Application note

Modelling the liver-brain axis using NANOSTACKS™

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Introduction

The liver plays an important role in the metabolism of compounds such as pharmaceutical drugs (1). The chemical modifications of compounds occurring during their metabolization lead to the generation of metabolites, which can exert toxic effects on human organs (2). Therefore, when assessing the toxicity risk of a drug on a particular target organ, it is advantageous to use *in vitro* models including a liver cellular component to simulate the metabolization of the compound.

In addition to its role in drug metabolism, the liver interacts with other organs of the human body such as the brain, particularly in pathological conditions such as Alzheimer's Disease (3) and Parkinson's Disease (4). To further improve our knowledge of such pathologies and develop novel therapies, the use of an *in vitro* model of the liver-brain axis would be advantageous.

In this work, we developed an *in vitro* model of the liver-brain axis using NANOSTACKS™. In particular, the neuronal cell line SH-SY5Y and the astrocytoma cell line 1321N1 were included to model respectively neurons and astrocytes, whilst the hepatic cell line HepaRG® was included to model the liver component. The model was characterised and then used as a platform to assess the neurotoxicity of the drug paclitaxel.

Methods

Collagen coating of NANOSTACKS™

NANOSTACKS™ were coated with collagen I (A10483-01; Gibco) by submerging their porous membrane with 60 µL of collagen I solution at a concentration of 27.5 µg/mL for 1 hour at room temperature. Collagen-coated NANOSTACKS™ were then washed 3 times for 5 min with distilled water, then dried overnight at room temperature.

HepaRG® culture on NANOSTACKS™

During the thawing and seeding procedures, HepaRG® cells (cryopreserved differentiated HepaRG® cells, HPR116010; Wepredic) were cultured using a cell culture medium termed "thawing medium", obtained by adding HepaRG® Thawing/Plating/General Purpose Medium supplement with antibiotics (ADD670C; Wepredic) to Williams E Medium with Glutamax™(32551020; ThermoFisher Scientific) according to the vendor's instructions. Cells were seeded on collagen-coated NANOSTACKS™ placed on wells of 24-well plates at a seeding density of 72 x 10^3 cells per NANOSTACK™. In particular, during the seeding procedure, a droplet of 75 µL of cell suspension was placed on the membrane of the NANOSTACKS™, which were then incubated at 37 °C, 5% CO_2 for 2.5 hours. Subsequently, 825 µL of thawing medium were added to the wells containing the cell-seeded NANOSTACKS™, reaching the working volume of 900 µL. The next day, and every 2-3 days, cell culture medium was changed using a medium termed "maintenance medium" obtained by adding HepaRG® Maintenance/Metabolism Medium Supplement with antibiotics (ADD620C; Wepredic) to Williams E Medium with Glutamax™ according to the vendor's instructions.

Cell viability and CYP activity assays on HepaRG®

Cell viability was assessed by performing a CellTiter-Glo® (G7571; Promega) assay, and CYP activity was assessed by performing a P450-Glo™ CYP3A4 Assay (V9001; Promega) following the instructions of the vendor. Both assays were performed in 24-well plates, making sure that sufficient volumes were added to the wells to completely submerge the NANOSTACKS™ at any point when cell submersion was necessary. The cell culture medium used throughout the assays was termed "induction medium" and was obtained by adding HepaRG induction medium supplement with antibiotics (ADD640C; Wepredic) to Williams E Medium with Glutamax™ according to the vendor's instructions.

