3D printed system for testing intestinal drug transport

Jepsen, Morten Leth; Nielsen, Line Hagner; Almdal, Kristoffer; Boisen, Anja; Dufva, Hans Martin

Publication date: 2018

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
3D printed system for testing intestinal drug transport

Morten Leth Jepsen1; Line Hagner Nielsen2; Kristoffer Almdal3: Anja Boisen4; Martin Dufva5

1The Danish National Research Foundation and Villum Foundation’s Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsteds plads 345C, 2800 Kgs. Lyngby, Denmark, mojep@dtu.dk,
2lihan@dtu.dk, 3kral@dtu.dk, 4anja.boisen@dtu.dk, 5martin.dufva@dtu.dk

INTRODUCTION
When in vitro investigating permeability of orally administrated drugs, Caco-2 cells grown on filter membranes are normally utilized1. However, these filters are far from the in vivo growth matrix for intestinal cells, which is very soft. We here present a method for casting a soft gelatin hydrogel in a 3D printed holder as growth matrix for intestinal cells. The method is easy to use and relies on the use of a commercially available Form 2 3D printer.

METHOD AND MATERIALS
Design of 3D prints were drawn in Fusion 360 and transported as an STL file for preparation for 3D printing in PreForm. The 3D printed inserts were printed in Dental SG resin and cleaned with isopropanol followed by UV cross-linking for 1 hour at 60°C and autoclaved. A 5 % (w/v) gelatin hydrogel in PBS was cross-linking into the 3D printed holder with 5 U/mL mTransglutaminase for 30 minutes. Followed by seeding Caco-2 cells in 500 µL (3×105 cells) apically in 3 mL medium basolaterally. The cells were grown for 28 days at 37°C and 5 % CO2 changing the medium every other day. For comparison, Caco-2 cells were seeded on a Corning® 12 transwell plate. transepithelial electrical resistance (TEER) values were measured at room temperature with a Millicell® ERS-2 Voltohmmeter. Moreover, Young’s modulus was measured at 37°C using s Discovery Hybrid Rheometer 2 with a 40 mm parallel plate and a steel Peltier plate. Mineral oil was added around the sample to ensure no evaporation of water from the hydrogel.

RESULTS
3D printed biocompatible inserts have been designed (Figure 1a, b) with a size of 11 mm in height and 10 mm in diameter. for culturing Caco-2 cells for testing permeability of drugs. A gelatin hydrogel was casted into the 3D printed inserts and Caco-2 cells were seeded on top of the hydrogel (Figure 1c). The 3D printed insert was inserted into a commercially available 12-well culturing plate (Figure 2).

Table 1. Characteristics of hydrogels in 3D printed inserts compared to commercial inserts with filters.
The softer growth matrix resulted in Caco-2 with a smaller diameter than Caco-2 cells grown on a tissue culture plate (Figure 3). Black spots on Figure 3a are due to cells adhering slightly worse on gelatin, thus the cells tend to grow more on top of each other. The smaller diameter of the cells resulted in a higher ratio of intercellular space to cells membrane hence, the resistance was lower. Growing Caco-2 cells on hydrogel resulted in lower TEER value (Table 1), however, the value of Caco-2 on the hydrogel is within the range reported for Caco-2 cells (62 to 1290 Ω·cm²). However, the hydrogel itself also raises the resistance.

CONCLUSION
The presented system is easy applicable for transport studies over a monolayer of Caco-2 cells. Moreover, the gelatin hydrogel is clear and suited for microscopy and can be cut out of the insert for fluorescent staining. The presented permeability test system also has the capability of being used for growth of cells on the underside of the hydrogel for basolateral to apical transport studies. Furthermore, the cells can be casted into the gel for advanced cocultures.

REFERENCES
