INTEGRATING CELL SHEETS FOR KIDNEY-ON-A-CHIP APPLICATIONS

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A drug being developed undergoes many stages of development to get to market. Information of the drug's absorption, distribution, excretion, metabolism, and systemic toxicology both short and long term are mandatory by regulatory agencies during clinical trials [1]. Drug-induced organ toxicity leads to 30% of all drugs failing to reach the market. Specifically, nephrotoxicity leads to 19% of all failures during phase III trials but only 2% during preclinical development stages. Current early stage tests for toxicity are widely perceived to be inadequate. 2D cell culture models can produce valuable data for drug discovery but do not accurately predict toxicity. A typical animal study to assess nephrotoxicity uses > 26 rodents, with substantially more animals if both sexes are required. An in vitro model that replaces or reduces animal use in toxicity testing is required for ethical reasons and to reduce species-specific effects. A drug can take 8-12 years and 0.8-1.2 billion US$ to get to market, hence there is a need for a more complex, human cell derived, in vitro model to accurately predict drug toxicity and reduce failure rates during the pre-clinical to clinical transition in drug development.

Renal damage from toxicity can occur in a number of kidney compartments, but most toxic and drug induced injuries affect the proximal tubules either as a primary or secondary effect. Such damage can lead to systemic consequences [2]. The proximal tubule is a single layer of epithelial cells bound together by its extracellular matrix (ECM) that mediates the blood's composition through defined paracellular and transcellular pathways. The challenge is to develop a microfluidic tissue assay that models injury to the proximal tubule that is observed in nephrotoxicity. Ideally the assay should mimic the tubular structure of the kidney and reproduce the tubular response to known nephrotoxicants.

Prior reports in the literature have proposed designs for microfluidic tissue assays similar to that illustrated in Fig. 1. Here a filtration structure that mimics the proximal tubule is placed on a membrane that separates two chambers in a trans-well cell culture configuration [3,4]. In all these cases proximal tubule epithelial cells are grown on a micro- or nanoporous polymer membrane within a microfluidic bioreactor. The need for a supporting substrate in these assays may reduce their biomimetic features of the cellular environment altering ECM construction, chemistry, complexity, and mechanical properties.

Here we present a study of a proximal tubule model that uses cell sheets [13] without the need for a parallel supporting structure other than that generated by the cells' ECM. We have utilized temperature responsive PIPAAm coated culture plates to harvest sheets of cells without digestive enzymes thus keeping the ECM intact. We then suspended this cell sheet (CS) over circle or rectangle holes in a Polydimethylsiloxane (PDMS) sheet. This process was developed in order to imbed a suspended cell sheet within a bioreactor.