Drug testing on the liver-brain axis model

On day 0, SH-SY5Y and 1321N1 cells were seeded on NANOSTACKS™ at seeding densities of respectively 6.5 x 10^3 cells per NANOSTACK™ and 9 x 10^3 cells per NANOSTACK™, whilst HepaRG® cells were seeded on NANOSTACKS™ at a seeding density of 72 x 10^3 cells per NANOSTACK™ as previously described, with the following modifications with regards to the culture of SH-SY5Y and 1321N1: the cell culture medium used during the seeding procedure and at day 0, termed "neural medium", was composed of Dulbecco's Modified Eagle's medium (11995065; ThermoFisher Scientific) including 10 % foetal bovine serum, 1X MEM Non-essential Amino Acids Solution (M7145-100ML; Merck) and 1X Penicillin-Streptomycin (P0781; Merck), and upon cell seeding the medium required to reach the working volume of 900 µL was added 2 hours later. On day 1, NANOSTACKS™ including the 3 cell types were combined to initiate the triculture, using maintenance medium as cell culture medium at a working volume of 1.5 mL for both tricultures and for monocultures of SH-SY5Y used as control, and including paclitaxel dissolved in dimethyl sulfoxide at concentrations: 0 (vehicle control), 0.0001, 0.001, 0.01, 0.1, 1, 10, 50 µM. On day 3, the viability of SH-SY5Y cells was assessed by performing a CellTiter-Glo® assay as previously described, using neural medium whenever the protocol required the use of cell culture medium.

Statistical analysis

Each datapoint reported in this application note was derived from measures obtained from a minimum of n = 3 cell-seeded NANOSTACKS™.

Results

Upon seeding, HepaRG® cells formed a confluent layer (Fig. 1D) and were viable throughout the entire experiment up to day 9 (Fig. 1A), thus indicating that NANOSTACKS™ are compatible with HepaRG® cells. The CYP3A4 activity of HepaRG® cells was also maintained throughout the entire experiment (Fig. 1B). Upon normalisation of the data obtained from the CYP3A4 activity assay with the data obtained from the viability assay, no statistically significant difference was found between different timepoints.

