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Organ-on-a-Chip Platforms for Studying Drug Delivery Systems

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Abstract

Novel microfluidic tools allow new ways to manufacture and test drug delivery systems. Organ-on-a-chip systems – microscale recapitulations of complex organ functions – promise to improve the drug development pipeline. This review highlights the importance of integrating microfluidic networks with 3D tissue engineered models to create organ-on-a-chip platforms, able to meet the demand of creating robust preclinical screening models. Specific examples are cited to demonstrate the use of these systems for studying the performance of drug delivery vectors and thereby reduce the discrepancies between their performance at preclinical and clinical trials. We also highlight the future directions that need to be pursued by the research community for these proof-of-concept studies to achieve the goal of accelerating clinical translation of drug delivery nanoparticles.

1. Introduction

The rapidly developing field of nanomedicine can significantly impact human disease therapy [1, 2]. The research progress accomplished in this field, over the last few decades,

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has led to the development of nanomaterials, useful for designing carriers that deliver therapeutic payload to diseased cells. An ideal drug delivery system should be easy to manufacture and scale-up, low cost, biocompatible, biodegradable, possess a high drug loading capacity and be targeted to the site-of-interest in the body. Nanocarriers, also routinely referred to as nanoparticles, are a class of drug delivery systems that range in size from about 50 to 200 nm, allowing them to efficiently translocate across the cell membrane barrier.

From a therapeutic standpoint, nanocarriers can prolong the systemic circulation time of the drug and significantly reduce adverse side effects caused by off-target delivery at healthy tissue sites. This controlled release of drugs reduces the magnitude of overall drug exposure required for a therapeutic effect, thus avoiding higher drug doses and consequent adverse effects. A wide variety of drugs, including hydrophobic and hydrophilic small molecules, as well as biomacromolecules, can be encapsulated within nanoparticles by tailoring the chemistry of nanomaterials, polymeric or inorganic/metallic, to achieve the desired encapsulation capability and release kinetics. The first use of nano-scale systems for drug delivery was reported in the 1970s, when liposomal Trojan horse nanoparticles were used for treating lysosomal storage disease [3, 4]. Nanoparticles have also been developed as diagnostic agents to enhance the sensitivity for imaging techniques, including X-ray computed tomography (CT) and magnetic resonance imaging (MRI). An increase in available techniques to engineer more precise and sophisticated nanomaterials, and a deeper understanding of disease biology has catapulted a new generation of nanotherapeutics with improved properties.

The above-mentioned advantages make nanoscale drug delivery systems appealing to the pharmaceutical companies and healthcare regulatory agencies. However, in spite of these rapid bench-side developments, the translation of therapeutic nanoparticles to the commercial pipeline has been less impressive [5]. Very few systems have been approved by the Food and Drug Administration (FDA), including Doxil, a liposomal formulation encapsulating the chemotherapeutic drug Doxorubicin, and Abraxane, based on the nanoparticle albumin-bound (nab) technology to deliver Paclitaxel, a widely used drug for breast and pancreatic câncer [6]. This slow pace of bench-to-bedside translation can be attributed to several challenges, the most critical being the lack of robust preclinical tissue culture platforms that can mimic *in vivo* conditions and predict the performance of these nanoparticles within the human body.

The development of microfluidic platforms for nanoparticle synthesis has shown to overcome several disadvantages of the traditional bulk synthesis methods such as scalability and batch-to-batch variability [7–9]. Microfluidic approaches have also been used as a tool for more sophisticated, faster and highly efficient characterization of the biophysical properties of nanoparticles [10, 11]. Additionally, the application of microfabrication techniques to tissue engineering aided in the creation of physiologically relevant disease models. Establishment of these techniques has paved the way for robust advances in tissue culture systems integrated with microfluidic networks [12]. More recently, the demand for high-throughput drug screening platforms with better preclinical predictability has translated into major developments in organ-on-a-chip systems [13–15]. This review presents recent

advances in *in vitro* tissue culture models by primarily emphasizing on organ-on-a-chip platforms useful for studying the performance of drug delivery nanotherapeutics. The current challenges in the development of drug delivery systems are highlighted and the use of organ-on-chips as a potential solution is discussed by presenting specific examples of relevant proof-of-concept studies.

2. Limitations of Current Culture Platforms used for Developing Drug Delivery Systems

Several parameters need to be studied for developing nanoparticles for clinical use. These include studying the fate of the nanoparticles inside the body and its toxicological effects, the mode of binding and internalization at the cellular level, the stability of the nanoparticles with respect to various physical and chemical conditions of the body, and, most importantly, the efficacy when compared to free drugs [5]. Large-batch synthesis, toxicity assessment and efficacy screening are the major levels at which clinical translation of nanotherapeutics faces set-back [16]. On the manufacturing front, scaling the small lab synthesis techniques to the large-scale production of nanoparticles has been challenging for the pharmaceutical companies [8]. Meanwhile, screening for the toxicity and efficacy suffers from the paucity of preclinical models that would robustly predict the nanoparticles' behavior inside the human body [16]. For simultaneous evaluation of the above-mentioned parameters, predictive *in vitro* platforms are essential while developing drug delivery vectors [13, 17].

The current gold standard for preclinical testing of nanotherapeutics is *in vivo* studies. These do not accurately predict human responses due to inter-species difference in genetic makeup, along with being extremely time-consuming, expensive, low-throughput and raising ethical concerns. The resolution for whole-animal imaging methods is limited, hindering visualization during transport of the theranostic agents in the target tissue. Being unable to reproduce its preclinical performance, many drug delivery systems which pass the preclinical phase fail to address the toxicity and efficacy effects when compared to their free drugs counterparts in human clinical trials [5]. Strikingly, the main reason cited for this effect is the use of animal models for optimization during drug carrier design [5], which brings back the obvious drawback of a certain degree of physiological irrelevance between human and animal models.

Animal models need to be complemented with sophisticated *in vitro* platforms to fill this gap. In current *in vitro* studies, drug delivery carriers are commonly tested in two-dimensional (2D) monolayer cell culture models. These 2D cultures involve growing on top of a flat substrate (*e.g.*, glass or polystyrene) a monolayer of single or multiple cell types that are either freshly isolated from human/animal tissues (primary cells) or are already established, immortalized cell lines. In these setups, drug delivery systems are usually mixed with culture media and directly applied on the cell monolayers, after which cellular responses are recorded. Among several published studies [18–21], the work of Xia and colleagues on cellular uptake of gold nanoparticles (AuNPs) by SK-BR-3 breast tumor cells [22], stands out by devising a novel testing method. After culturing the cells on a piece of glass, the substrate was carefully reversed and placed upside down before AuNPs, with different shapes and sizes, were added in the culture media. Such an approach successfully

avoided the issues caused by rapid sedimentation of nanoparticles. Indeed, the amount of cellular uptake of nanoparticles in upright and inverted cultures was found to significantly depend on the rate of diffusion/sedimentation of the nanoparticles.

In spite of these novel approaches for 2D cell culture, it is gradually realized that there are many shortcomings with these “flat” models to mimic the complex three-dimensional (3D) *in vivo* microenvironment, wherein the cells and extracellular matrix (ECM) exist in well-organized architectures. Moreover, the nanoparticle delivery efficacy differs considerably between 2D and 3D culture platforms [23]. Primary cells usually have a limited lifespan, undergo rapid phenotypic alterations, and show large variability over different batches of isolation; on the other hand, although established cell lines are more stable, many times they do not present genuine tissue-specific functions [24]. In this regard, efforts were shifted towards developing multiple 3D culture systems that can better recapitulate *in vivo* tissue functions. Multicellular spheroids are important 3D models for researchers [25–29]. These spheroids are formed by spontaneous aggregation of multiple cells held together by ECM secreted by residing cells. The apoptotic/necrotic core of the spheroids contrasts with the proliferative cell layers on the periphery, providing a better mimic of *in vivo* tumor environment. Due to the importance and long-time usage of multicellular spheroids in both pharmaceutical studies and regenerative medicine, researchers have developed sophisticated methods that allow efficient fabrication of uniform spheroids at relatively large scales, including the use of hanging drops, non-adhesive microwells, rotation cultures, or 3D porous scaffolds [30–37]. Multicellular tumor cylindroids have been used to study the effect of charge on the uptake of fluorescein isothiocyanate (FITC) or doxorubicin (DOX)-conjugated AuNPs loaded with drugs, where diffusion is permitted only from the periphery to the center [38]. Kotov and co-workers directly utilized tumor spheroids for toxicity testing of CdTe quantum dots and AuNPs [39]. The toxic effects of these nanoparticles was compared with conventional 2D cultures, to reveal different responses of cells in terms of morphology, particle distribution, membrane integrity, mitochondrial activity, and apoptosis (Fig. 1).

Besides multicellular spheroids, hydrogels and porous scaffolds have also been widely employed for constructing 3D tissue models at larger size scales [40–42]. There are a number of advantages associated with 3D cultures within a matrix. For example, the mechanical properties of the gels can be precisely modulated, which have been shown to determine the phenotypic behaviors of the cells [43–45]; the matrices can be fabricated to possess various hierarchical structures and any desired shape to accommodate specific target tissues. As an example, Huang and co-workers demonstrated that cancer cells became more tumorigenic when cultured in a fibrin gel with a stiffness of approximately 90 Pa, as shown by *in vivo* tumor formation in mice even when only very few (10 or 100) tumor cells were injected, whereas the same number of tumor cells from stiff 2D substrates could not induce the formation of tumors [46]. Moreover, Mooney *et al.* cultured OSCC-3 oral squamous carcinoma cells within porous poly(lactide-*co*-glycolide) (PLG) scaffolds to create an *in vitro* tumor model [47]. They argued that tumor cells cultured in PLG scaffolds could better recapitulate their *in vivo* states than in 3D Matrigel or 2D substrates as shown by their morphological appearances, proliferation rates, distribution of oxygen concentrations, and secretion patterns of biomolecules.

Although static culture systems based on multicellular spheroids or 3D matrices can recapitulate the *in vivo* functionality of tissues much better than 2D cultures, they fail to present dynamic flow conditions that the cells usually experience in the body. The absence of homogenous perfusion results in improper gas and nutrient exchange through the core of the constructs. Additionally, the gravitational settling of nanoparticles in static conditions affects the outcome of dosage optimization studies. Evaluating the drug carrier in static *in vitro* assays without considering mechanical forces and fluid flow has shown instances of false positives [13, 17, 22].

Hence, perfusion-based dynamic culture systems in bioreactors have become increasingly popular. At the same time, owing to the rapid development of microfluidics, the size of these bioreactors is being constantly reduced while the functionality is improved, enabling the development of improved systems for drug toxicity and efficacy studies [48–52]. For example, Lee and co-workers constructed microfluidic chambers containing arrays of hydrodynamic traps, which was used to immobilize around ten tumor cells per trap (Fig. 2A). The entrapped tumor cells aggregated in the traps to form small spheroids (<50 μm) within several hours (Fig. 2B,C) [53]. Similarly, Cheung and co-workers developed a type of cell culture chip with arrays of microsieves for efficient trapping of larger cell aggregates [54]. Treating the multicellular spheroids of LCC6/Her2 breast tumor cells in the microsieves with increasing concentrations of DOX, resulted in a reduction of the size of spheroid and viability of the cells. In addition a microfluidic platform to study the interaction of nanoparticle-aptamer conjugates with prostate cancer cells that expressed (LNCaP) or did not express (PC3) prostate specific surface antigen (PSMA) was developed [11]. For LNCaP cells, the binding of nanoparticles was higher in presence of aptamer and decreased with increasing flow rate, whereas in the case of PC3 the nanoparticles did not bind with or without aptamers (Fig. 3). Such advanced microfluidic culture models, where essentially any form of cell/tissue constructs (*e.g.*, spheroids, hydrogels, and porous scaffolds) can be directly utilized on-chip for testing, can potentially be used for cytotoxicity and efficacy assessment of drug delivery systems.

3. Organ-on-a-Chip Platforms as a Potential Solution

Recent innovations in microfluidic technologies can provide novel synthesis and screening methods to address issues of scalability, batch-to-batch variability, and poor predictability that limit the pace of clinical translation of nanotherapeutics [16]. The integration of advanced 3D tissue engineered constructs with microfluidic network systems, termed ‘organ-on-a-chip’, provides a novel platform for better preclinical testing of drug delivery systems. Easy manipulation of micro-liter volume of liquids has made these models a platform where scaling and dynamic crosstalk between cells can be achieved [15]. The system geometry and structures recreate physiological length scales, concentration gradients, and the fluid flow generates mechanical forces that recapitulate the *in vivo* microenvironment experienced by cells [13, 55]. Thus, these highly biomimetic platforms overcome the drawbacks with conventional tissue culture models as highlighted in the previous section [16]. By better mimicking the physiological conditions and more accurately predicting the effect of drug carriers, these sophisticated *in vitro* screening models can fill the gap between the outcomes of animal studies and human clinical trials [16].

