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Promises, challenges and future directions of μCCAs

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ABSTRACT

Micro-cell culture analogs (μCCAs) are a class of in vitro tissue analogs that combine multiple organ analogs on one microfluidic platform in physiologically correct volume ratios. The microfluidic platform also provides fluid flow rates and substance residence times close to those present in the human body. Several advantages arise from the microfluidic format that can be exploited for realistic simulations of drug absorption, metabolism and action. We envision that, together with theoretical modeling, μCCAs may produce reliable predictions of the efficacy of newly developed drugs. Advantages, challenges, and future directions of μCCAs are discussed and examples of systems are provided.

1. Introduction

Drug discovery has greatly benefitted from the development of in vitro tissues that can be used to screen libraries of chemicals for new drug candidates. Screening experiments are typically carried out in multiwell plates, in which the cell culture based tissues are exposed to drug candidates. Such assays are easy to carry out and allow for quick testing of drug candidates. Because immortalized cells as well as isolated primary cells can change their behavior significantly when cultured in an environment outside the body, the results obtained from these tests are only an estimate of what might happen inside the body. For example, Caco-2 cell systems simulate the uptake of nutrients and drugs through the intestine with sufficient predictability for passively absorbed compounds, but the data obtained with some of these systems for carrier-mediated and paracellularly transported compounds indicate that they do not always accurately reflect the in vivo uptake of these compounds (Stewart et al., 1995). Further, the multi-cellular, three-dimensional architecture of organs necessary for authentic cell function is not present in multiwell plates. On the other end of the spectrum of drug screening tools are whole, perfused animal organs. Technologies have been developed that allow whole organs to stay intact ex vivo and maintain a viable state for a reasonable period of time (for example, see Bekersky and Colburn, 1981; Feldhoff et al., 1977). In comparison to in vitro tissues, perfused, whole organs provide testing conditions that are often much closer to those present in vivo. Their preserved three-dimensional structure, native cellular architecture, and the presence of vasculature and substances such as hormones all support a more authentic cell behavior. However, dynamic regulatory changes due to nervous and immune regulations may not be captured even with these pseudo organs (Leung, 2009). Perfused organs are also typically isolated and hence do not provide a realistic simulation of drug metabolism if several organs participate in a particular drug's metabolism.

In this paper we are reviewing a relatively new, in vitro drug screening tool that provides an intermediate between cell-based assays and experimentation with perfused organs. Micro-cell culture analogs (μCCAs) are a particular class of in vitro tissue models in which a drug can challenge a combination of several tissues arranged in physiologically correct order. Microfabrication has provided the opportunity to integrate these tissues into microfludic devices. Because of the small dimensions of microfluidic structures, the cells cultured within μCCAs experience physiological conditions such as realistic liquid to cell ratios, fluid residence times that approach those seen in vivo, and physiological shear forces, typically below 2 dynes/cm^2. Assumptions made for theoretical, physiologically based pharmacokinetic (PBPK) models provide guidance to correctly design the geometry of μCCAs (see Fig. 1). According to these assumptions, organs can be represented as compartments that absorb, metabolize, and excrete substances. Differential equations describe these processes in the theoretical model. In μCCAs, which can be thought of as physical representations of PBPK models, microfluidic chambers represent each organ, fluidic channels provide in and outflow, and the cells cultured within provide the metabolic function. If the cells have retained most of their original function, they exhibit their complete metabolism while challenged with a chemical or drug. Hence they include reactions unknown to us and therefore not included in

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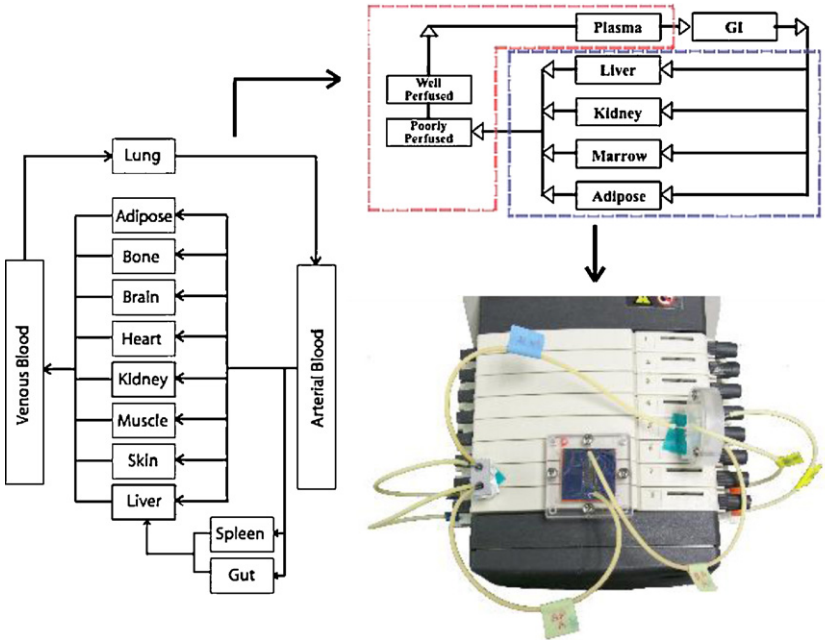

