) 1025–1033, https://doi.org/10.1016/J.JOCA.2007.03.008.

[107] C.Y. Huang, Y.G. Wei, Effects of Tension-Compression Nonlinearity on Solute Transport in Charged Hydrated Fibrous Tissues Under Dynamic Unconfined Compression, J. Biomech. Eng. 129 (2007) 423–429, https://doi.org/10.1115/1.2720920.

[108] P.I.K. Wu, E.R. Edelman, Structural biomechanics modulate intramuscular distribution of locally delivered drugs, J. Biomech. 41 (2008) 2884–2891, https://doi.org/10.1016/J.JBIOMECH.2008.06.025.

[109] P.I. Wu, S. Minisini, E.R. Edelman, Intramuscular drug transport under mechanical loading: resonance between tissue function and uptake, J. Control. Release. 136 (2009) 99–109, https://doi.org/10.1016/J.JCONREL.2009.01.016.

[110] M.A. Lovich, A.E. Wei, M.Y. Maslov, P.I. Wu, E.R. Edelman, Local epicardial inotropic drug delivery allows targeted pharmacologic intervention with preservation of myocardial loading conditions, J. Pharm. Sci. 100 (2011) 4993–5006, https://doi.org/10.1002/JPS.22681.

[111] J. Ihlemann, T. Ploug, H. Galbo, Effect of force development on contraction induced glucose transport in fast twitch rat muscle, Acta Physiol. Scand. 171 (2001) 439–444, https://doi.org/10.1046/J.1365-201X.2001.00807.X.

[112] K.A. Abraham, R.L. Terjung, Phosphate uptake in rat skeletal muscle is reduced during isometric contractions, J. Appl. Physiol. 97 (2004) 57–62, https://doi.org/10.1152/JAPPLPHYSIOL.01294.2003.

[113] H. Gissel, T. Clausen, Excitation-induced Ca²⁺ influx in rat soleus and EDL muscle: Mechanisms and effects on cellular integrity, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 279 (2000), https://doi.org/10.1152/AJPREGU.2000.279.3.R917.

[114] A.J. Primeau, A. Rendon, D. Hedley, L. Lilge, I.F. Tannock, The distribution of the anticancer drug Doxorubicin in relation to blood vessels in solid tumors, Clin. Cancer Res. 11 (2005) 8782–8788, https://doi.org/10.1158/1078-0432.CCR-05-1664.

[115] Q. Chang, O.I. Ornatsky, I. Siddiqui, R. Straus, V.I. Baranov, D.W. Hedley, Biodistribution of cisplatin revealed by imaging mass cytometry identifies extensive collagen binding in tumor and normal tissues, Sci. Reports 2016 61. 6 (2016) 1–11. 10.1038/srep36641.

[116] C.M. Ghajar, X. Chen, J.W. Harris, V. Suresh, C.C.W. Hughes, N.L. Jeon, A.J. Putnam, S.C. George, The effect of matrix density on the regulation of 3-D capillary morphogenesis, Biophys. J. 94 (2008) 1930–1941, https://doi.org/10.1529/BIOPHYSJ.107.120774.

[117] R.W. Sirianni, J. Kremer, I. Guler, Y.L. Chen, F.W. Keeley, W.M. Saltzman, Effect of extracellular matrix elements on the transport of paclitaxel through an arterial wall tissue mimic, Biomacromolecules 9 (2008) 2792–2798, https://doi.org/10.1021/BM800571S.

[118] O. Trédan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug Resistance and the Solid Tumor Microenvironment, JNCI J. Natl. Cancer Inst. 99 (2007) 1441–1454, https://doi.org/10.1093/JNCI/DJM135.

[119] P.A. Netti, D.A. Berk, M.A. Swartz, A.J. Grodzinsky, R.K. Jain, Role of Extracellular Matrix Assembly in Interstitial Transport in Solid Tumors 1, Cancer Res. 60 (2000) 2497–2503.

[120] J.K. Saggar, M. Yu, Q. Tan, I.F. Tannock, The tumor microenvironment and strategies to improve drug distribution, Front. Oncol. 3 (2013) 154, https://doi.org/10.3389/FONC.2013.00154.

[121] L. Eikenes, Ø.S. Bruland, C. Brekken, C. De Lange Davies, Collagenase Increases the Transcapillary Pressure Gradient and Improves the Uptake and Distribution of Monoclonal Antibodies in Human Osteosarcoma Xenografts, Cancer Res. 64 (2004) 4768–4773, https://doi.org/10.1158/0008-5472.CAN-03-1472.

[122] M.A. Jacobetz, D.S. Chan, A. Neesse, T.E. Bapiro, N. Cook, K.K. Frese, C. Feig, T. Nakagawa, M.E. Caldwell, H.I. Zecchini, M.P. Lolkema, P. Jiang, A. Kultti, C.B. Thompson, D.C. Maneval, D.I. Jodrell, G.I. Frost, H.M. Shepard, J.N. Skepper, D. A. Tuveson, Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer, Gut 62 (2013) 112–120, https://doi.org/10.1136/GUTJNL-2012-302529.

[123] C.B. Thompson, H.M. Shepard, P.M. O'Connor, S. Kadhim, P. Jiang, R.J. Osgood, L.H. Bookbinder, X. Li, B.J. Sugarman, R.J. Connor, S. Nadjsombati, G.I. Frost, Enzymatic Depletion of Tumor Hyaluronan Induces Antitumor Responses in Preclinical Animal Models, Mol. Cancer Ther. 9 (2010) 3052–3064, https://doi.org/10.1158/1535-7163.MCT-10-0470.