Since lung-on-a-chip [13, 56], the field of biomimetic organs-on-a-chip has expanded rapidly to encompass several organs including liver, kidney, heart, gut, breast, and blood vessels [55, 57–59]. These studies have already shown that organ-on-a-chip platforms can generate responses similar to those observed *in vivo* towards nanoparticles [13, 17]. Furthermore, multiple organ modules can be interconnected in a physiologically relevant scale and organized to form a human-on-a-chip platform [60]. These systems can be used to study the fate of nanoparticles. The information obtained from such studies would allow rapid testing of new therapeutic designs. Thus, the use of physiologically relevant organ-on-a-chip models as a studying tool for drug delivery systems can accelerate the clinical translation of nanoparticles. The following sections of this review, ordered by organ-type, will discuss the progress, advantages and challenges of existing organ-on-a-chip platforms, in the context of testing of drug delivery systems.

3.1 Vascular platforms

Nanoparticles introduced intravenously have to traverse through the vascular endothelial barrier to reach the target tissue. It is thus important to study the interactions of these carriers with the vasculature with regards to their vascular transport and toxicity. Some studies have already reproduced geometric features of vasculature, including straight channels [61–64], bifurcations [65, 66], and a mixture of more complex features [67]. By mapping and replicating a microvascular network from a hamster muscle, a small microfluidic platform was created, reproducing several geometric features including bifurcations and tortuosities, as well as accounting for different shear stresses along the branches [67]. Another important parameter to be considered is particle size. A microfluidic vascular model was developed to study the differences in accumulation of micro-and nanosized spheres inside microchannels. It was reported that microspheres tend to locate more to the margins when compared to nanospheres [68]. This effect of size on accumulation, as well as hemodynamics and hemorheology, should be taken into account when designing drug delivery carriers targeted for vascular diseases and cancer. Additionally, these carriers also interact with blood components. A study using high concentrations of mesoporous silica nanoparticles showed that despite having no effects on platelet viability, it greatly increased platelet aggregation and adhesion to endothelium [62]. This emphasizes the evaluation of other parameters apart from viability when studying particle interaction with blood components.

Another key aspect that vascular models are able to mimic is the influence of shear stress [63, 65, 69]. This is particularly important for nanoparticle delivery as it affects the endocytic uptake of nanoparticles by endothelial cells [69]. Narrowing in particular regions of the vascular system exhibits increased shear stress, which is an important factor in the development of thrombosis. A microfluidic model was developed to study the potential of shear responsive nanoparticles for targeting and treating embolic occlusions. The tissue plasminogen activator (tPA) loaded nanoparticles were able to dissolve preformed fibrin clots inside this microfluidic channel. This *in vitro* observation was then validated in an ex-vivo mouse pulmonary embolism model, a shear-sensitive particle system allowed the use of a 100-fold lower dose of tPA to obtain the same lysing effect as achieved by free tPA (Fig. 4A) [63]. Such smart particles, which respond to biophysical cues, can target drugs more efficiently, reduce dosage and concomitantly lessen undesired secondary effects [63].

Vascular microfluidic models also take into account other aspects, such as particle functionalization [61, 65]. Platforms can model the influence of particle functionalization combined with either shear stress [61] or geometric features as straight versus bifurcating channels [65]. Some studies have highlighted the effect of particle shape on adhesion to the channel walls (Fig. 4B) [66, 70]. In case of an endothelialized channel, rod-shaped nanoparticles showed high specific targeting and lower non-specific accumulation as compared to sphere-shaped nanoparticles [70]. When engineering and designing novel carriers, one should take these shape effects into consideration.

Microfluidic technologies have the potential for scalability and high-throughput analysis, and can characterize populations of nanoparticles in a quick and low cost manner [10], or even evaluate endothelial permeability [64]. Moreover, inherent characteristics of microfluidics, such as laminar flow, can be exploited to build superior models. For instance, hydrodynamic focusing [71] could be used for precisely targeting drug carriers to specific regions in microfluidic chips, and possibly study their subsequent transport to neighboring cells from a non-exposed region. Several of the above discussed models have the potential to leverage drug delivery by probing the efficacy and mode of action of drug carriers. A future challenge that still remains to be elucidated is the prioritization of parameters, and, perhaps, how to combine multiple features in a single, high-throughput capable, device. On-chip vasculature models are thus a relevant tool when considering the targeting of vascular diseases, tumor angiogenesis and usage of injectable drug delivery systems.

3.2 Cardiac platforms

The need for targeting drugs for cardiovascular disease has become very critical [72]. Several drugs used for cardiovascular diseases have been subjected to FDA withdrawals [73]. Also a few commercial drugs used to treat other organ disorders have been identified to have adverse side effects on the cardiac tissue [74]. Inadequate screening models have become one of the major accountable factors for these failures [74, 75]. With multitude of features to be researched for a drug and its carrier to successfully translate to clinics, testing them in the appropriate *in vitro* conditions has turned out to be the key aspect [16]. However, persisting difficulties in mimicking the physiology of the heart *in vitro* have hampered this process. Nevertheless, there are several reports on cardiac tissue engineering [76] and heart-on-a-chip [75] that promise to potentially emerge as physiologically relevant models for *in vitro* drug testing. Most of the developed platforms use primary cells derived from rats, however, more focus on integration of human cells with these models is required for mimicking human physiological response. Among the human cell sources, cardiomyocytes generated from human induced pluripotent stem cells have enormous potential to be pathophysiologically relevant [77]. They also facilitate creation of platforms for mimicking genetic disorder in patient-specific cases, thereby paving the way for individualized therapy [77].

The culture of functional cardiomyocytes can benefit from stimulation with electrical and mechanical forces [78], which cannot be reproduced in a simple cell culture setting. Additionally, to recreate a functional cardiac tissue, scaffolds can be used to recreate 3D tissue architecture [79]. The use of cardiomyocyte cell sheets has offered a possible platform

for drug testing that exhibits an important physiological parameter of heart: a uniform contractile function. Stevens *et al.* [80] developed a scaffold free cardiac patch derived from human embryonic stem cells (hESCs) that showed electromechanical coupling with each beat. Shimizu *et al.* [81] fabricated these sheets with primary rat myocytes. When two of these sheets were overlaid, they exhibited a simultaneous and spontaneous beating after a week. Other attempts to record electrical stimulation have involved the use of cardiac tissue slices. Bussek *et al.* [82] implemented the use of guinea pig cardiac tissue slices to evaluate the performance of a potassium channel blocker. Even though different potentials were recorded when the slice was exposed to the drug, the experiment does not completely reproduce the effects in the human patient. Naturally, these reconstructions do not perform as the organ itself and fail to reproduce *in vivo* conditions but they can offer an advantage for further improvements by means of microfluidic platforms.

Classically, most of the microfluidic platforms are fabricated using poly(dimethylsiloxane) (PDMS). To tune the stiffness of the PDMS surface for cardiomyocyte culture the microfluidic channels can be coated with a hydrogel of controllable matrix stiffness [14]. In a study by Annabi *et al.* two types of hydrogels, methacrylated gelatin (GelMA) and methacrylated tropoelastin (MeTro) were compared [14]. The cell attachment and alignment was dependent on the type of hydrogel used, whereas the beating was dependent on the stiffness of the matrix. In another report, cardiomyocytes were seeded on thin PDMS films patterned with fibronectin and cultured within a bioreactor [83]. The high-throughput format enabled testing of different geometric patterns of fibronectin and monitoring up to forty thin films in real-time in a single bioreactor. As the calcium dynamics of the cardiomyocytes are directly affected by disease conditions, Martewicz *et al.*, developed a microfluidic platform where the calcium dynamics of the cardiomyocytes can be observed in real-time [84]. This platform could also control the oxygen concentration experienced by the cells, thus allowing induction of hypoxia. Concurrently, the differential response of calcium dynamics was monitored with varying oxygen concentration. These *in vitro* microfluidic platforms, with high-throughput capability, can accelerate the translation of a drug carrier to the clinic by recreating cardiovascular environment that better mimics human physiological conditions [75].

3.3 Liver platforms

Drug hepatotoxicity is one of the main concerns in identifying new drugs [85], thus studying hepatoxicity of nanoparticles is very important for developing novel drug delivery platforms. Also with animal models failing to better predict outcomes of drug intake in humans [85], there is an extensive body of research focused towards developing *in vitro* models for evaluating hepatotoxity [86–89]. *In vitro* hepatic models aim at emulating functionality and mimicking the normal as well as pathophysiological architecture of liver. These models include 2D/3D mono- and co-cultures, hydrogel based engineered models, and organ-on-a-chip platforms of normal and diseased cells. Since most of the drug delivery and nanotoxicity studies are restricted to 2D cell culture plates [90–92], some important studies that describe the testing of nanoparticles in these platforms that more accurately predict *in vivo* behavior are highlighted.

A recent study using lipid nanoparticles for siRNA delivery demonstrated the importance of using primary hepatocytes instead of immortalized cell lines for better translation of screening results to *in vivo* outcomes [93]. Assessment of nanoparticles should be evaluated within such clinically relevant models that can maintain the functionality of primary hepatocytes for long-term culture [86, 94]. One such model used micropatterned collagen substrate for culturing primary hepatocytes in a high-throughput 24-well plate format [86]. In this model, primary hepatocytes were co-cultured with fibroblasts to augment homotypic interactions with heterotypic interactions for maintaining the functionality of hepatocytes for a long period. This model represents a potentially useful platform for long-term analysis of liver nanotoxicity. In a study by Dragoni *et al.*, the uptake and toxicity of AuNPs was investigated using precision cut rat liver slices [95]. The tissue slice model was used to evaluate the uptake of AuNPs by hepatocytes, Kupffer and endothelial cells present in the liver slice. However, tissue biopsies or slices cannot be used for high-throughput studies and show rapid loss of functionality within days during *in vitro* culturing, thus limiting their application for long-term studies [96].

With extensive research on developing 3D hepatic spheroids for drug testing [87, 88, 97, 98] and also with availability of high-throughput commercial platforms for formation of spheroids, liver spheroids represent a promising 3D construct for rapid clinically relevant evaluation of nanoparticles. Spheroids formed from primary human hepatocytes have been used for long-term drug studies [97]. In a work by Lee *et al.*, the toxicity of gold nanoparticles and CdTe nanoparticles in 2D cell cultures and 3D spheroids were compared (Fig. 1) [39]. Results showed considerably reduced toxicity in spheroids when compared to 2D cultures and the reduction in toxicity was attributed to change in phenotype and complex cell-cell interaction that altered the transport of nanoparticles. Spheroids of cancerous hepatocytes have also been used as a model for mimicking *in vivo* tumor microenvironments as shown by England *et al.* [99]. They studied nanoparticle delivery to avascular regions in the spheroid core and used surface modification strategy to enhance nanoparticle penetration within the tumor core.

Nanoparticles exhibit differential behavior in static and flow conditions [13, 17], thus necessitating the development of dynamic microfluidic platforms for toxicity evaluation. Multiple biomimetic liver-on-a-chip platforms have been long established for drug toxicity testing [15, 89, 96, 100]. Since liver is the major organ for drug metabolism, it is important to integrate a liver module with other organ modules to assess the cytotoxicity of prodrugs. In such models, the prodrugs are first metabolized by the liver module before reaching the target organ of drug action. For example, Shuler and Sung developed a micro cell culture analog (μCCA) with 3D cultures of HepG2/C3A cells (as the liver module) and HCT-116 cells (as the tumor module), both in Matrigel, and Kasumi-1 myeloblasts (as the bone marrow module) in stiffer alginate gel (Fig. 5) [101]. The cytotoxic effect of Tegafur, an oral prodrug of 5-fluorouracil (5-FU), on each organ analogue was then tested using such a three organs-on-a-chip platform, where they found that the μCCA was capable of reproducing the metabolism of Tegafur to 5-FU in the liver module, whereas the metabolic conversion of Tegafur could not be accomplished with 2D cultures in 96-well plates.

In a work by Wagner *et al.*, a multi organ-on-a-chip model was developed for long-term culture by combining liver microtissues made of spheroids of HepaRG and hepatic stellate cells with skin biopsies [15]. Their microfluidic system integrated an inbuilt peristaltic pump with a total media volume of 300 μL, thus enabling cross-talk between skin and liver compartments proven by the dynamic level of albumin in mono- and co-culture systems. Such models could be channeled towards evaluating the toxicity of nanoparticles used in the cosmetic industry. Infectious disease models that recreate the hepatic stage of infections, such as hepatitis C viral infection, will also prove significantly important for clinical translation of nanotherapeutics [102, 86,103].

