Electronic Supplementary Material (ESI) for Analyst.

Supplementary Data

A Novel Approach for Precisely Controlled Multiple Cell Patterning in Microfluidic Chip by Inkjet Printing and the Study of Drug Metabolism and Diffusion

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Chemical transformation schematic of tegafur.

Tegafur was transformed into 5'-hydroxytegafur and then 5-FU in the liver cells by CYP enzyme. 5-FU diffused out from liver cells and got into cancer cells. 5-FU exhibits cytotoxity by incorporation into RNA and DNA after transformed into FUTP or FdUTP respectively, as well as by inhibition of thymidylate synthase.

5-FU: 5-fluorouracil

FUTP: fluorouridine triphosphate

FdUTP: fluorodeoxyuridine triphosphate

FdUMP: fluorodeoxyuridine monophosphate

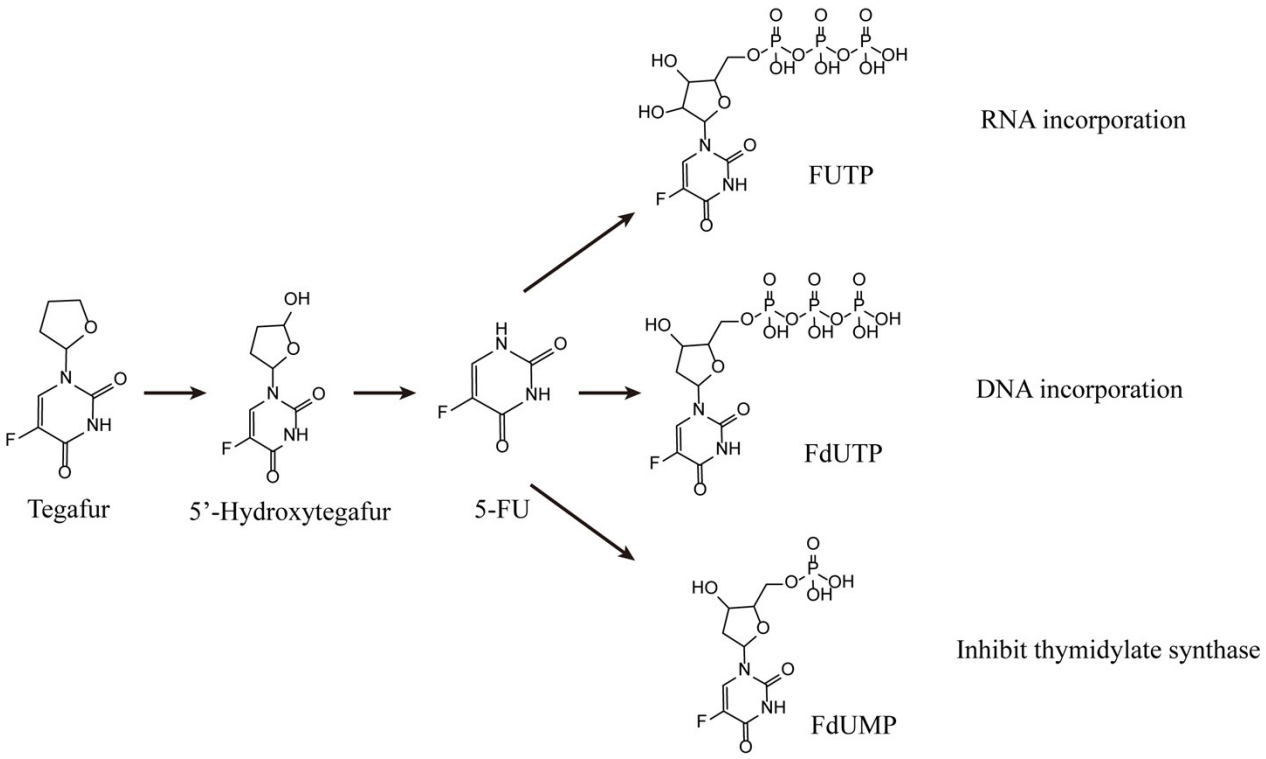

Fig. S1 Chemical transformation schematic of tegafur.

Design and size of the microchip.