[124] A. Albanese, A.K. Lam, E.A. Sykes, J. V. Rocheleau, W.C.W. Chan, Tumour-on-a-chip provides an optical window into nanoparticle tissue transport, Nat. Commun. 2013 41. 4 (2013) 1–8. 10.1038/ncomms3718.

[125] A. Tchoryk, V. Taresco, R.H. Argent, M. Ashford, P.R. Gellert, S. Stolnik, A. Grabowska, M.C. Garnett, Penetration and uptake of nanoparticles in 3D tumor spheroids, Bioconjug. Chem. 30 (2019) 1371–1384, https://doi.org/10.1021/ACS.BIOCONJCHEM.9B00136.

[126] M.W. Dewhirst, T.W. Secomb, Transport of drugs from blood vessels to tumour tissue, Nat. Rev. Cancer 2017 1712. 17 (2017) 738–750. 10.1038/nrc.2017.93.

[127] T. Stylianopoulos, L.L. Munn, R.K. Jain, Reengineering the Physical Microenvironment of Tumors to Improve Drug Delivery and Efficacy: From

Mathematical Modeling to Bench to Bedside, Trends Cancer. 4 (2018) 292–319, https://doi.org/10.1016/J.TRECAN.2018.02.005.

[128] C.F. Guimarães, L. Gasperini, A.P. Marques, R.L. Reis, The stiffness of living tissues and its implications for tissue engineering, Nat. Rev. Mater. 2020 55. 5 (2020) 351–370. 10.1038/s41578-019-0169-1.

[129] B. Piersma, M.K. Hayward, V.M. Weaver, Fibrosis and cancer: A strained relationship, Biochim. Biophys. Acta. Rev. Cancer. 1873 (2020), https://doi.org/10.1016/J.BBCAN.2020.188356.

[130] J. Schrader, T.T. Gordon-Walker, R.L. Aucott, M. van Deemter, A. Quaas, S. Walsh, D. Benten, S.J. Forbes, R.G. Wells, J.P. Iredale, Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells, Hepatology 53 (2011) 1192–1205, https://doi.org/10.1002/HEP.24108.

[131] X. Qin, X. Lv, P. Li, R. Yang, Q. Xia, Y. Chen, Y. Peng, L. Li, S. Li, T. Li, Y. Jiang, H. Yang, C. Wu, C. Zheng, J. Zhu, F. You, H. Wang, J. Chen, Y. Liu, Matrix stiffness modulates ILK-mediated YAP activation to control the drug resistance of breast cancer cells, Biochim. Biophys. Acta. Mol. Basis Dis. 1866 (2020), https://doi.org/10.1016/J.BBADIS.2019.165625.

[132] C.-H. Lin, F.A. Pelissier, H. Zhang, J. Lakins, V.M. Weaver, C. Park, M.A. LaBarge, K. Luo, Microenvironment rigidity modulates responses to the HER2 receptor tyrosine kinase inhibitor lapatinib via YAP and TAZ transcription factors, Mol. Biol. Cell. 26 (22) (2015) 3946–3953.

[133] S.H. Medina, B. Bush, M. Cam, E. Sevcik, F.W. DelRio, K. Nandy, J.P. Schneider, Identification of a mechanogenetic link between substrate stiffness and chemotherapeutic response in breast cancer, Biomaterials 202 (2019) 1–11, https://doi.org/10.1016/J.BIOMATERIALS.2019.02.018.

[134] J.W. Shin, D.J. Mooney, Extracellular matrix stiffness causes systematic variations in proliferation and chemosensitivity in myeloid leukemias, Proc. Natl. Acad. Sci. USA 113 (2016) 12126–12131, https://doi.org/10.1073/PNAS.1611338113.

[135] T.V. Nguyen, M. Sleiman, T. Moriarty, W.G. Herrick, S.R. Peyton, Sorafenib resistance and JNK signaling in carcinoma during extracellular matrix stiffening, Biomaterials 35 (2014) 5749–5759, https://doi.org/10.1016/J.BIOMATERIALS.2014.03.058.

[136] E.Y. Tokuda, J.L. Leight, K.S. Anseth, Modulation of matrix elasticity with PEG hydrogels to study melanoma drug responsiveness, Biomaterials 35 (2014) 4310–4318, https://doi.org/10.1016/J.BIOMATERIALS.2014.01.063.

[137] M. Deng, J. Lin, S. Nowsheen, T. Liu, Y. Zhao, P.W. Villalta, D. Sicard, D.J. Tschumperlin, S.B. Lee, J.J. Kim, Z. Lou, Extracellular matrix stiffness determines DNA repair efficiency and cellular sensitivity to genotoxic agents, Sci. Adv. 6 (2020), https://doi.org/10.1126/SCIADV.ABB2630.

[138] A. Abramson, M.R. Frederiksen, A. Vegge, B. Jensen, M. Poulsen, B. Mouridsen, M.O. Jespersen, R.K. Kirk, J. Windum, F. Hubálek, J.J. Water, J. Fels, S.B. Gunnarsson, A. Bohr, E.M. Straarup, M.W.H. Ley, X. Lu, J. Wainer, J. Collins, S. Tamang, K. Ishida, A. Hayward, P. Herskind, S.T. Buckley, N. Roxhed, R. Langer, U. Rahbek, G. Traverso, Oral delivery of systemic monoclonal antibodies, peptides and small molecules using gastric auto-injectors, Nat. Biotechnol. 2021 (2021) 1–7, https://doi.org/10.1038/s41587-021-01024-0.