Microfluidic Cell Culture Analog Devices to Mimic Animal Exposures to Toxins and Drugs

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Principal Investigator(s): Michael L. Shuler
User(s): Mandy B. Esch, Jong Hwan Sung, Changhao (Bobby) Yu

Abstract:
Our group has developed microfluidic in vitro devices that mimic the response of humans or animals to drugs, toxins, or nanoparticles. Each device, or cell culture analog (CCA), contains an array of pseudo tissues that are interconnected by microfluidic channels [1]. The recirculation of blood surrogate through the microchannels allows us to study tissue-tissue interactions, such as the breakdown of a parent compound in the liver and subsequent transport and reaction in the lung. We combine these in vitro device experiments with physiologically-based pharmacokinetic model simulations to predict toxin and drug dynamics in humans [2].

Summary of Results:
Using µCCAs to Test the Toxicity of Nanoparticles.
We have used micro cell culture analogs (µCCAs) to test the toxicity of nanoparticles. Because of their small size and surface to volume ratio, nanoparticles possess unique properties, which can be utilized in medical applications such as diagnostics and drug delivery [3]. Particles might, for example, carry drugs or alter the absorption of drugs and nutrients that are administered orally. However, little is known of the particle’s fate within the body and tissues. We have recently developed a µCCA that combines cell culture models of the liver and the intestinal epithelium of the gastrointestinal tract in a physiologically realistic way [4]. This model can be used to simulate the oral uptake of nanoparticles and other drugs.

To take full advantage of the capabilities of µCCAs, it is desirable to be able to continuously monitor cellular stress in addition to measuring cell viability. To this end oxygen sensors that report on the cell’s metabolic activity have been developed and were included in past devices [5]. Our recent efforts have focused on identifying and measuring marker molecules that indicate the condition of liver and gastrointestinal cells. For example, when the liver is damaged, liver cells release enzymes such as alanine transaminase and aspartate transaminase into the bloodstream. We have found that liver cells (HepG2/C3A) maintained in cell culture release cytosolic enzymes such as aspartate transaminase (AST) in significant amounts when stressed (results not shown).

We have used the liver/gastrointestinal µCCA together with AST measurements to simulate the absorption and first pass metabolism of carboxylated latex nanoparticles. The AST release indicates that both cell lines are stressed in a dose-dependant manner when exposed to nanoparticles. The AST release increases when the device is operated with liver and gastrointestinal cells as compared to operating it with liver cells only. It is possible that significant cellular stress in the gastrointestinal module is responsible for the increase. It is also conceivable that while crossing the gastrointestinal barrier, nanoparticles become modified.

Figure 1: Schematic of the µCCA concept. The human body can be described as a series of interconnected compartments as has been done for physiologically based pharmacokinetic (PBPK) models. On a µCCA device, microfluidic chambers physically represented these compartments in physiologically relevant order and with physiologically relevant fluid flow rates and residence times.
(through the modification of their carboxyl tags) so that they subsequently are more toxic toward liver cells.

**Development of Microfluidic GI Tract Module.** To obtain more detailed information from simulations with first pass metabolism µCCAs, we have developed a microfabricated gastrointestinal tract model that incorporates an on-chip membrane and integrated electrodes for transepithelial resistance measurements. The transepithelial resistance (TER) of the gastrointestinal epithelium is a measure for the intactness of its barrier function. To simulate the barrier function, gastrointestinal cells are cultured on membranes that allow access to either side (apical and basolateral) of the cell layer. Using GI-tract epithelial cells (Caco-2) and mucous-producing cells (HT-29), co-cultured and grown to confluence on commercially available transwell membranes, we were able to simulate the GI-tract barrier and measure the transepithelial resistance (TER) [4,6]. From experiments with these static cell culture models of the intestinal epithelium, we know that nanoparticles alter the TER. To be able to measure the TER on chip, we have developed microfabricated electrodes and membranes (see Figure 1) that allow us to include the gastrointestinal tract model on a microfluidic chip that contains the systemic circulation.

**Pumpless Operation of µCCAs.** A critical issue in microfluidics, including µCCA development, is that they require specialized techniques for assembly and operation, limiting their usability to non-experts. To simplify µCCA operation, we employed a novel, multi-layer design, which enhanced usability while allowing hydrogel-cell cultures of multiple types. Gravity-induced flow enabled pumpless operation and prevented bubble formation. Three cell lines representing the liver, tumor and marrow were cultured in the three-chamber µCCA to test the toxicity of the anticancer drug, 5-fluorouracil. The result was analyzed with a PK-PD model of the device, and compared with the result in static conditions. Each cell type exhibited differential responses to 5-FU, and the responses in microfluidic environment were different from those in static environment. A combination of a mathematical modeling approach (PK-PD modeling) and an *in vitro* experimental approach (µCCA) may provide a novel platform with improved predictability for drug toxicity and help researchers gain a better insight into the drug’s mechanism of action.

**References:**