3.4 Lung platforms

The respiratory tract is one of the most significant ports of entry in the human body. This can result in a number of infectious diseases as well as occupational pathology, but it also offers an appealing route of drug administration. The alveoli itself offers a thin mucosal barrier (200–800 nm) with low enzymatic action, vast absorptive surface area (100 m^2) and rapid access to the bloodstream while avoiding invasive procedures [104]. Aerosol nanocomplexes for gene therapy have proven to diffuse through the alveoli-capillary barrier in an efficient manner compared to other particles due to their small size [105]. Other drug delivery systems like zinc oxide nanoparticles (ZnONP) [106], silver nanoparticles (AgNP) [107] and doxorubicin micelles [108] have been used with 2D culture platforms to study the effect on cell function and apoptotic response. Multi-well plate cultures of MCF-7 and A549 lung cancer cell lines have been used to evaluate nanoparticles [109]. But these systems fail to reproduce a physiological environment for drug testing. To recreate the complexity of the human lung's structure, the use of 3D microfluidic systems that can introduce fluid and solid mechanical forces [110] are essential to imitate the human alveoli environment.

In an effort to develop biomimetic 'lung-like' microsystems, Huh *et al.* reproduced the alveolar-capillary interface *in vitro* by creating a layer of alveolar epithelial cells and endothelial cells both seeded on opposite sides of a porous ECM-coated membrane (Fig. 6A) [13]. The inflammatory and toxic effect of silica nanoparticles was evaluated by particle absorption through a vacuum strain model that mimics the physiological breathing motion. This cyclic mechanical force augmented the nanoparticle translocation across the alveolar-capillary barrier, enhancing the toxic and inflammatory response as indicated by increased reactive oxygen species (ROS) generation and intercellular adhesion molecule-1 (ICAM-1) expression. The nanoparticle translocation behavior was also evaluated in a whole mouse lung ventilation-perfusion model to demonstrate the significance of using lung-on-a-chip systems to better predict physiological response. Yu *et al.* fabricated a 'lung-like-compartment' in one of the four multichannel 3D microfluidic cell culture platform that also incorporated kidney, liver and adipose cells [111]. They evaluated gelatin microspheres as a controlled-release system for TGF-β1 encapsulated within the microspheres, and used A549 cells to recreate a human lung microenvironment for drug testing. In the device, common media goes through this first chamber of A549 cells and TGF-β1 encapsulated microspheres to continue to the other 'organs'. The 'lung' compartment revealed an enhanced cellular function compared to the other uncompromised 'organs' showing a compartmental

separation between the chambers and imitating the partial cross-talk between organs that occurs in the human body.

Future endeavors must be oriented to combine the multiple cell types in the alveoli and the mechanical forces involved in ventilation. The reconstruction of a reliable alveolar-capillary barrier without a complex culturing process [112] as well as developing novel particles with effective transport properties are also upcoming challenges. A system that combines all of the above would be a reliable model that represents the inner organ conditions in the human body.

3.5 Other organ platforms

Other organs that are important for studying nanocarrier mediated drug delivery are the kidney, spleen and gut [113–115]. Kidney and spleen play an important role in clearance of systemically delivered nanoparticles. The kidney is one of the major excretion pathways in the human body, playing a key role in blood filtration and water metabolism [116]. A study indicated that the glomerular filtration barrier contributed towards the dissociation of siRNA from its cationic cyclodextrin carrier. Thus it is important to study the behavior of nanoparticles in the renal system especially when administered intravenously [113]. To replicate renal conditions, such as fluid flow and shear stress [117], Jang *et al.* created a microfluidic device that mimics the *in vivo* renal tubular environment [118]. The device consists of a PDMS platform designed to contain both ‘luminal’ and ‘tubular’ chambers separated by a porous membrane. Human primary kidney proximal tubular epithelial cells were grown on the ‘tubular’ side of the membrane while media was perfused into the ‘luminal’ side (Fig. 6B) [118]. The device demonstrated differential cell histologic arrangement and protein expression when using static and dynamic flow conditions. The dynamic condition revealed an augmentation in the reabsorption function of the kidney. In the work by Shintu *et al.*, a microfluidic device was used to measure the ‘metabolomic’ fingerprint of the kidney using high-throughput screening approach [119]. This method provides for rapid screening of a compound target library that can be of further use in the drug delivery and discovery research.

Spleen plays an important role in filtering the bloodstream and is a vital part of the body’s immunological response [120]. It forms a fundamental defense component against certain bacteria such as *Streptococcus pneumoniae*. Yung *et al.* [121] created a blood cleansing platform that uses magnetic opsonins to target *Candida albicans* [121]. The pathogen is then removed by a micromagnetic–microfluidic separator and cleared from the blood. Such models are yet to be exploited for drug studies and can be of great use for optimizing drug delivery systems.

Among other organs that could be used for drug testing is the gut-on-a-chip created by Kim *et al.* [55] This device recreates intestinal human environment and could be used to test oral drugs absorption. The device consists of two chambers divided by a porous membrane (Fig. 6C). The membrane separates a columnar epithelium of Caco-2 cells from another chamber that lies below the porous structure. In addition, *Lactobacillus rhamnosus* GG bacteria was cultured together with the epithelial cells to enhance the barrier function of cells during this period. The inclusion of a *Lactobacillus* that resembles the intestinal flora offers a step

forward towards simulating the intestinal lumen. Future design strategies ought to consider the development of an organ-like platform that can mimic the *in vivo* complexity of the gut environment in a simple and efficient way.

3.6 Tumor platforms

The complex *in vivo* microenvironment of a tumor presents a unique challenge for the efficient delivery of nanotherapeutics to the tumor site. The key features of this complex microenvironment include heterogenous vascularization, increased interstitial pressure and inadequate lymphatic drainage [122]. Significant advances have been made in the chemical discovery, design and synthesis of chemotherapeutic drugs and diagnostic agents. However, their adverse side effects in healthy tissues greatly limit the maximum tolerated dose and thereby reduce their therapeutic efficacy [123–125]. Researchers and pharmaceutical companies are pursuing the use of nanoparticles for encapsulating anti-cancer drugs and delivering them specifically to the tumor site as a potential solution to reduce the side effects [126]. Nanoparticles with surface targeting moieties have shown efficacy in *in vitro* screening studies, but fail to show the same performance during *in vivo* trials. Several delivery barriers make nanotherapeutics ineffective within the human body and hinder successful clinical translation of these anti-cancer drug carriers. To solve this delivery problem, it is important to gain an understanding of the nanoparticle transport through the bloodstream, distribution at the target tissue and subsequent uptake by the cells. Preclinical models that mimic the *in vivo* 3D tumor architecture and dynamic flow conditions are important for studying these delivery parameters and for toxicology assessment.

The integration of microfluidic networks with 3D tissue engineered cultures offers a unique opportunity to probe nanoparticle transport barriers in a controlled manner. Additionally, these tumor-on-a-chip platforms can be used for studies to optimize nanoparticle dosage, to generate gradient drug concentrations and to develop personalized drug screening and treatment strategies [127]. For example, in a recent report by Albanese *et al.*, a tumor-on-a-chip model was developed to investigate the transport behavior of AuNPs through 3D tumor-like spheroids of human melanoma cells immobilized in a PDMS chamber (Fig. 7A) [128]. Their microfluidic device allowed precise control of the media flow conditions and the spheroids were coated with a laminin layer that acted as a barrier to AuNPs transport, thus mimicking conditions found *in vivo* at the tumor site. The tissue penetration and accumulation of fluorescent NPs under physiological flow conditions was monitored in real-time. The nanoparticle diameter, surface functionalization and the flow conditions in the microenvironment were shown to affect the accumulation of AuNPs near the tumor tissue (Fig. 7B,C). The nanoparticles functionalized with targeting groups accumulated in the periphery and failed to penetrate deep inside the core. Such studies provide critical insights to help design better nanoparticles for improved *in vivo* targeting. Pellegrino *et al.* have also designed a microfludic platform to evaluate magnetic thermoresponsive polymers for controlled release of chemotherapeuitc drugs. This preliminary study was conducted without cells in PBS, but this system can be further developed for studying drug delivery to cells using thermoresponsive carriers [129].

Several other tumor-on-a-chip platforms have been reported to study the complex tumor microenvironment and to screen drugs [127, 130–135]. In a recent study by Kim *et al.*, a fully automated microfluidic system was developed for high-throughput screening of different concentrations and combinations of anti-cancer drugs on a parallel culture of PC3 prostate cancer cells. Their platform could capture the synergy between different sensitizer drugs (DOX or mitoxantrone in this case) and TRAIL (TNF-alpha Related Apoptosis Inducing Ligand). Such high-throughput systems can be used for screening and optimizing combinatorial delivery strategies using nanocarriers for cancer therapeutics before testing in humans [135, 136]. Further, microfluidic systems that allow for testing anti-cancer drug sensitivity to a specific patient are promising for developing clinically relevant nanotherapeutics for personalized treatments [137]. The study by Zhao *et al.* addressed a key aspect of anticancer drug assessment, namely the integration of quantitative data acquisition methods with the on-chip platform for analyzing the cell apoptosis induced by the chemotherapeutic drug. This was achieved by using Annexin V conjugated quantum dots and Calcein AM as dual apoptotic probes [138]. Such real-time optical probing methods will be useful for monitoring the behavior of nanotherapeutics in the tumor-on-a-chip platforms.

New delivery systems have been proposed in recent years to meet the demand for developing safer chemotherapeutic strategies for breast cancer, one of the commonest type of tumor in women [139]. These include liposomal nanoparticles used to deliver DOX [140] and albumin-bound Paclitaxel delivery system [141]. The anatomy of the mammary gland presents a number of challenges when it comes to clinical translation of these theranostics. Two of the major barriers are: the presence of a system of ducts in a branched manner with different sizes [142] and high pressure buildup during a diagnostic liquid procedure like the fluid nipple aspiration or ductal lavage. Due to the increased pressure in the smaller ducts, the delivered theranostics fail to reach their target and in 20% of the patients these methods provide an inadequate number of cells to determine a diagnosis [143]. In their effort to reproduce the ductal system in a mammary gland and address the difficulties mentioned above, Grafton *et al.* developed a microfluidic breast-on-a-chip system. This system consisted of laminin coated microfluidic channels with decreasing ductal size to culture a polarized monolayer of human mammary epithelial cells. In this model, they proposed the use of iron oxide superparamagnetic sub-micron particles (SMPs) with an external magnetic field guidance for theranostic purposes of mammary gland neoplasias [144]. To further investigate breast neoplasia models, the same group attempted the reconstruction of tumor growth in the mammary gland.

Knowing that most of the human mammary lesions ascend from the terminal lobular ductal units [145], they used a platform to reproduce the disease on a chip. Human breast epithelial cells and nodules (3–5 cells) of tumorigenic human mammary carcinoma cells were seeded in an acrylic hemichannel system leading to a model that mimics the tumor’s organization in human biopsies [146]. Using patient biopsies in future studies, the tumor-on-a-chip models described above could serve as a personalized medicine platform to optimize an individualized chemotherapy regimen.

4. Future Directions & Conclusion

Pharmaceutical companies involved in drug discovery continuously aim to both reduce the cost of their research and speed up the development of new drugs. In this process, pre-clinical animal studies are the most expensive and time-consuming part, while not assuring human *in vivo* significance. *In vitro* organ-on-a-chip platforms can revolutionize this process by making it less expensive, while representing a reliable way for drug discovery. However, further advances in the field are needed to overcome engineering and biological challenges before entirely assessing their value as an efficient and robust platform for screening nanotherapeutics.

The final goal is to develop a body-on-a-chip platform for systemic evaluation of drug delivery vectors. But current efforts are directed towards the development of individual organ platforms limiting their range of applications to a specific organ's functions. For example, liver being the most relevant organ for drug toxicity and metabolism, the development of a functional liver-on-a-chip system is the most important challenge currently faced by researchers. As mentioned in section 3.3, several attempts have been developed in this direction, but the available platforms are yet unable to fully capture the complexities of *in vivo* drug metabolism in a robust manner. For developing a universal platform, the flexibility and modularity of this technology, based on extremely versatile microfabrication techniques, should be exploited. The possibility of culturing different cell types employing the identical standard setups could lead to the development of several organ models on demand. The results obtained with such systems could be easily compared, and relevant drug-specific cell responses can be quickly noticed. In this context, the simplicity of the setup and the implementation of several parallel experiments will be a key aspect (*e.g.*, for testing drug-dose responses as well as different target organs). Indeed, in many cases the platforms proposed so far have been optimized for one or two main parameters and the comparison between them is highly complicated. A crucial challenge is to build platforms that deliver comparable results, both to other systems and among different research groups.

Furthermore, the inherent modularity of these systems allows for the development of several on-chip modules by only changing cell type. Interconnection of such modules would mimic the communication between different organs, making the search for potential new drugs a more effective and comprehensive process. However, high-throughput and automation, which are intrinsic capabilities of these systems, yet remain a challenge. Developments and efforts on the field of biosensors should be coupled to organs-on-a-chip, so that evaluation of outcomes could be done in a quantitative and real-time manner. An ideal platform should include these sensors to monitor, *in situ*, various physical/chemical parameters, such as O_2, pH or even proteins. A robust system has to show stable experimental conditions in order to correlate any significant change in those parameters to the direct effect of the candidate drug. Commercially available sensors, and microfabricated ones, that allow a quick integration in a microfluidic system, will be combined to previously developed organ-on-a-chip platforms.