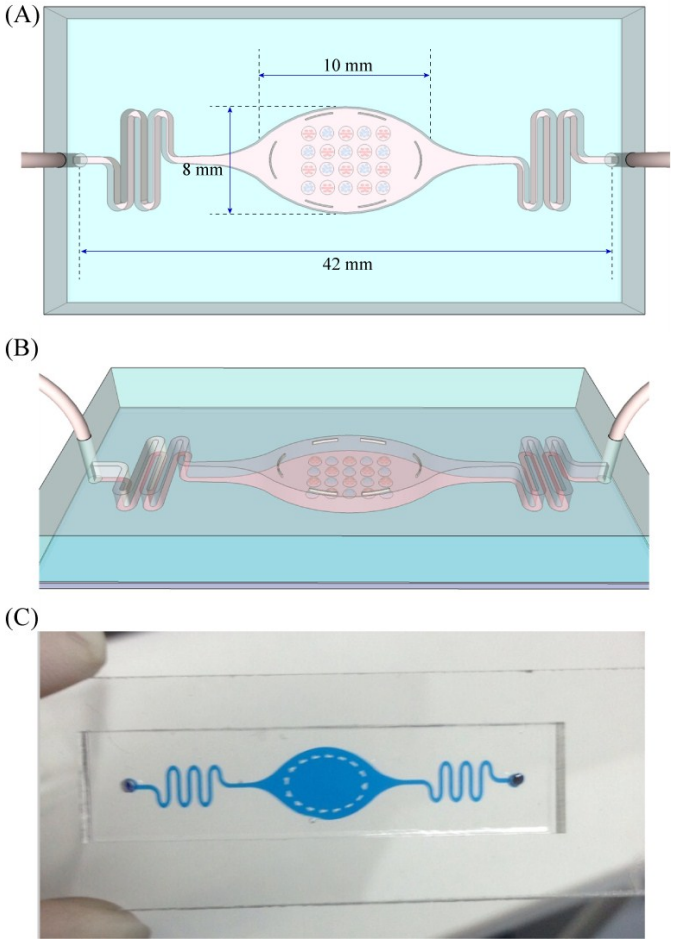

Fig. S2 Design of the microchip. (A) size of the microchannel of the PDMS layer; (B) side view of the microchip; (C) real photo of the manufactured microchip.

Influence of alginate sodium solid content.

The viscosity of commonly used alginate sodium for 3D cell culture was relatively too high for electronic inkjet printers, the 1.0% hydrogel drops failed to separate from the nozzle and accumulated at the outlet, while 0.5% alginate drops could be ejected smoothly.

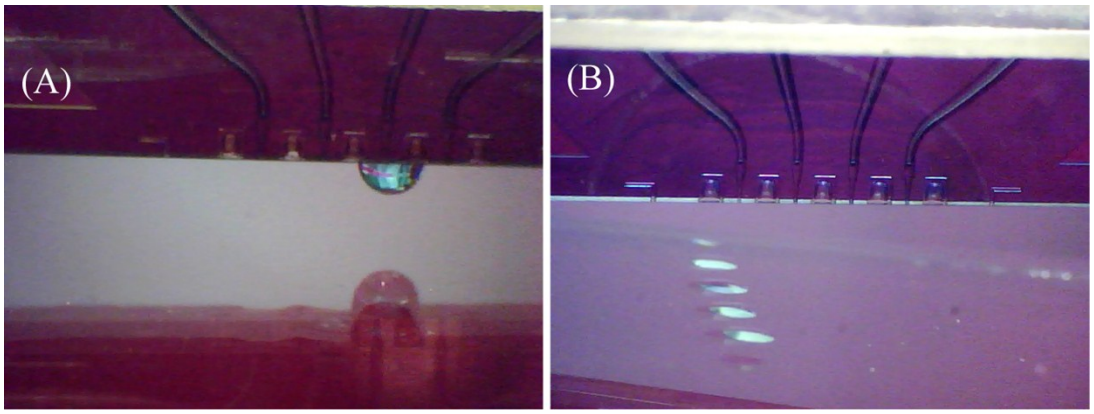

Fig. S3 Inkjet printing of different solid content alginate sodium: (A) 1.0%; (B) 0.5%.

Influence of glass surface.

