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Integrating cell sheets for organ-on-a-chip applications

[a]William Loewenhardt, [b]Rachel Saunders [c]Rachel Lennon, [a,b]Brian Derby*

[a]*School of Materials, University of Manchester, Manchester M13 9PL, UK*
[b]*Manchester Centre for Digital Fabrication, University of Manchester, Manchester M13 9PL, UK*
[c]*School of Biological Sciences, University of Manchester, Manchester M13 9PL, UK*

* Corresponding Author Tel.: +44-1613063569. *E-mail address:* brian.derby@ manchester.ac.uk

Abstract

To improve on attrition rates during drug development due to nephrotoxicity, a better *in vitro* model of the kidney is needed. We demonstrate a model of the kidney proximal tubule in which a sheet of proximal tubule epithelial cells is fixed as a membrane separating two fluid chambers without the presence of a supporting membrane. Cell sheets were prepared using a Poly(N-isopropylacrylamide) (PIPAAm) temperature responsive surface and a bespoke bioreactor was constructed using a 3D printer to produce an appropriate mould. Cell sheets were suspended using a fibronectin functionalized surface seeded with cells. Further testing is needed to determine viability in bioreactor.

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Keywords: cell sheet engineering; PIPAAm; temperature responsive; PDMS; bioreactor; kidney; microfluidics; organ on a chip

Nomenclature

PIPAAm	Poly(N-isopropylacrylamide)
ECM	Extracellular matrix
PDMS	Polydimethlysiloxane
CS	Cell sheet
PBS	Phosphate buffered saline

1. Introduction

A drug being developed undergoes many stages of development to get to market. Information of the drugs 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 they cannot accurately predict toxicity. A typical animal study to assess nephrotoxicity uses at least 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 also to reduce species-specific effects. Considering that a drug can take 8-12 years and 0.8-1.2 billion US$ to get to market, [2] there is a requirement 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. This need is fulfilled by organ-on-a-chip systems which utilize microfluidic technologies and tissue engineering to control the *in vitro* microenvironment of cells in a bioreactor, mimicking the real tissues characteristics.

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 [3]. The proximal tubule is a single layer of

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epithelial cells bound together by its extracellular matrix (ECM) that mediates the bloods composition through defined paracellular and transcellular pathways [4]. The challenge is to develop a microfluidic tissue assay that models injury to the proximal tubule that is observed in nephrotoxicity. Ideally

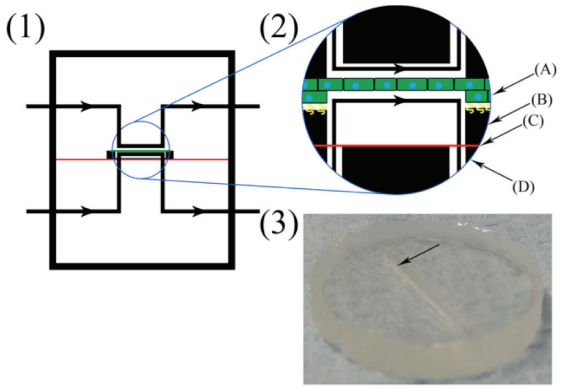

Fig. 1. (1) Schematic of membrane placement in bioreactor; (2) enlargement of the (A) cell sheet, seeder layer, and fibronectin (B) PDMS sheet (C) line showing gap between bioreactor halves (D) the bottom bioreactor: (3) test run of the PDMS sheet.

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 [5, 6, 7, 8, 9, 10, 11]. 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 [12].

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.

2. Materials and methods

2.1. Cell sheet suspension

2.1.1. Cell type

The cell type we used was 3T3 Swiss Albino (ECACC 85022108, Salisbury, UK) and human proximal tubule HK-2 cells (ATCC® CRL-2190™, Middlesex, UK).

2.1.2. Cell culture

Dulbecco's Modified Eagle's Medium- Low glucose (Sigma Aldrich, Gillingham, UK), supplemented with fetal bovine serum to 5% was used to culture cells in T75 tissue culture flasks (Greiner Bio-one GmbH, Frickenhausen, Germany) for 1 - 1.5 weeks.

2.1.3. Cell sheet harvesting

PIPAAm surface coated UpCell Nunc Muiltidishes® (Scientific Laboratory Supplies, Nottingham, UK) were seeded with 3T3 cells and grown to confluence (1−1.5 weeks). All media was removed and a further 500µl added to nourish the cells through this step.

2.1.4. PDMS sheet functionalization

First a PDMS sheet was washed with PBS and then human plasma fibronectin purified protein (Millipore Limited, Watford, UK) at 25µg a PDMS sheet was added for 20 minutes. The PBS was then removed and media added. 3T3 cells were then seeded and grown for 1-1.5 weeks. Multiple attachment protocols were attempted as seen in Table. 1.

Table 1. PDMS sheet preparation determining cell sheet attachment.

Group	Fibronectin	Cells	Cell sheet?
1	-	-	-
2	X	-	-
3	-	X	-
4	X	-	X
5	X	X	X

2.1.5. Cell sheet application

PDMS sheets were placed inverted on the UpCell dishes and incubated for 50 minutes. The plates were removed and 1ml cold media added. The cells were allowed to detach for another 45 minutes and the PDMS sheet removed to another empty culture dish and unflipped. To determine if immobilization had occurred, excess media was added to submerge the cell sheet whereby non-attached sheets would lift up and wash away. The media was then removed and the sheets stained.

2.2. Bioreactor construction

Two bioreactor designs were constructed with windows for cell sheet attachment fabricated in a 1 cm diameter PDMS sheet: i) 2 mm diameter window in 1.5 mm thick sheet, ii) a 1 x 5 mm rectangle in a 1 mm thick sheet. An OBJET30-Pro 3D printer (Stratasys, Eden Prairie, USA) was used to print moulds using VeroBlue RGD840 (Stratasys) build material. The moulds power washed with water and allowed to air dry for 24 hours in a closed but ventilated container to reduce dust accumulation. The first PDMS casting from each mould was discarded because of interactions between the build material and the initial polymerization reaction.

Silicone elastomer (SYLGARD 184 Dow Corning, Seneffe, Belgium) was weighed at 10:1 ratio base:curing agent and mixed by hand for 10 minutes using a pipette tip.