Future platforms should integrate parenchymal and non-parenchymal cells, and allow long-term experiments without experiencing irreversible changes in cell function and behavior.

Parameters such as cell proliferation and viability are quite immediate to assess, but changes in proteomic, genomic and epigenomic levels, that can dramatically affect drug metabolism, can be more difficult to monitor. A stable and robust system to address these issues is a mandatory challenge that will be faced in the next generation organ-on-a-chip systems.

New organ-on-a-chip platforms for drug discovery should also consider any problems arising from the interaction between a candidate drug vector and the microfluidic systems. Mathematical and computational fluid dynamics (CFD) studies can help in this direction, providing a mechanistic understanding of drug carrier attachment in organ-on-a-chip systems. The known differential behavior of nanoparticle shape in terms of cell adhesion and distribution can be assessed by CFD, and potentially aid in the generation of better and more comprehensive models. For instance, in a computational study nanorods were shown to contact and adhere to the wall much better than spherical counterparts [147]. Moreover, a CFD model of a branched blood vessel showed that bifurcating sections had higher nanoparticle accumulation when compared to straight ones. In the same study, authors were able to model the trajectory and adhesive forces of nanoparticles under shear flow [148]. Together, these and other works [149], set the stage for future CFD studies on particle-particle, particle-cell and particle-microfluidic device interaction (accumulation and adhesion). More complex CFD studies on these phenomena, especially within organ-on-a-chip, can help building next generation drug delivery systems, by better understanding their underlying biophysical aspects.

From a materials point of view, the devices developed in this field show important differences that can determine different cell responses as well as affect drug concentration into the system, due to drug nonspecific adsorption to the channel walls. Though PDMS and polystyrene are the most popular materials due to several advantages [150], such as optical transparency and cell compatibility, other plastic materials have been used and a detailed comparison of their advantages/drawbacks in drug screening tests is missing. If an ideal material will be not found, research groups could decide to cover the microchannels with a cell layer for preventing drug adsorption, as successfully reported by Schimek *et al.* [151].

In the long run, organ-on-a-chip systems will be used also for personalized drug screening. As these systems will show strong standardization and reliability, the possibility to use patient-derived iPSCs, produced *in vitro* and then terminally differentiated, can solve one of the most important problems in disease treatment – patient-to-patient drug response variability – avoiding long and ineffective drug treatments and minimizing drug toxicity. In summary, organ-on-a-chip platforms for studying drug delivery systems hold great promise and some aspects of them have been investigated, but their full applicability and potential have yet to be realized.

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References

1. Farokhzad OC, Langer R. Nanomedicine: developing smarter therapeutic and diagnostic modalities. Adv Drug Deliv Rev. 2006; 58:1456–1459. [PubMed: 17070960]
2. Peppas NA. Intelligent therapeutics: biomimetic systems and nanotechnology in drug delivery. Adv Drug Deliv Rev. 2004; 56:1529–1531. [PubMed: 15350286]
3. Gregoriadis G. Drug entrapment in liposomes. FEBS Lett. 1973; 36:292–296. [PubMed: 4763309]
4. Gregoriadis G, Buckland RA. Enzyme-containing liposomes alleviate a model for storage disease. Nature. 1973; 244:170–172. [PubMed: 4583499]
5. Stirland DL, Nichols JW, Miura S, Bae YH. Mind the gap: A survey of how cancer drug carriers are susceptible to the gap between research and practice. J Control Release. 2013; 172:1045–1064. [PubMed: 24096014]
6. Fu Q, Sun J, Zhang W, Sui X, Yan Z, He Z. Nanoparticle albumin-bound (NAB) technology is a promising method for anti-cancer drug delivery. Recent Pat Anticancer Drug Discov. 2009; 4:262–272. [PubMed: 19538176]
7. Hakimi N, Tsai SS, Cheng CH, Hwang DK. One-Step Two-Dimensional Microfluidics-Based Synthesis of Three-Dimensional Particles. Adv Mater. 2013
8. Valencia PM, Pridgen EM, Rhee M, Langer R, Farokhzad OC, Karnik R. Microfluidic platform for combinatorial synthesis and optimization of targeted nanoparticles for cancer therapy. ACS Nano. 2013; 7:10671–10680. [PubMed: 24215426]
9. Andreas J, Joseph ER, Wyatt NV, Don LD, Laurie EL, Michael G. Preparation of nanoparticles by continuous-flow microfluidics. Journal of Nanoparticle Research. 2008
10. Fraikin JL, Teesalu T, McKenney CM, Ruoslahti E, Cleland AN. A high-throughput label-free nanoparticle analyser. Nature Nanotechnology. 2011; 6:308–313.
11. Farokhzad OC, Khademhosseini A, Jon S, Hermmann A, Cheng J, Chin C, Kiselyuk A, Teply B, Eng G, Langer R. Microfluidic system for studying the interaction of nanoparticles and microparticles with cells. Anal Chem. 2005; 77:5453–5459. [PubMed: 16131052]
12. Whitesides GM. The origins and the future of microfluidics. Nature. 2006; 442:368–373. [PubMed: 16871203]
13. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science. 2010; 328:1662–1668. [PubMed: 20576885]
14. Annabi N, Selimovic S, Acevedo Cox JP, Ribas J, Afshar Bakooshli M, Heintze D, Weiss AS, Cropek D, Khademhosseini A. Hydrogel-coated microfluidic channels for cardiomyocyte culture. Lab Chip. 2013; 13:3569–3577. [PubMed: 23728018]
15. Wagner I, Materne EM, Brincker S, Sussbier U, Fradrich C, Busek M, Sonntag F, Sakharov DA, Trushkin EV, Tonevitsky AG, Lauster R, Marx U. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip. 2013; 13:3538–3547. [PubMed: 23648632]
16. Valencia PM, Farokhzad OC, Karnik R, Langer R. Microfluidic technologies for accelerating the clinical translation of nanoparticles. Nature Nanotechnology. 2012; 7:623–629.
17. Mahto SK, Yoon TH, Rhee SW. A new perspective on in vitro assessment method for evaluating quantum dot toxicity by using microfluidics technology. Biomicrofluidics. 2010; 4
18. Jiang W, KimBetty YS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nature Nanotechnology. 2008; 3:145–150.
19. Chithrani BD, Ghazani AA, Chan WCW. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. Nano Letters. 2006; 6:662–668. [PubMed: 16608261]
20. Baselga J, Norton L, Albanell J, Kim Y-M, Mendelsohn J. Recombinant Humanized Anti-HER2 Antibody (Herceptin™) Enhances the Antitumor Activity of Paclitaxel and Doxorubicin against HER2/neu Overexpressing Human Breast Cancer Xenografts. Cancer Research. 1998; 58:2825–2831. [PubMed: 9661897]
21. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M. Feasibility of a High-Flux

Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. Journal of the National Cancer Institute. 1991; 83:757–766. [PubMed: 2041050]

22. Cho EC, Zhang Q, Xia Y. The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. Nature Nanotechnology. 2011; 6:385–391.
23. Bhise NS, Gray RS, Sunshine JC, Htet S, Ewald AJ, Green JJ. The relationship between terminal functionalization and molecular weight of a gene delivery polymer and transfection efficacy in mammary epithelial 2-D cultures and 3-D organotypic cultures. Biomaterials. 2010; 31:8088–8096. [PubMed: 20674001]
24. Wilkening S, Stahl F, Bader A. Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties. Drug Metab Dispos. 2003; 31:1035–1042. [PubMed: 12867492]
25. Hamilton G. Multicellular spheroids as an in vitro tumor model. Cancer letters. 1998; 131:29–34. [PubMed: 9839617]
26. Ong S-M, Zhao Z, Arooz T, Zhao D, Zhang S, Du T, Wasser M, van Noort D, Yu H. Engineering a scaffold-free 3D tumor model for< i> in vitro drug penetration studies. Biomaterials. 2010; 31:1180–1190. [PubMed: 19889455]
27. Dubessy C, Merlin J-L, Marchal C, Guillemin F. Spheroids in radiobiology and photodynamic therapy. Critical Reviews in Oncology/Hematology. 2000; 36:179–192. [PubMed: 11033305]
28. Kunz-Schughart LA. Multicellular tumor spheroids: intermediates between monolayer culture and in vivo tumor. Cell Biology International. 1999; 23:157–161. [PubMed: 10562436]
29. Mueller-Klieser W. Multicellular spheroids. Journal of Cancer Research and Clinical Oncology. 1987; 113:101–122. [PubMed: 3549738]
30. Yuhas JM, Li AP, Martinez AO, Ladman AJ. A Simplified Method for Production and Growth of Multicellular Tumor Spheroids. Cancer Research. 1977; 37:3639–3643. [PubMed: 908012]
31. Tung Y-C, Hsiao AY, Allen SG, Torisawa Y-s, Ho M, Takayama S. High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. Analyst. 2011; 136:473–478. [PubMed: 20967331]
32. Karp JM, Yeh J, Eng G, Fukuda J, Blumling J, Suh K-Y, Cheng J, Mahdavi A, Borenstein J, Langer R, Khademhosseini A. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. Lab Chip. 2007; 7:786–794. [PubMed: 17538722]
33. Hardelauf H, Frimat J-P, Stewart JD, Schormann W, Chiang Y-Y, Lampen P, Franzke J, Hengstler JG, Cadenas C, Kunz-Schughart LA, West J. Microarrays for the scalable production of metabolically relevant tumour spheroids: a tool for modulating chemosensitivity traits. Lab Chip. 2011; 11:419–428. [PubMed: 21079873]
34. Kinney MA, Saeed R, McDevitt TC. Systematic analysis of embryonic stem cell differentiation in hydrodynamic environments with controlled embryoid body size. Integrative Biology. 2012; 4:641–650. [PubMed: 22609810]
35. Zhang Y, Xia Y. Formation of Embryoid Bodies with Controlled Sizes and Maintained Pluripotency in Three-Dimensional Inverse Opal Scaffolds. Advanced Functional Materials. 2012; 22:121–129.
36. Torisawa YS, Takagi A, Nashimoto Y, Yasukawa T, Shiku H, Matsue T. A multicellular spheroid array to realize spheroid formation, culture, and viability assay on a chip. Biomaterials. 2007; 28:559–566. [PubMed: 16989897]
37. Zhang YS, Choi SW, Xia YN. Inverse opal scaffolds for applications in regenerative medicine. SOFT MATTER. 2013; 9:9747–9754.
38. Kim B, Han G, Toley BJ, Kim C-k, Rotello VM, Forbes NS. Tuning payload delivery in tumour cylindroids using gold nanoparticles. Nature Nanotechnology. 2010; 5:465–472.
39. Lee J, Lilly GD, Doty RC, Podsiadlo P, Kotov NA. In vitro Toxicity Testing of Nanoparticles in 3D Cell Culture. Small. 2009; 5:1213–1221. [PubMed: 19263430]
40. Gurski LA, Jha AK, Zhang C, Jia X, Farach-Carson MC. Hyaluronic acid-based hydrogels as 3D matrices for in vitro evaluation of chemotherapeutic drugs using poorly adherent prostate cancer cells. Biomaterials. 2009; 30:6076–6085. [PubMed: 19695694]

