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3D printed system for testing intestinal drug transport

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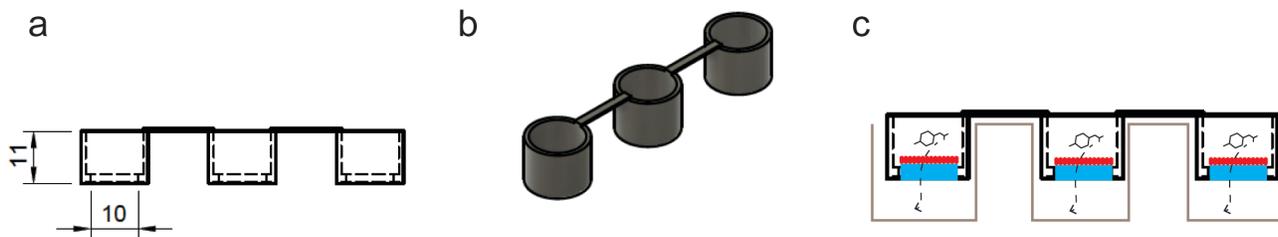


Figure 1. (a) Drawing of 3D printed insert design. Numbers in mm. (b) Graphic rendered image of 3D print design. (c) Illustration of transport principle for 3D printed inserts with a hydrogel matrix.

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.



Figure 2. Image of casted hydrogel in 3D printed inserts.

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 μ L (3×10^5 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). Once the cells had proliferated and differentiated to a tight monolayer (after 28 days), drug transport and permeability was investigated as with a commercially available Transwell system (Figure 1c). This makes the method easy applicable in ordinary laboratory settings. Caco-2 cells grown on a gelatin hydrogel have a softer matrix to grow on (Table 1), thus, the growth is closer to the *in vivo* situation.

	Hydrogel	Polyester membrane
TEER ($\Omega \times \text{cm}^2$)	310.7 \pm 42.1 (day 28)	983.6 \pm 226.5 (day 22)
Young's modulus (Pa)	1080	N.A.

Table 1. Characteristics of hydrogels in 3D printed inserts compared to commercial inserts with filters.

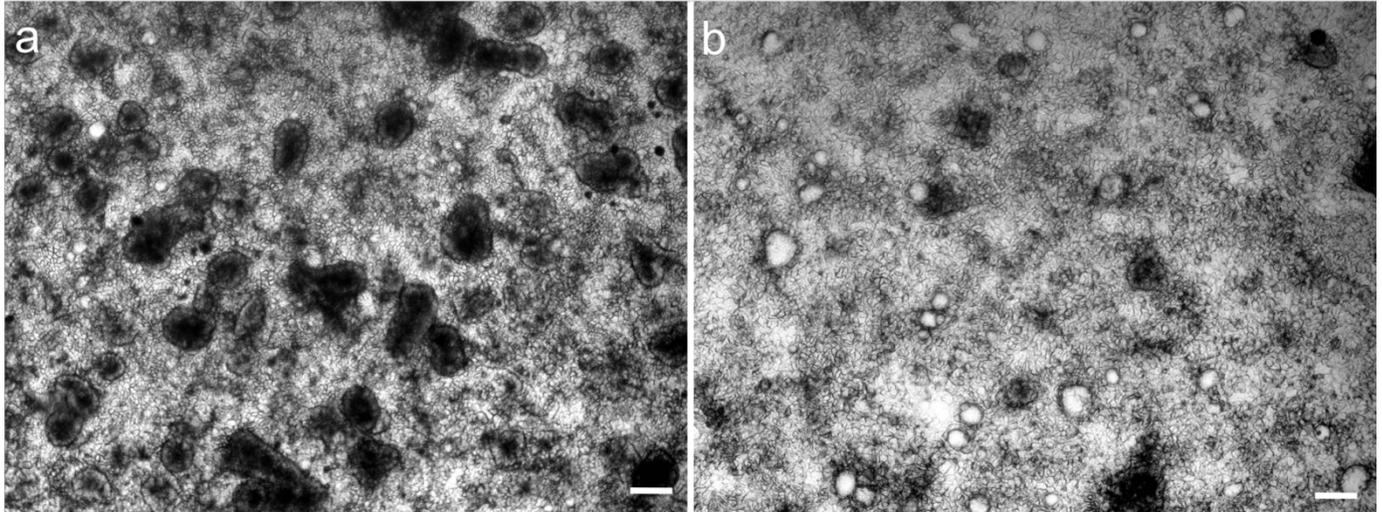


Figure 3. (a) Caco-2 cells grown on a gelatin hydrogel. scale bar: 100 μm . (b) Caco-2 cells grown on tissue culture plate. scale bar: 100 μm .

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 $1290 \Omega \times \text{cm}^2$). 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

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