Contact angle of alginate sodium on glass slides became smaller along with the increase of hydrophilicity.

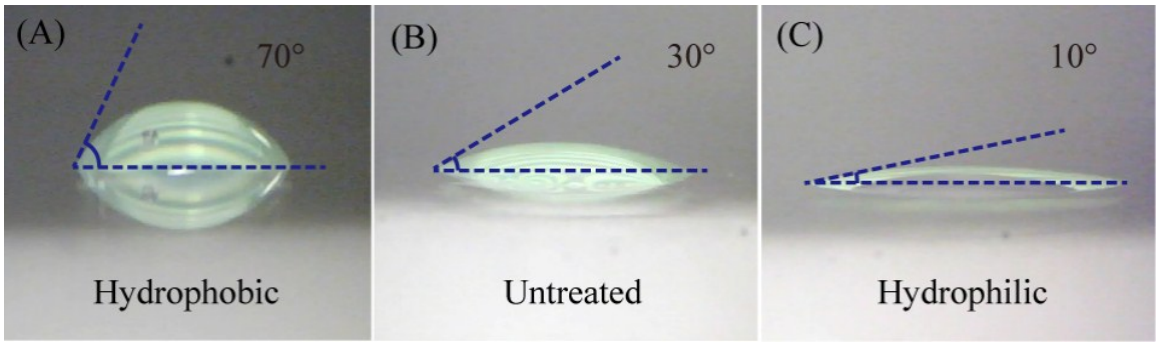

Fig. S4 Contact angle of alginate sodium on glass slides of different hydrophilic-hydrophobic property.

Co-patterning of two kinds of cells.

Two kinds of cells stained with different color were co-patterned in a cross way.