41. Kievit FM, Florczyk SJ, Leung MC, Veiseh O, Park JO, Disis ML, Zhang M. Chitosan-alginate 3D scaffolds as a mimic of the glioma tumor microenvironment. Biomaterials. 2010; 31:5903–5910. [PubMed: 20417555]
42. Hutmacher DW. Biomaterials offer cancer research the third dimension. Nature Materials. 2010; 9:90–93.
43. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. 2006; 126:677–689.
44. Discher DE, Janmey P, Wang Y-l. Tissue Cells Feel and Respond to the Stiffness of Their Substrate. Science. 2005; 310:1139–1143. [PubMed: 16293750]
45. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nature Materials. 2010; 9:518–526.
46. Liu J, Tan Y, Zhang H, Zhang Y, Xu P, Chen J, Poh Y-C, Tang K, Wang N, Huang B. Soft fibrin gels promote selection and growth of tumorigenic cells. Nature Materials. 2012; 11:734–741.
47. Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, Polverini PJ, Mooney DJ. Engineering tumors with 3D scaffolds. Nature Methods. 2007; 4:855–860. [PubMed: 17767164]
48. Wu M-H, Huang S-B, Lee G-B. Microfluidic cell culture systems for drug research. Lab Chip. 2010; 10:939–956. [PubMed: 20358102]
49. Wu M-H, Huang S-B, Cui Z, Cui Z, Lee G-B. Development of perfusion-based micro 3-D cell culture platform and its application for high throughput drug testing. Sensors and Actuators B: Chemical. 2008; 129:231–240.
50. Baudoin R, Griscom L, Prot JM, Legallais C, Leclerc E. Behavior of HepG2/C3A cell cultures in a microfluidic bioreactor. Biochemical Engineering Journal. 2011; 53:172–181.
51. Yang ST, Zhang X, Wen Y. Microbioreactors for high-throughput cytotoxicity assays. Current opinion in drug discovery & development. 2008; 11:111–127. [PubMed: 18175274]
52. Robert C, Kamal E, Honglu W, Wei S. Biofabrication of a three-dimensional liver micro-organ as an in vitro drug metabolism model. Biofabrication. 2010; 2:045004. [PubMed: 21079286]
53. Wu LY, Di Carlo D, Lee LP. Microfluidic self-assembly of tumor spheroids for anticancer drug discovery. Biomedical Microdevices. 2008; 10:197–202. [PubMed: 17965938]
54. Yu L, Chen MC, Cheung KC. Droplet-based microfluidic system for multicellular tumor spheroid formation and anticancer drug testing. Lab Chip. 2010; 10:2424–2432. [PubMed: 20694216]
55. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip. 2012; 12:2165–2174. [PubMed: 22434367]
56. Huh D, Fujioka H, Tung YC, Futai N, Paine R 3rd, Grotberg JB, Takayama S. Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. Proc Natl Acad Sci U S A. 2007; 104:18886–18891. [PubMed: 18006663]
57. Sung J, Shuler M. In vitro microscale systems for systematic drug toxicity study. Bioprocess and Biosystems Engineering. 2010; 33:5–19. [PubMed: 19701779]
58. Sin A, Chin KC, Jamil MF, Kostov Y, Rao G, Shuler ML. The Design and Fabrication of Three-Chamber Microscale Cell Culture Analog Devices with Integrated Dissolved Oxygen Sensors. Biotechnology Progress. 2004; 20:338–345. [PubMed: 14763861]
59. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. Trends in Cell Biology. 2011; 21:745–754. [PubMed: 22033488]
60. Moraes C, Labuz JM, Leung BM, Inoue M, Chun TH, Takayama S. On being the right size: scaling effects in designing a human-on-a-chip. Integr Biol (Camb). 2013; 5:1149–1161. [PubMed: 23925524]
61. Kusunose J, Zhang H, Gagnon MK, Pan T, Simon SI, Ferrara KW. Microfluidic system for facilitated quantification of nanoparticle accumulation to cells under laminar flow. Ann Biomed Eng. 2013; 41:89–99. [PubMed: 22855121]
62. Kim D, Finkenstaedt-Quinn S, Hurley KR, Buchman JT, Haynes CL. On-chip evaluation of platelet adhesion and aggregation upon exposure to mesoporous silica nanoparticles. Analyst. 2014

63. Korin N, Kanapathipillai M, Matthews BD, Crescente M, Brill A, Mammoto T, Ghosh K, Jurek S, Bencherif SA, Bhatta D, Coskun AU, Feldman CL, Wagner DD, Ingber DE. Shear-activated nanotherapeutics for drug targeting to obstructed blood vessels. Science. 2012; 337:738–742. [PubMed: 22767894]
64. Young EW, Watson MW, Srigunapalan S, Wheeler AR, Simmons CA. Technique for real-time measurements of endothelial permeability in a microfluidic membrane chip using laser-induced fluorescence detection. Anal Chem. 2010; 82:808–816. [PubMed: 20050596]
65. Lamberti G, Tang Y, Prabhakarpandian B, Wang Y, Pant K, Kiani MF, Wang B. Adhesive interaction of functionalized particles and endothelium in idealized microvascular networks. Microvasc Res. 2013; 89:107–114. [PubMed: 23557880]
66. Doshi N, Prabhakarpandian B, Rea-Ramsey A, Pant K, Sundaram S, Mitragotri S. Flow and adhesion of drug carriers in blood vessels depend on their shape: a study using model synthetic microvascular networks. J Control Release. 2010; 146:196–200. [PubMed: 20385181]
67. Rosano JM, Tousi N, Scott RC, Krynska B, Rizzo V, Prabhakarpandian B, Pant K, Sundaram S, Kiani MF. A physiologically realistic in vitro model of microvascular networks. Biomed Microdevices. 2009; 11:1051–1057. [PubMed: 19452279]
68. Namdee K, Thompson AJ, Charoenphol P, Eniola-Adefeso O. Margination propensity of vascular-targeted spheres from blood flow in a microfluidic model of human microvessels. Langmuir. 2013; 29:2530–2535. [PubMed: 23363293]
69. Samuel SP, Jain N, O'Dowd F, Paul T, Kashanin D, Gerard VA, Gun'ko YK, Prina-Mello A, Volkov Y. Multifactorial determinants that govern nanoparticle uptake by human endothelial cells under flow. Int J Nanomedicine. 2012; 7:2943–2956. [PubMed: 22745555]
70. Kolhar P, Anselmo AC, Gupta V, Pant K, Prabhakarpandian B, Ruoslahti E, Mitragotri S. Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium. Proc Natl Acad Sci U S A. 2013; 110:10753–10758. [PubMed: 23754411]
71. Wang F, Wang H, Wang J, Wang H-Y, Rummel PL, Garimella SV, Lu C. Microfluidic delivery of small molecules into mammalian cells based on hydrodynamic focusing. Biotechnology and Bioengineering. 2008; 100:150–158. [PubMed: 18078299]
72. Ruiz-Esparza GU, Flores-Arredondo JH, Segura-Ibarra V, Torre-Amione G, Ferrari M, Blanco E, Serda RE. The physiology of cardiovascular disease and innovative liposomal platforms for therapy. Int J Nanomedicine. 2013; 8:629–640. [PubMed: 23413209]
73. Nordt SP, Minns A, Tomaszewski C, Cantrell FL, Clark RF. Retrospective review of digoxin exposures to a poison control system following recall of Digitek(R) tablets. Am J Cardiovasc Drugs. 2010; 10:261–263. [PubMed: 20653332]
74. Natarajan A, Stancescu M, Dhir V, Armstrong C, Sommerhage F, Hickman JJ, Molnar P. Patterned cardiomyocytes on microelectrode arrays as a functional, high information content drug screening platform. Biomaterials. 2011; 32:4267–4274. [PubMed: 21453966]
75. Grosberg A, Alford PW, McCain ML, Parker KK. Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip. Lab Chip. 2011; 11:4165–4173. [PubMed: 22072288]
76. Shin SR, Jung SM, Zalabany M, Kim K, Zorlutuna P, Kim SB, Nikkhah M, Khabiry M, Azize M, Kong J, Wan KT, Palacios T, Dokmeci MR, Bae H, Tang XS, Khademhosseini A. Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. ACS Nano. 2013; 7:2369–2380. [PubMed: 23363247]
77. Zanella F, Lyon RC, Sheikh F. Modeling heart disease in a dish: From somatic cells to disease-relevant cardiomyocytes. Trends Cardiovasc Med. 2014; 24:32–44. [PubMed: 24054750]
78. Cimetta E, Godier-Furnemont A, Vunjak-Novakovic G. Bioengineering heart tissue for in vitro testing. Curr Opin Biotechnol. 2013; 24:926–932. [PubMed: 23932513]
79. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nature Medicine. 2008; 14:213–221.
80. Stevens KR, Pabon L, Muskheli V, Murry CE. Scaffold-free human cardiac tissue patch created from embryonic stem cells. Tissue Eng Part A. 2009; 15:1211–1222. [PubMed: 19063661]

81. Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res. 2002; 90:e40. [PubMed: 11861428]
82. Bussek A, Schmidt M, Bauriedl J, Ravens U, Wettwer E, Lohmann H. Cardiac tissue slices with prolonged survival for in vitro drug safety screening. J Pharmacol Toxicol Methods. 2012; 66:145–151. [PubMed: 22245702]
83. Agarwal A, Goss JA, Cho A, McCain ML, Parker KK. Microfluidic heart on a chip for higher throughput pharmacological studies. Lab Chip. 2013; 13:3599–3608. [PubMed: 23807141]
84. Martewicz S, Michielin F, Serena E, Zambon A, Mongillo M, Elvassore N. Reversible alteration of calcium dynamics in cardiomyocytes during acute hypoxia transient in a microfluidic platform. Integr Biol (Camb). 2012; 4:153–164. [PubMed: 22158991]
85. Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov. 2005; 4:489–499. [PubMed: 15931258]
86. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. Nature Biotechnology. 2008; 26:120–126.
87. Leite SB, Wilk-Zasadna I, Zaldivar JM, Airola E, Reis-Fernandes MA, Mennecozzi M, Guguen-Guillouzo C, Chesne C, Guillou C, Alves PM, Coecke S. Three-dimensional HepaRG model as an attractive tool for toxicity testing. Toxicol Sci. 2012; 130:106–116. [PubMed: 22843569]
88. Yip D, Cho CH. A multicellular 3D heterospheroid model of liver tumor and stromal cells in collagen gel for anti-cancer drug testing. Biochem Biophys Res Commun. 2013; 433:327–332. [PubMed: 23501105]
89. Toh YC, Lim TC, Tai D, Xiao G, van Noort D, Yu H. A microfluidic 3D hepatocyte chip for drug toxicity testing. Lab Chip. 2009; 9:2026–2035. [PubMed: 19568671]
90. Smith WE, Brownell J, White CC, Afsharinejad Z, Tsai J, Hu X, Polyak SJ, Gao X, Kavanagh TJ, Eaton DL. In vitro toxicity assessment of amphiphillic polymer-coated CdSe/ZnS quantum dots in two human liver cell models. ACS Nano. 2012; 6:9475–9484. [PubMed: 23039050]
91. Tzeng SY, Higgins LJ, Pomper MG, Green JJ. Student award winner in the Ph.D. category for the 2013 society for biomaterials annual meeting and exposition, april 10-13, 2013, Boston, Massachusetts : biomaterial-mediated cancer-specific DNA delivery to liver cell cultures using synthetic poly(beta-amino ester)s. J Biomed Mater Res A. 2013; 101:1837–1845. [PubMed: 23559534]
92. Arunraj TR, Sanoj Rejinold N, Ashwin Kumar N, Jayakumar R. Bio-responsive chitin-poly(l-lactic acid) composite nanogels for liver cancer. Colloids Surf B Biointerfaces. 2013; 113C:394–402. [PubMed: 24129331]
93. Whitehead KA, Matthews J, Chang PH, Niroui F, Dorkin JR, Severgnini M, Anderson DG. In vitro-in vivo translation of lipid nanoparticles for hepatocellular siRNA delivery. ACS Nano. 2012; 6:6922–6929. [PubMed: 22770391]
94. Hui EE, Bhatia SN. Micromechanical control of cell-cell interactions. Proc Natl Acad Sci U S A. 2007; 104:5722–5726. [PubMed: 17389399]
95. Dragoni S, Franco G, Regoli M, Bracciali M, Morandi V, Sgaragli G, Bertelli E, Valoti M. Gold nanoparticles uptake and cytotoxicity assessed on rat liver precision-cut slices. Toxicol Sci. 2012; 128:186–197. [PubMed: 22539612]
96. van Midwoud PM, Merema MT, Verpoorte E, Groothuis GM. A microfluidic approach for in vitro assessment of interorgan interactions in drug metabolism using intestinal and liver slices. Lab Chip. 2010; 10:2778–2786. [PubMed: 20835427]
97. Tostoes RM, Leite SB, Serra M, Jensen J, Bjorquist P, Carrondo MJ, Brito C, Alves PM. Human liver cell spheroids in extended perfusion bioreactor culture for repeated-dose drug testing. Hepatology. 2012; 55:1227–1236. [PubMed: 22031499]
98. Wong SF, No da Y, Choi YY, Kim DS, Chung BG, Lee SH. Concave microwell based size-controllable hepatosphere as a three-dimensional liver tissue model. Biomaterials. 2011; 32:8087–8096. [PubMed: 21813175]