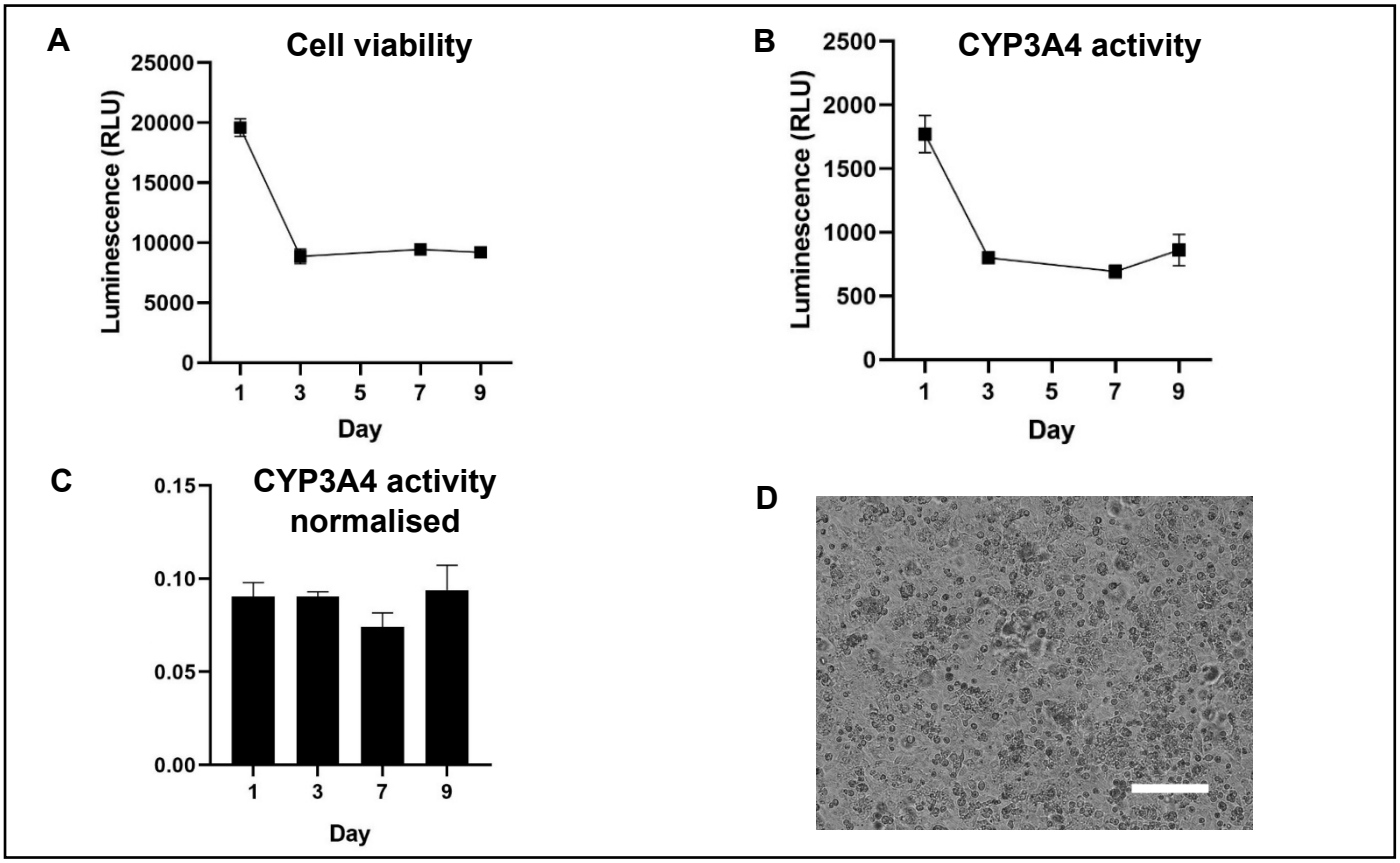

Figure 1. A: *Viability of HepaRG® cells expressed in relative lights units (RLU).* ***B:*** *CYP activity of HepaRG® cells expressed in RLU.* ***C:*** *CYP activity normalised with viability data.* ***D:*** *Representative widefield image of HepaRG® cells on NANOSTACKS™ on day 1. Magnification: 10X. Scale bar: 250 µm. Data are expressed as mean ± SEM.*

HepaRG®, 1321N1, and SH-SY5Y cells were combined on NANOSTACKS™ to obtain a liver-brain model (Fig. 2B). The effect on SH-SY5Y viability of the drug paclitaxel was tested on both the liver-brain model and on a monoculture of SH-SY5Y on NANOSTACKS™ (Fig. 1C). In particular, the concentration of paclitaxel used in this experiment ranged between 0.0001 µM and 50 µM, and the cells were exposed to the compound from day 1 to day 3, when the viability assays were performed and widefield images were acquired (Fig 2A). The IC50 value obtained from the dose response curve associated to the liver-brain model was 0.0684 µM. The IC50 associated to the triculture was 26.8 % lower than the IC50 associated with the SH-SY5Y monoculture, which was 0.0935 µM.