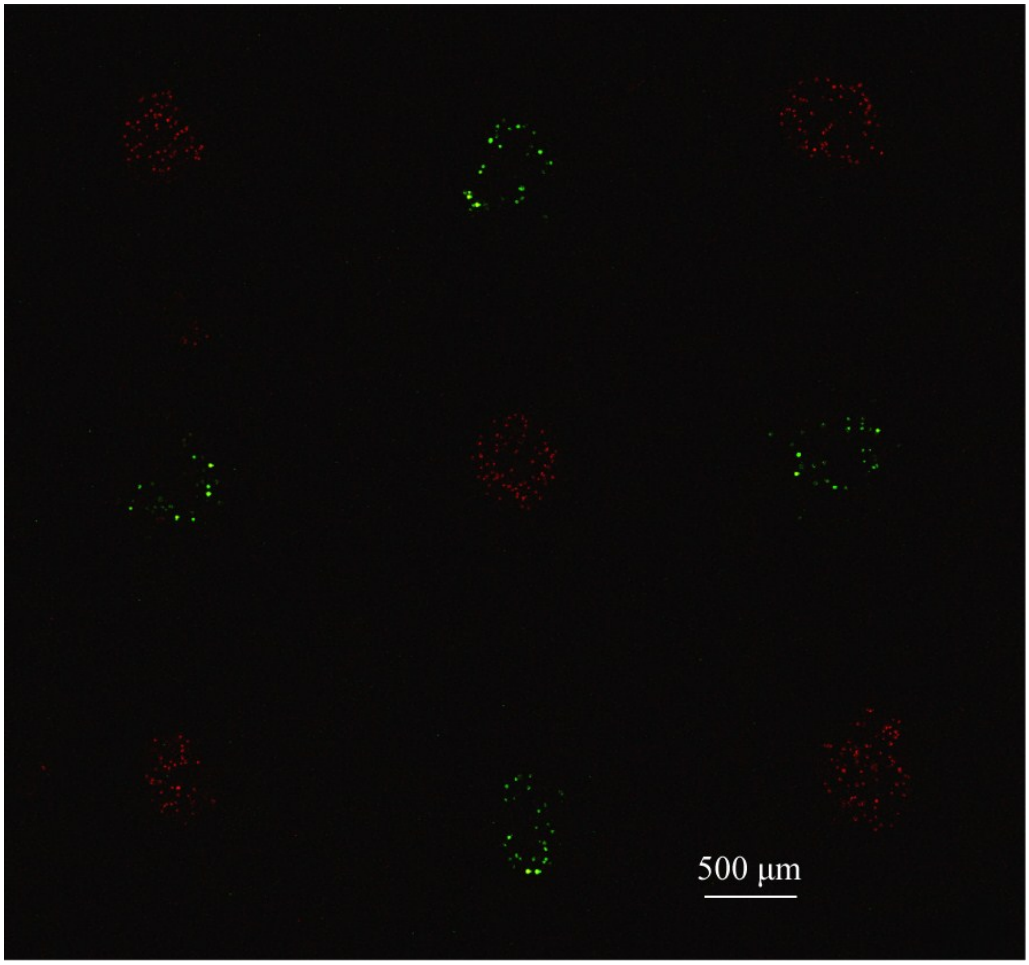

Fig. S5 Co-patterning of two kinds of cells.

Gelation of alginate sodium.

After printing of cells and integration with a PDMS layer. 100 mM $CaCl_2$ dissolved in 1640 cell culture medium was injected into the microchip for the alginate gelation. Then normal 1640 cell culture medium was inject into the microchip to replace $CaCl_2$ solution. **Fig. S6** showed the situation before and after hydrogel gelation.

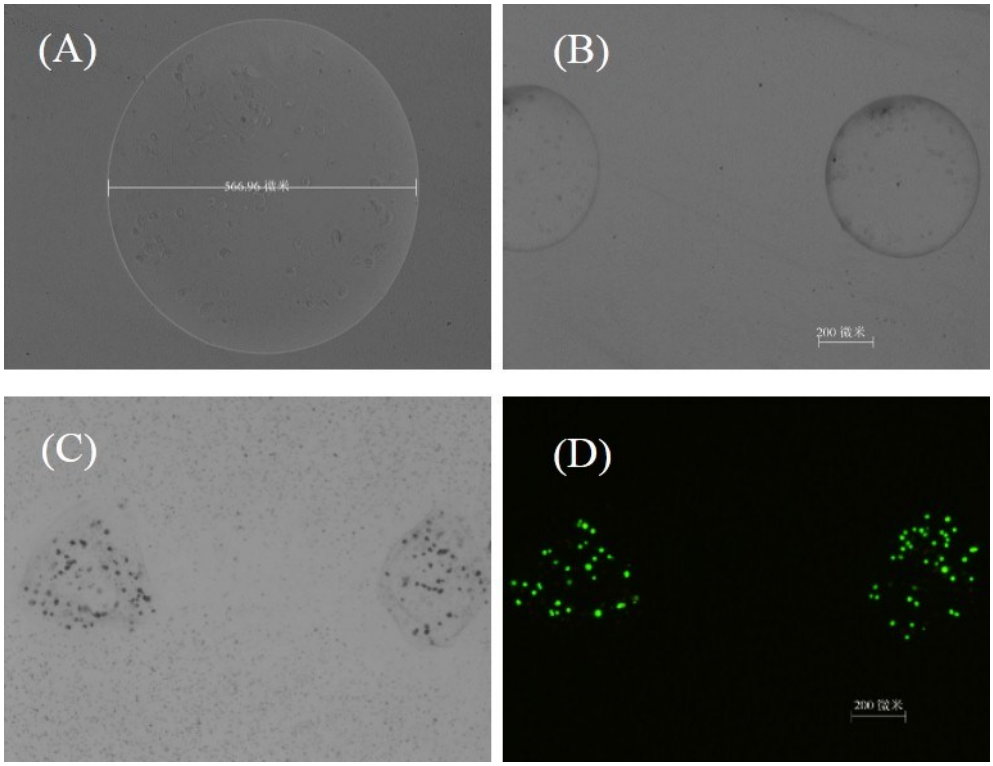

Fig. S6 Inkjet printing alginate hydrogel drops (A) (B), and the situation after gelation (C) (D).

Cell proliferation in alginate.

The cells kept good viability cultured in alginate hydrogel in the microchip and cell clusters were observed.