99. England CG, Priest T, Zhang G, Sun X, Patel DN, McNally LR, van Berkel V, Gobin AM, Frieboes HB. Enhanced penetration into 3D cell culture using two and three layered gold nanoparticles. Int J Nanomedicine. 2013; 8:3603–3617. [PubMed: 24124360]
100. Goral VN, Hsieh YC, Petzold ON, Clark JS, Yuen PK, Faris RA. Perfusion-based microfluidic device for three-dimensional dynamic primary human hepatocyte cell culture in the absence of biological or synthetic matrices or coagulants. Lab Chip. 2010; 10:3380–3386. [PubMed: 21060907]
101. Sung JH, Shuler ML. A micro cell culture analog (μCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip. 2009; 9:1385–1394. [PubMed: 19417905]
102. March S, Ng S, Velmurugan S, Galstian A, Shan J, Logan DJ, Carpenter AE, Thomas D, Sim BK, Mota MM, Hoffman SL, Bhatia SN. A microscale human liver platform that supports the hepatic stages of Plasmodium falciparum and vivax. Cell Host Microbe. 2013; 14:104–115. [PubMed: 23870318]
103. Ploss A, Khetani SR, Jones CT, Syder AJ, Trehan K, Gaysinskaya VA, Mu K, Ritola K, Rice CM, Bhatia SN. Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures. Proc Natl Acad Sci U S A. 2010; 107:3141–3145. [PubMed: 20133632]
104. Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 2003; 56:588–599. [PubMed: 14616418]
105. Zarogoulidis P, Karamanos NK, Porpodis K, Domvri K, Huang H, Hohenforst-Schmidt W, Goldberg EP, Zarogoulidis K, Zarogouldis p, et al. vectors for inhaled gene therapy in lung cancer. Application for nano oncology and safety of bio nanotechnology. Int J Mol Sci. 2012; 13:10828–10862. Int. J. Mol. Sci. 2012, 13, 17290-17291. [PubMed: 23109824]
106. Vandebriel RJ, De Jong WH. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol Sci Appl. 2012; 5:61–71. [PubMed: 24198497]
107. Govender R, Phulukdaree A, Gengan RM, Anand K, Chuturgoon AA. Silver nanoparticles of Albizia adianthifolia: the induction of apoptosis in human lung carcinoma cell line. J Nanobiotechnology. 2013; 11:5. [PubMed: 23418790]
108. Wu Y, Shih EK, Ramanathan A, Vasudevan S, Weil T. Nano-sized albumin-copolymer micelles for efficient doxorubicin delivery. Biointerphases. 2012; 7:5. [PubMed: 22589048]
109. Ding D, Tang X, Cao X, Wu J, Yuan A, Qiao Q, Pan J, Hu Y. Novel Self-assembly Endows Human Serum Albumin Nanoparticles with an Enhanced Antitumor Efficacy. AAPS PharmSciTech. 2013
110. Douville NJ, Zamankhan P, Tung YC, Li R, Vaughan BL, Tai CF, White J, Christensen PJ, Grotberg JB, Takayama S. Combination of fluid and solid mechanical stresses contribute to cell death and detachment in a microfluidic alveolar model. Lab Chip. 2011; 11:609–619. [PubMed: 21152526]
111. Zhang C, Zhao Z, Abdul Rahim NA, van Noort D, Yu H. Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. Lab Chip. 2009; 9:3185–3192. [PubMed: 19865724]
112. Kumar Mahto S, Tenenbaum-Katan J, Sznitman J. Respiratory Physiology on a Chip. Scientifica (Cairo). 2012; 2012:364054. [PubMed: 24278686]
113. Naeye B, Deschout H, Caveliers V, Descamps B, Braeckmans K, Vanhove C, Demeester J, Lahoutte T, De Smedt SC, Raemdonck K. In vivo disassembly of IV administered siRNA matrix nanoparticles at the renal filtration barrier. Biomaterials. 2013; 34:2350–2358. [PubMed: 23261216]
114. Anselmo AC, Gupta V, Zern BJ, Pan D, Zakrewsky M, Muzykantov V, Mitragotri S. Delivering Nanoparticles to Lungs while Avoiding Liver and Spleen through Adsorption on Red Blood Cells. ACS Nano. 2013; 7:11129–11137. [PubMed: 24182189]
115. des Rieux A, Fievez V, Theate I, Mast J, Preat V, Schneider YJ. An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle transport by M cells. Eur J Pharm Sci. 2007; 30:380–391. [PubMed: 17291730]

116. Johns EJ. The autonomic nervous system and pressure-natriuresis in cardiovascular-renal interactions in response to salt. Clin Auton Res. 2002; 12:256–263. [PubMed: 12357279]

117. Grabias BM, Konstantopoulos K. The Physical Basis of Renal Fibrosis: Effects of Altered Hydrodynamic Forces on Kidney Homeostasis. Am J Physiol Renal Physiol. 2013

118. Jang KJ, Mehr AP, Hamilton GA, McPartlin LA, Chung S, Suh KY, Ingber DE. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol (Camb). 2013; 5:1119–1129. [PubMed: 23644926]

119. Shintu L, Baudoin R, Navratil V, Prot JM, Pontoizeau C, Defernez M, Blaise BJ, Domange C, Pery AR, Toulhoat P, Legallais C, Brochot C, Leclerc E, Dumas ME. Metabolomics-on-a-chip and predictive systems toxicology in microfluidic bioartificial organs. Anal Chem. 2012; 84:1840–1848. [PubMed: 22242722]

120. Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, Berner R, Peters A, Boehm T, Plebani A, Quinti I, Carsetti R. Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen. J Exp Med. 2003; 197:939–945. [PubMed: 12682112]

121. Yung CW, Fiering J, Mueller AJ, Ingber DE. Micromagnetic-microfluidic blood cleansing device. Lab Chip. 2009; 9:1171–1177. [PubMed: 19370233]

122. Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. Nat Rev Cancer. 2004; 4:806–813. [PubMed: 15510161]

123. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012; 161:505–522. [PubMed: 22353619]

124. Young EW. Cells, tissues, and organs on chips: challenges and opportunities for the cancer tumor microenvironment. Integr Biol (Camb). 2013; 5:1096–1109. [PubMed: 23799587]

125. Feng X, Du W, Luo Q, Liu BF. Microfluidic chip: next-generation platform for systems biology. Anal Chim Acta. 2009; 650:83–97. [PubMed: 19720178]

126. Bhise NS, Shmueli RB, Sunshine JC, Tzeng SY, Green JJ. Drug delivery strategies for therapeutic angiogenesis and antiangiogenesis. Expert Opin Drug Deliv. 2011; 8:485–504. [PubMed: 21338327]

127. Wlodkowic D, Cooper JM. Tumors on chips: oncology meets microfluidics. Curr Opin Chem Biol. 2010; 14:556–567. [PubMed: 20832352]

128. Albanese A, Lam AK, Sykes EA, Rocheleau JV, Chan WC. Tumour-on-a-chip provides an optical window into nanoparticle tissue transport. Nature Communications. 2013; 4:2718.

129. Pernia Leal M, Torti A, Riedinger A, La Fleur R, Petti D, Cingolani R, Bertacco R, Pellegrino T. Controlled release of doxorubicin loaded within magnetic thermo-responsive nanocarriers under magnetic and thermal actuation in a microfluidic channel. ACS Nano. 2012; 6:10535–10545. [PubMed: 23116285]

130. Kirby BJ, Jodari M, Loftus MS, Gakhar G, Pratt ED, Chanel-Vos C, Gleghorn JP, Santana SM, Liu H, Smith JP, Navarro VN, Tagawa ST, Bander NH, Nanus DM, Giannakakou P. Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. PLoS One. 2012; 7:e35976. [PubMed: 22558290]

131. Mauk MG, Ziober BL, Chen Z, Thompson JA, Bau HH. Lab-on-a-chip technologies for oral-based cancer screening and diagnostics: capabilities, issues, and prospects. Ann N Y Acad Sci. 2007; 1098:467–475. [PubMed: 17435153]

132. Li P, Stratton ZS, Dao M, Ritz J, Huang TJ. Probing circulating tumor cells in microfluidics. Lab Chip. 2013; 13:602–609. [PubMed: 23306378]

133. Elliott NT, Yuan F. A microfluidic system for investigation of extravascular transport and cellular uptake of drugs in tumors. Biotechnol Bioeng. 2012; 109:1326–1335. [PubMed: 22124930]

134. Nawy T. Receptive cells feel the squeeze. Nature Methods. 2013; 10:198. [PubMed: 23570045]

135. Kim C, Bang JH, Kim YE, Lee SH, Kang JY. On-chip anticancer drug test of regular tumor spheroids formed in microwells by a distributive microchannel network. Lab Chip. 2012; 12:4135–4142. [PubMed: 22864534]

136. Yang CG, Wu YF, Xu ZR, Wang JH. A radial microfluidic concentration gradient generator with high-density channels for cell apoptosis assay. Lab Chip. 2011; 11:3305–3312. [PubMed: 21842069]

137. Xu Z, Gao Y, Hao Y, Li E, Wang Y, Zhang J, Wang W, Gao Z, Wang Q. Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials. 2013; 34:4109–4117. [PubMed: 23473962]

138. Zhao L, Caot JT, Wu ZQ, Li JX, Zhu JJ. Lab-on-a-Chip for anticancer drug screening using quantum dots probe based apoptosis assay. J Biomed Nanotechnol. 2013; 9:348–356. [PubMed: 23620989]

139. Jin S, Ye K. Targeted drug delivery for breast cancer treatment. Recent Pat Anticancer Drug Discov. 2013; 8:143–153. [PubMed: 23394116]

140. Batist G, Ramakrishnan G, Rao CS, Chandrasekharan A, Gutheil J, Guthrie T, Shah P, Khojasteh A, Nair MK, Hoelzer K, Tkaczuk K, Park YC, Lee LW. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. J Clin Oncol. 2001; 19:1444–1454. [PubMed: 11230490]

141. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol. 2005; 23:7794–7803. [PubMed: 16172456]

142. Love SM, Barsky SH. Anatomy of the nipple and breast ducts revisited. Cancer. 2004; 101:1947–1957. [PubMed: 15382093]

143. Dooley WC. Ductal lavage, nipple aspiration, and ductoscopy for breast cancer diagnosis. Curr Oncol Rep. 2003; 5:63–65. [PubMed: 12493152]

144. Grafton MM, Wang L, Vidi PA, Leary J, Lelievre SA. Breast on-a-chip: mimicry of the channeling system of the breast for development of theranostics. Integr Biol (Camb). 2011; 3:451–459. [PubMed: 21234506]

145. Wellings SR. A hypothesis of the origin of human breast cancer from the terminal ductal lobular unit. Pathol Res Pract. 1980; 166:515–535. [PubMed: 6253967]

146. Vidi PA, Maleki T, Ochoa M, Wang L, Clark SM, Leary JF, Lelievre SA. Disease-on-a-chip: mimicry of tumor growth in mammary ducts. Lab Chip. 2014; 14:172–177. [PubMed: 24202525]

147. Shah S, Liu Y, Hu W, Gao J. Modeling particle shape-dependent dynamics in nanomedicine. J Nanosci Nanotechnol. 2011; 11:919–928. [PubMed: 21399713]

148. Tan J, Shah S, Thomas A, Ou-Yang HD, Liu Y. The influence of size, shape and vessel geometry on nanoparticle distribution. Microfluid Nanofluidics. 2013; 14:77–87. [PubMed: 23554583]

149. Kim MJ, Rhee K. Computational analysis of nanoparticle adhesion to endothelium: effects of kinetic rate constants and wall shear rates. Med Biol Eng Comput. 2011; 49:733–741. [PubMed: 21556956]

150. Berthier E, Young EW, Beebe D. Engineers are from PDMS-land, Biologists are from Polystyrenia. Lab Chip. 2012; 12:1224–1237. [PubMed: 22318426]

151. Schimek K, Busek M, Brincker S, Groth B, Hoffmann S, Lauster R, Lindner G, Lorenz A, Menzel U, Sonntag F, Walles H, Marx U, Horland R. Integrating biological vasculature into a multi-organ-chip microsystem. Lab Chip. 2013; 13:3588–3598. [PubMed: 23743770]

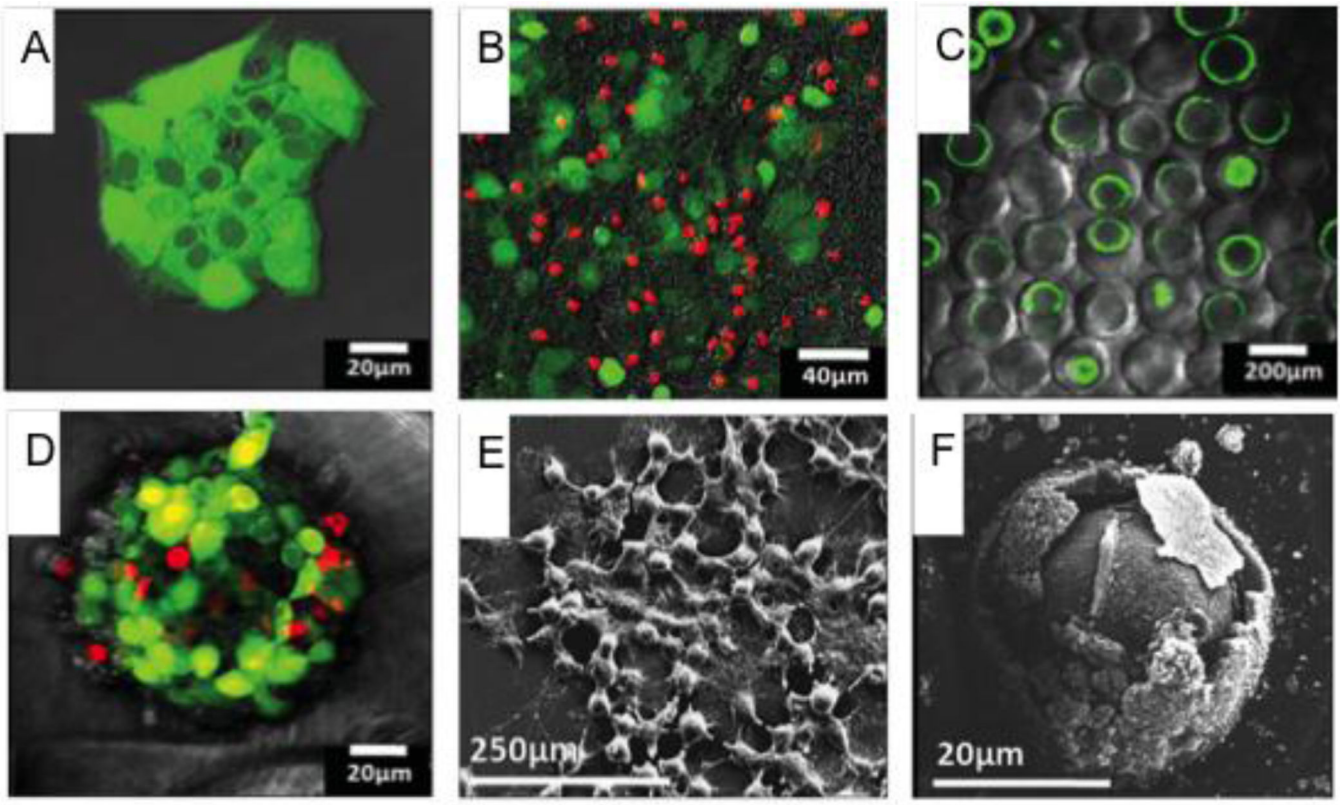

Fig. 1.

