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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, 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, possesses a high drug loading capacity and can 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 nanoscale 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 have 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 cancer [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.

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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 the 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 drug 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 the 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 toward 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 were 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

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