Fig. 1. A physiologically based pharmacokinetic (PBPK) model of the human body is used as the basis for the design of μCCAs. In this example, four organ analogs are placed on a microfluidic chip, while the remaining organs are combined in off-chip wells. A gastrointestinal tract module is added as an off-chip module to simulate the absorption of orally taken drugs. Microfluidic channels and interconnects allow for the recirculation of a medium.

theoretical models. The physiologic design of μCCAs with its combination of organs and microfluidic characteristics provides μCCA with advantages over other in vitro tissue models.

Since the first successful design of a μCCA (Sin et al., 2004), several other μCCAs have been developed. They demonstrate the described concept and show that complex mechanisms can be simulated with them. Here we provide a brief review of their advantages as well as an outlook into the future of μCCA development.

2. Advantages

Drug development is an expensive undertaking that benefits from screening identified drug candidates with cell culture based in vitro assays. Such assays provide data that inform researchers whether to pursue the development of a particular drug candidate or not. In addition to conventional in vitro tissue models, microcell culture analogs can provide an opportunity to test drugs in a more complex environment that includes analogs of several types of tissues. Advantages of μCCAs that derive from their microfluidic format are outlined below and examples of devices that demonstrate these are provided.

The presence of several organ analogs in μCCAs allows us to investigate their synergistic response to a drug. Often, a drug will produce the desired therapeutic effect in the target organ, but its metabolites cause unwanted side effects in other organs. In μCCAs, the systemic circulation represented by microfluidic channels carries metabolites to other tissue compartments where cell stress can be monitored. We can reproduce such interactions in μCCAs that contain several relevant organ compartments. To demonstrate this, we have challenged these μCCAs with known toxins/drugs such as naphthalene (Viravaidya et al., 2004; Viravaidya and Shuler, 2004) and acetaminophen (Mahler et al., 2009). When the μCCA contained both liver and lung compartments, liver cells metabolized naphthalene to reactive metabolites (1,2-naphthoquinone and 1,2-naphthalenediol), which subsequently travelled to the lung compartment. Here the metabolites caused cell stress and decreased lung cell viability. Another μCCA designed with compartments for liver and gastrointestinal tract analogs, simulated the absorption of acetaminophen and its conversion within epithelial cells of the digestive system analog as well as the liver analog. The experiment replicated a dose-dependent toxic effect of acetaminophen and its metabolites on liver cell viability (Mahler et al., 2009). The results obtained with the μCCA was comparable to those published for in vivo studies with mice (Kola and Landis, 2004). These experiments have demonstrated that toxicological problems that involve two interacting tissues can be reliably addressed with μCCAs.

Since μCCAs are designed with an emphasis on recreating physiological relationships between organ compartments, and physiological fluid flow and drug residence times, it is possible to design experiments in which realistic drug concentrations can be tested. Compared to other in vitro systems, the liquid to cell ratio in μCCAs is closer to that found in vivo and the tested drugs come into contact with a realistic number of cells. Therefore, drug absorption and metabolism take place at a rate, closer to those seen in vivo. Potentially, results obtained from such experiments will let us eliminate those concentration ranges that cause harmful side effects, and estimate concentrations that are potentially of benefit to humans. Therefore the parameter space that is investigated further with animal experiments is reduced, potentially leading to fewer experiments needed to identify successful drug candidates and their effective concentrations. This is particularly useful if combinations of drugs need to be tested, as might be the case for the development of drug combinations needed to treat multidrug resistant cancer. When combining drugs, their concentrations as well as the order in which they are administered play an important role in the effect they cause. Knowledge about the effective concentrations ranges, eliminates unsuccessful drug combinations. Hence the required number of experiments could potentially be decreased.

We have also observed that testing combinations of drugs with μCCAs can reveal unexpected synergistic effects that cannot be seen in conventional multiwall plates. Experimenting with multidrug resistant (MDR) cancer cell lines (uterine cancer cell line MES-SA and its multidrug resistant variant, MES-SA/DX-5), we have found that combinations of three drugs that are not typically used in combination with each other are more effective in diminishing