The application of 3D scaffold-based multicellular tumor spheroids in drug testing. A–D) Confocal micrographs of live/dead staining showing (A) 2D culture and (C) 3D spheroid culture. B) 2D culture revealed significant cell death after CdTe NP exposure. D) Spheroids showed much lower cell damage (especially in the central area) than the cells in 2D culture. E, F) SEM images of (E) 2D culture and (F) 3D spheroid culture at 24 h post CdTe treatment. E) In 2D culture most cells were dead with a large amount of cells detached. F) Cells in spheroids experienced much lower cellular damage than those in 2D culture. Reproduced with permission from Ref. [39].

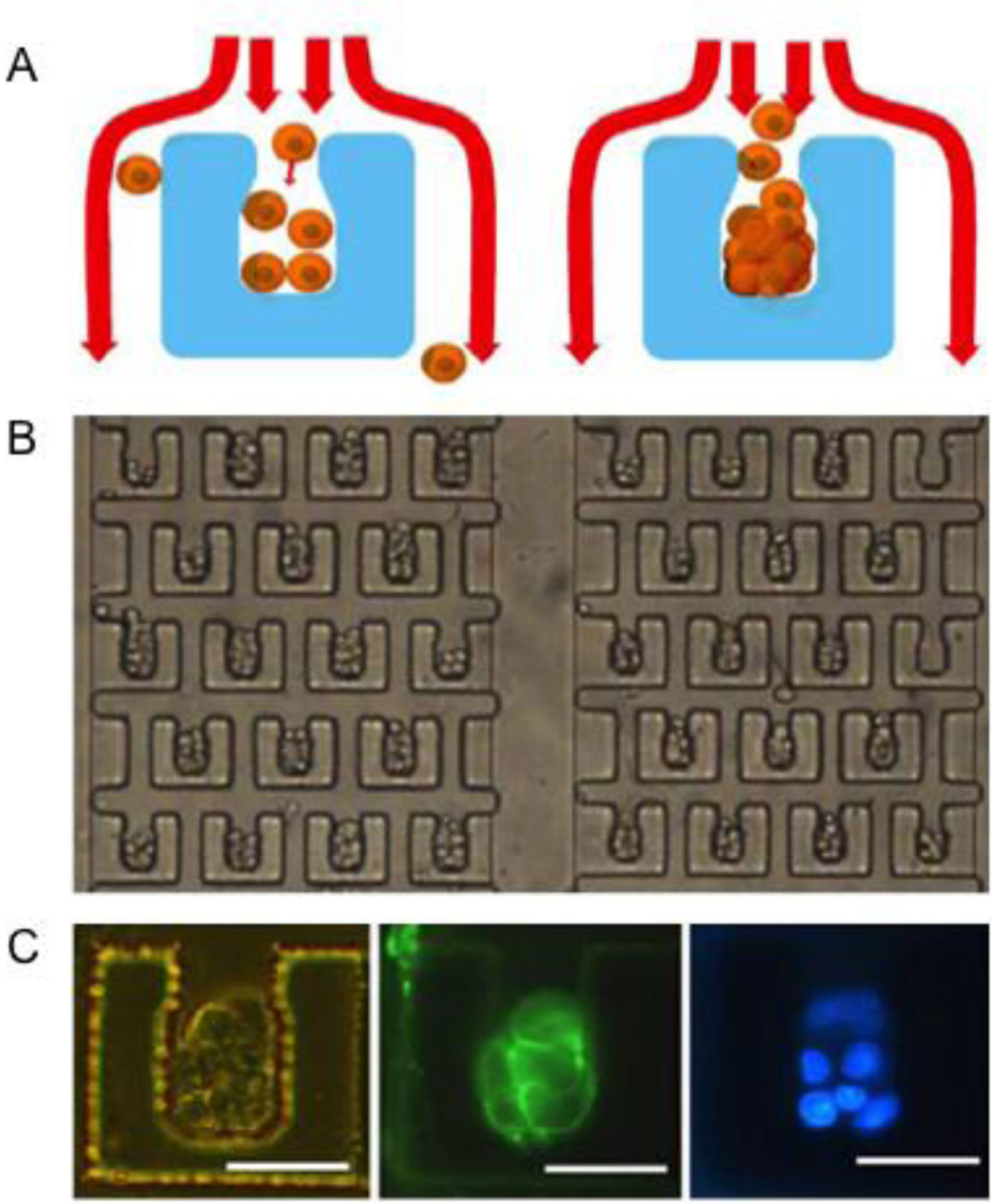

Fig. 2.

The application of microfluidic bioreactors for drug testing. A) Schematic showing the tumor spheroid formation and culture in a microfluidic flow chamber. B) Optical micrograph showing that MCF-7 cells could uniformly fill all the traps. C) Optical micrographs showing the structure of the tumor spheroids: (left to right) phase contrast image and fluorescence images showing cell membrane and nuclei. Reproduced with permission from Ref. [53].

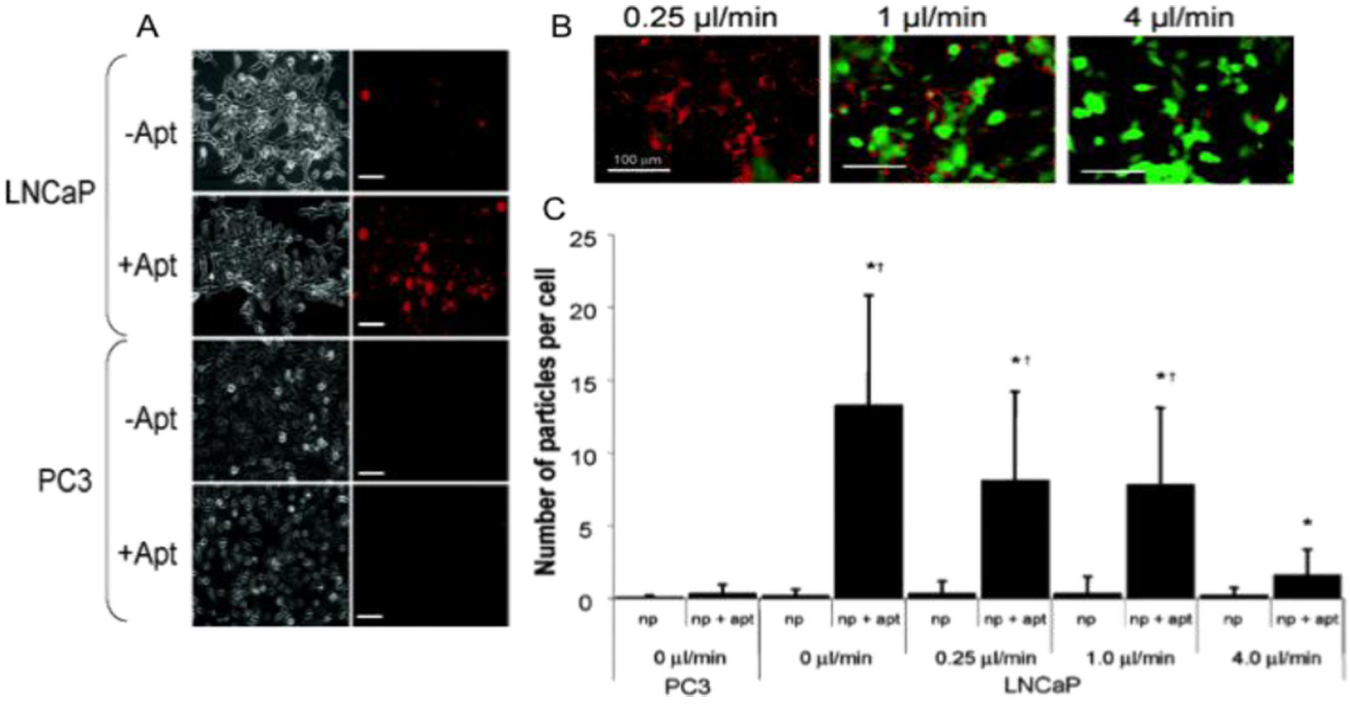

Fig. 3.

The application of microfluidic system for studying nanoparticles and cells interactions. A) Fluorescence images showing binding of rhodamine-labeled nanoparticles, without or with PSMA aptamer, to PSMA-positive LNCaP cell and PSMA-negative PC3 cells under static conditions. The left column shows phase contrast images of the cells and corresponding fluorescence images of the nanoparticles are indicated in the right column. B) Fluorescence images showing binding of rhodamine-labeled nanoparticle-aptamer conjugates (red) to LNCaP cells under fluid flow conditions at flow rates of 0.25, 1, and 4 µL/min. The cells were stained with Calcein AM (green) and DAPI (blue) to quantify cell viability. C) Quantification of the number of nanoparticles bound to PC3 cells under static condition and to LNCaP cells under both static and fluid flow conditions. Reproduced with permission from Ref. [11].

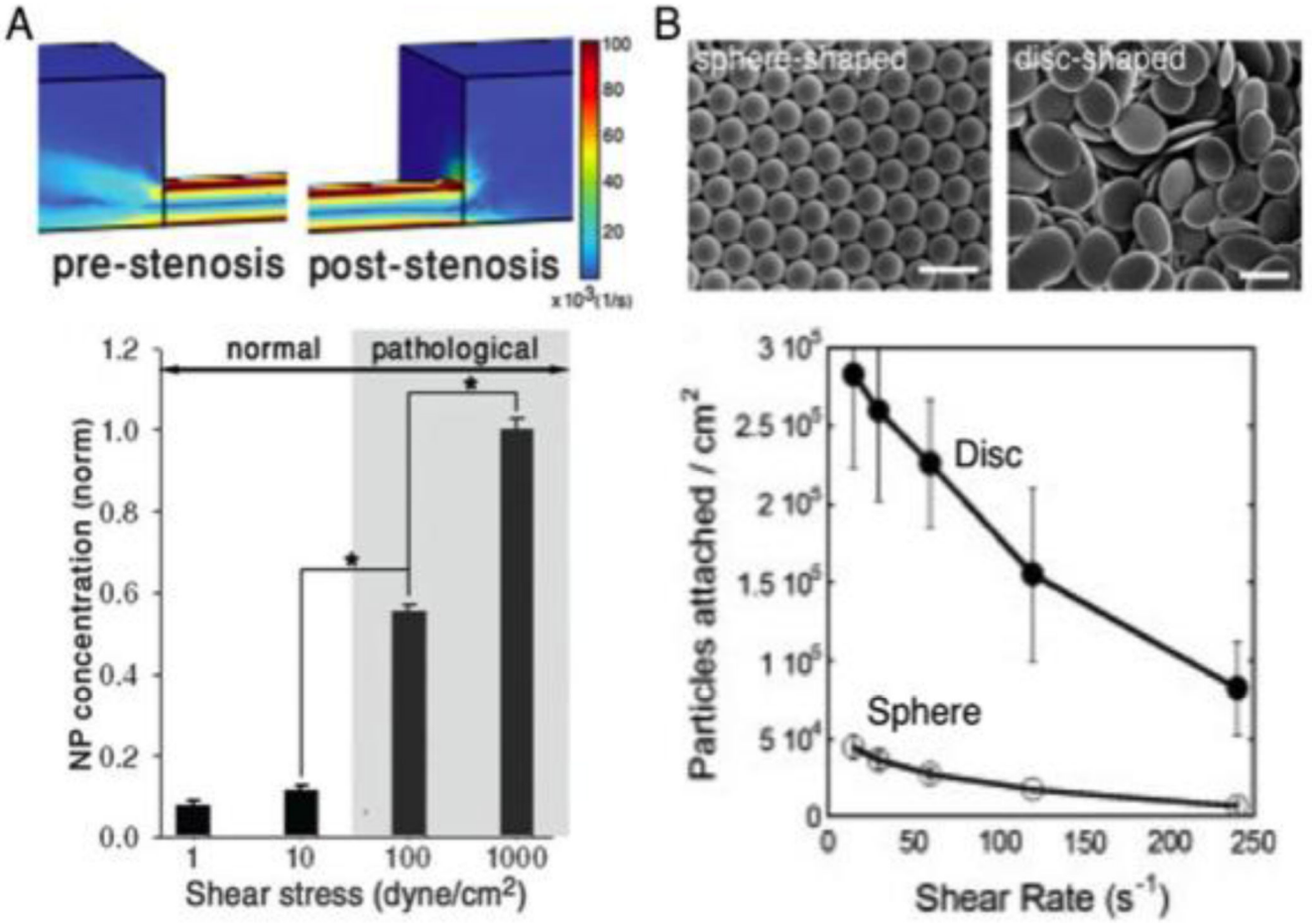

Fig. 4.