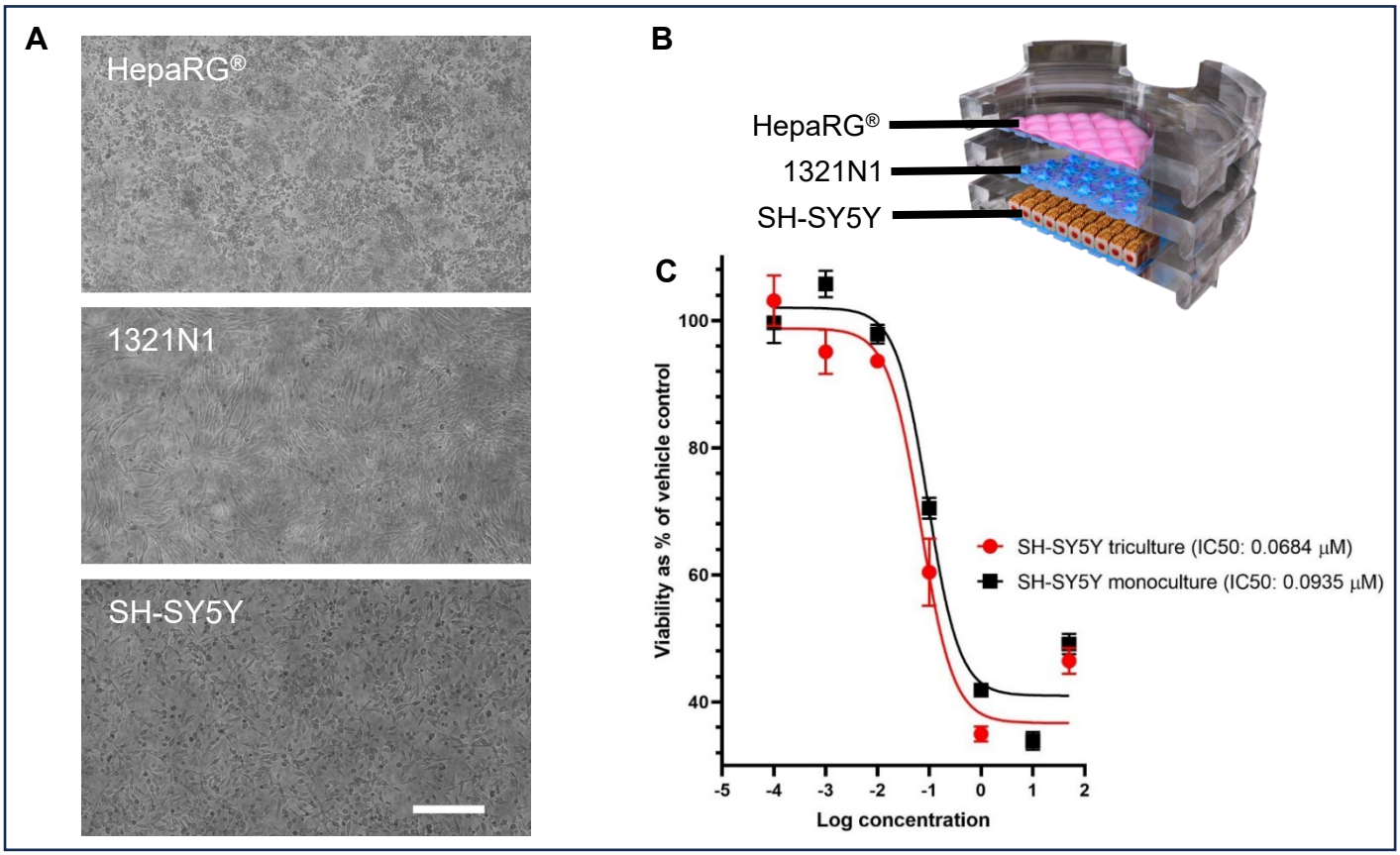

Figure 2. A: *Representative widefield images of HepaRG®, 1321N1 and SH-SY5Y cells in triculture on NANOSTACKS™ on day 3, associated with the vehicle control. Magnification: 10X. Scale bar: 250 µm.* ***B:*** *Triculture scheme.* ***C:*** *Paclitaxel dose-response curve and IC50 values for the triculture and the monoculture setups. Data are expressed as mean ± SEM.*

Conclusions

Differentiated HepaRG® cells adhered to NANOSTACKS™ and maintained their viability for 9 days of cell culture on the platforms. Additionally, HepaRG® cells were metabolically active, as CYP3A4 was detected across all timepoints. Therefore, HepaRG® cells can successfully be incorporated into NANOSTACKS™.

HepaRG® were cocultured with the neural cell lines SH-SY5Y and 1321N1, recapitulating the liver-brain axis. The drug paclitaxel was tested on the liver-brain model, and the IC50 associated with the viability of SH-SY5Y cells in the triculture model was 26.8 % lower than the IC50 associated with the SH-SY5Y monoculture, suggesting that the liver component increased the model's sensitivity.

This work demonstrates the feasibility of using a NANOSTACKS™-based *in vitro* model of the liver-brain axis for drug testing. However, this model could also be used to study the liver-brain crosstalk in physiological and pathological conditions.

References

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