Microfluidic platforms provide insights on flow dynamics and shape influence for carrier attachment. A) Stenotic regions have higher shear rate (top) and accumulate more nanoparticles (NP; bottom). B) Influence of particle shape (top) on attachment and accumulation in a 45° bifurcation, showing that discshaped particles attach more than spherical particles (bottom). Reproduced with permission from Ref. [63] & [66].

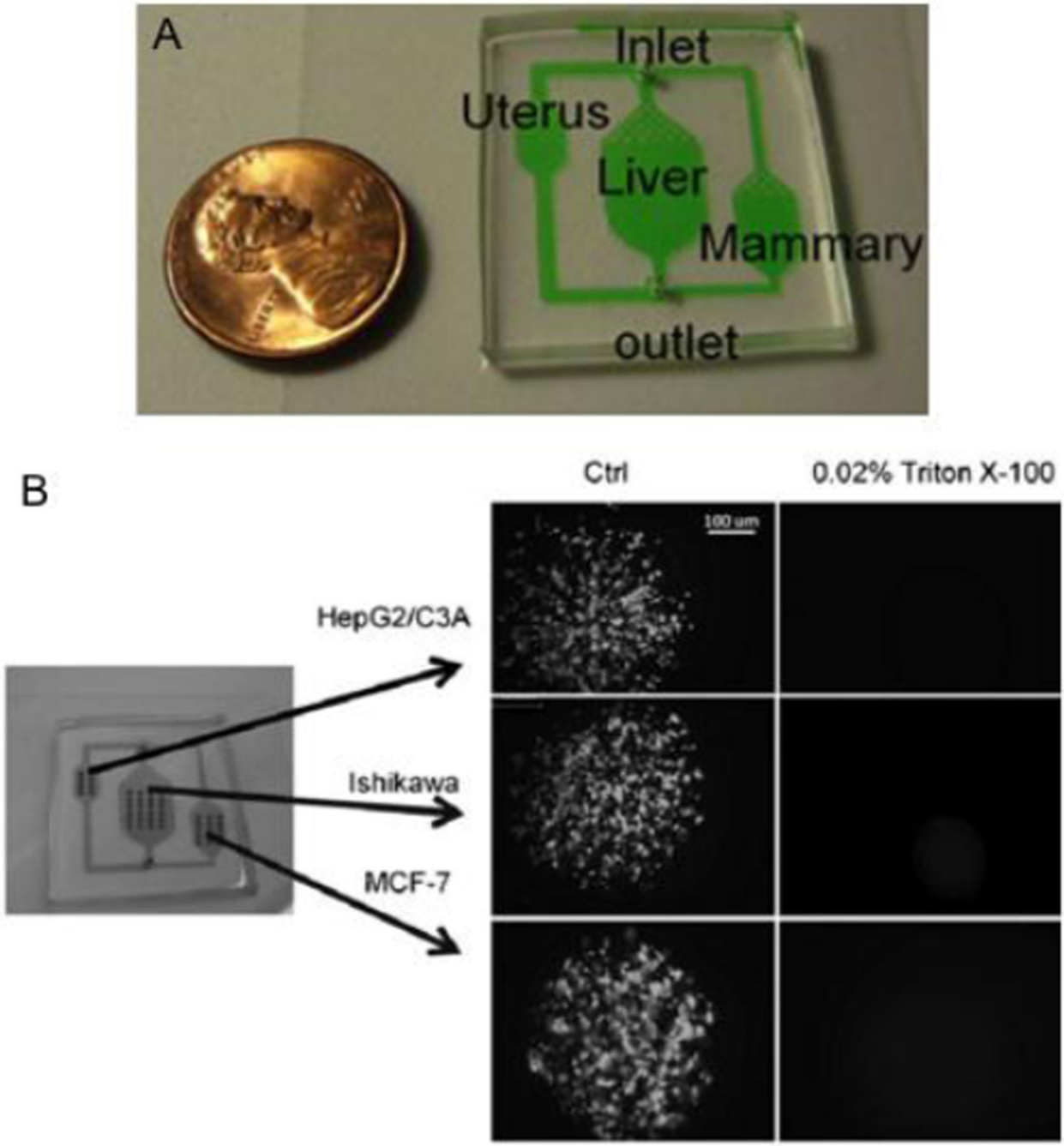

Fig. 5.

The multi-chamber μCCA device. A) A PDMS μCCA device consisting of three chambers, which represented uterus, liver, and mammary tissues, respectively. B) Measurement of cell viability after treatment with 0.02% Triton. Triton treatment led to full mortality for all cell types. Reproduced with permission from Ref. [101].

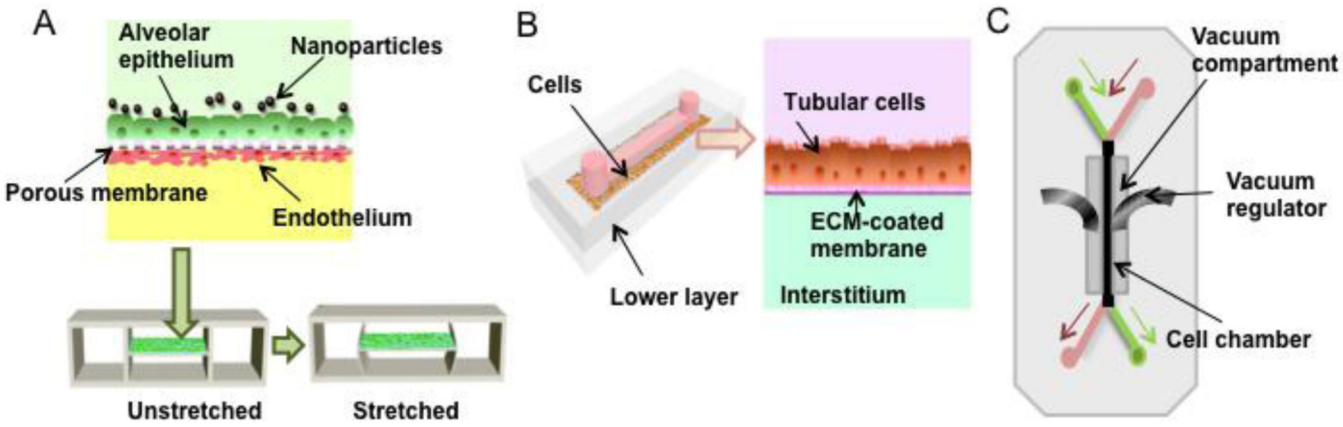

Fig. 6.

A) Schematic of the lung-on-a-chip platform developed to study nanoparticle transport. The transport of nanoparticles across the alveolar-capillary interface is augmented significantly when vacuum is applied to the side channels of the lung platform. Adapted from Ref. [13]. B) Schematic of the Kidney-on-chip platform with two chambers divided by a porous membrane that separates the 'luminal' from the 'tubular' space recreating the in vivo conditions in the kidney. Adapted from Ref. [118]. C) The gut-on-a-chip platform includes two compartments divided by a porous membrane separating Caco-2 cells from the bottom chamber. A vacuum regulator is attached to the side chambers to assess the behavior of the cells under the effect of strain. Adapted from Ref. [55].

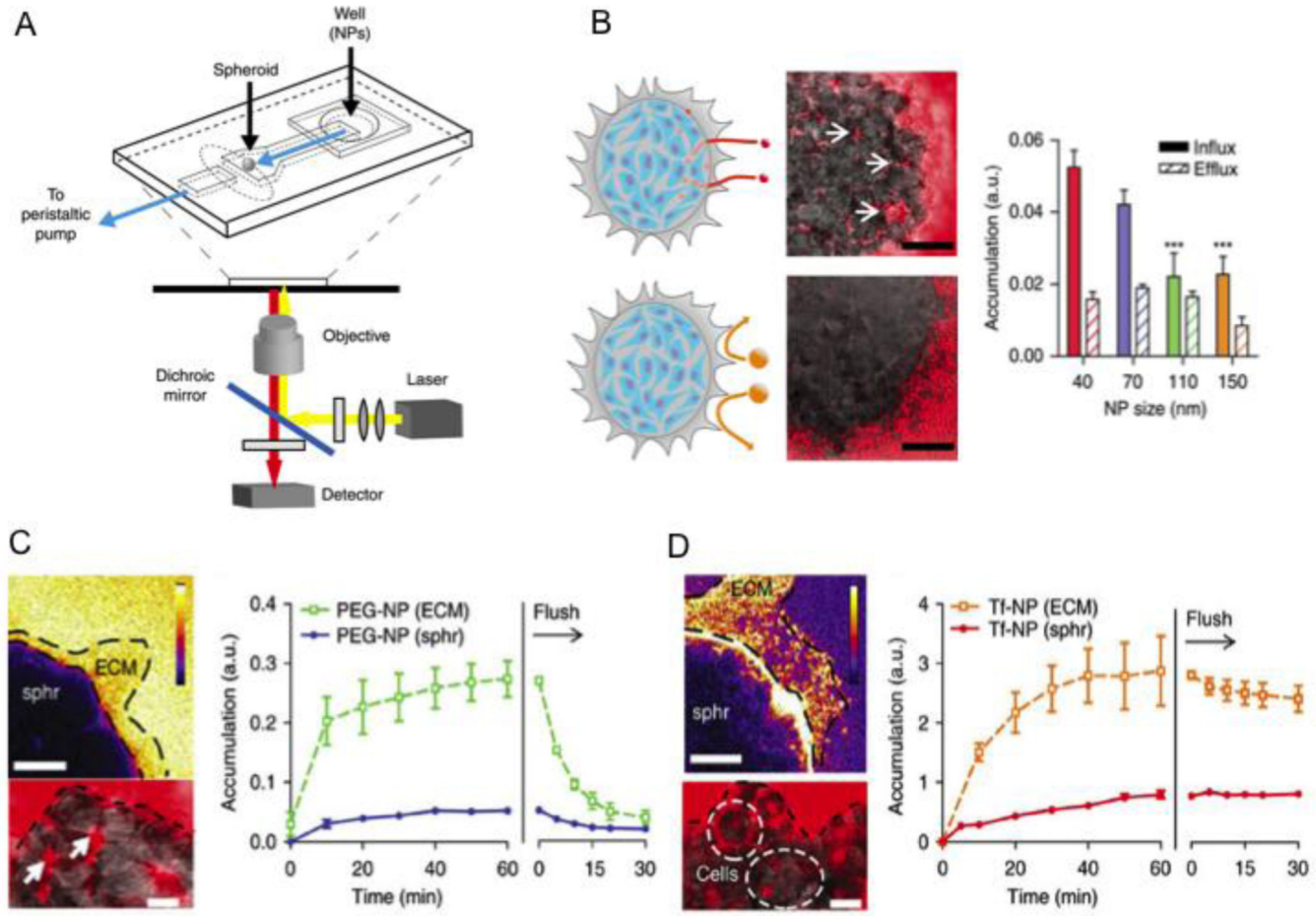

Fig. 7.

Studying the nanoparticle (NP) tissue transport behavior using tumor-on-chip platform. A) The schematic of the microfluidic chip assembled on top of a microscope stage. B) The schematic (left), image (center) and graph (right) showing the effect of NP size on tissue accumulation. Four different sizes, 40 (red), 70 (blue), 110 (green) and 150 (orange) of PEGylated NPs were investigated. C–D) The effect of NP functionalization on tissue accumulation: C) PEGylated NPs and D) iron-transporting transferring (Tf) protein functionalized NP. Reproduced with permission from Ref. [128].