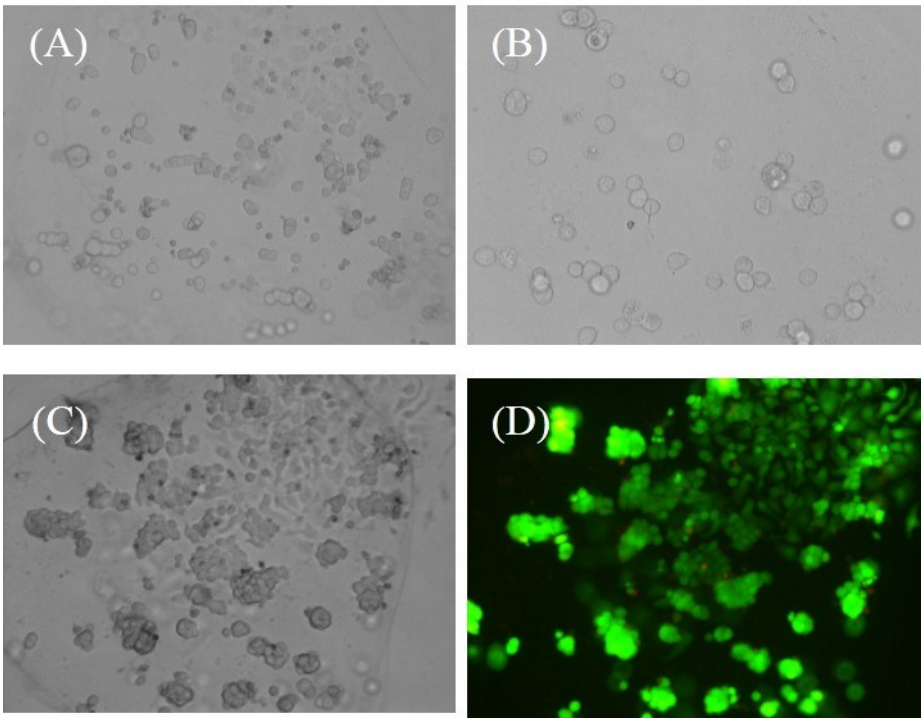

Fig. S7 Cell proliferation in alginate hydrogel of HepG2 (A) cells and U251 (B) cells, and an Live/Dead assay comparison (C) (D).

Cells just printed and after culture

Images of cells just printed and after a period of culture.

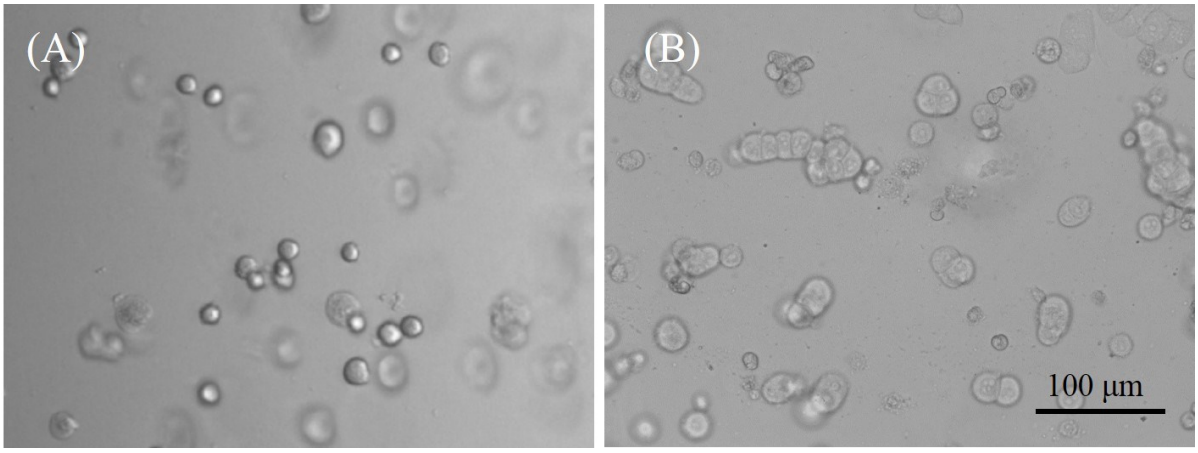

Fig. S8 Cells just printed (A) and after a period of culture (B).

Drug metabolism experiment

Supplementary figure of **Fig. 5** with separate colour channels indicating live cells and dead cells stained by Live/Dead assay kit.

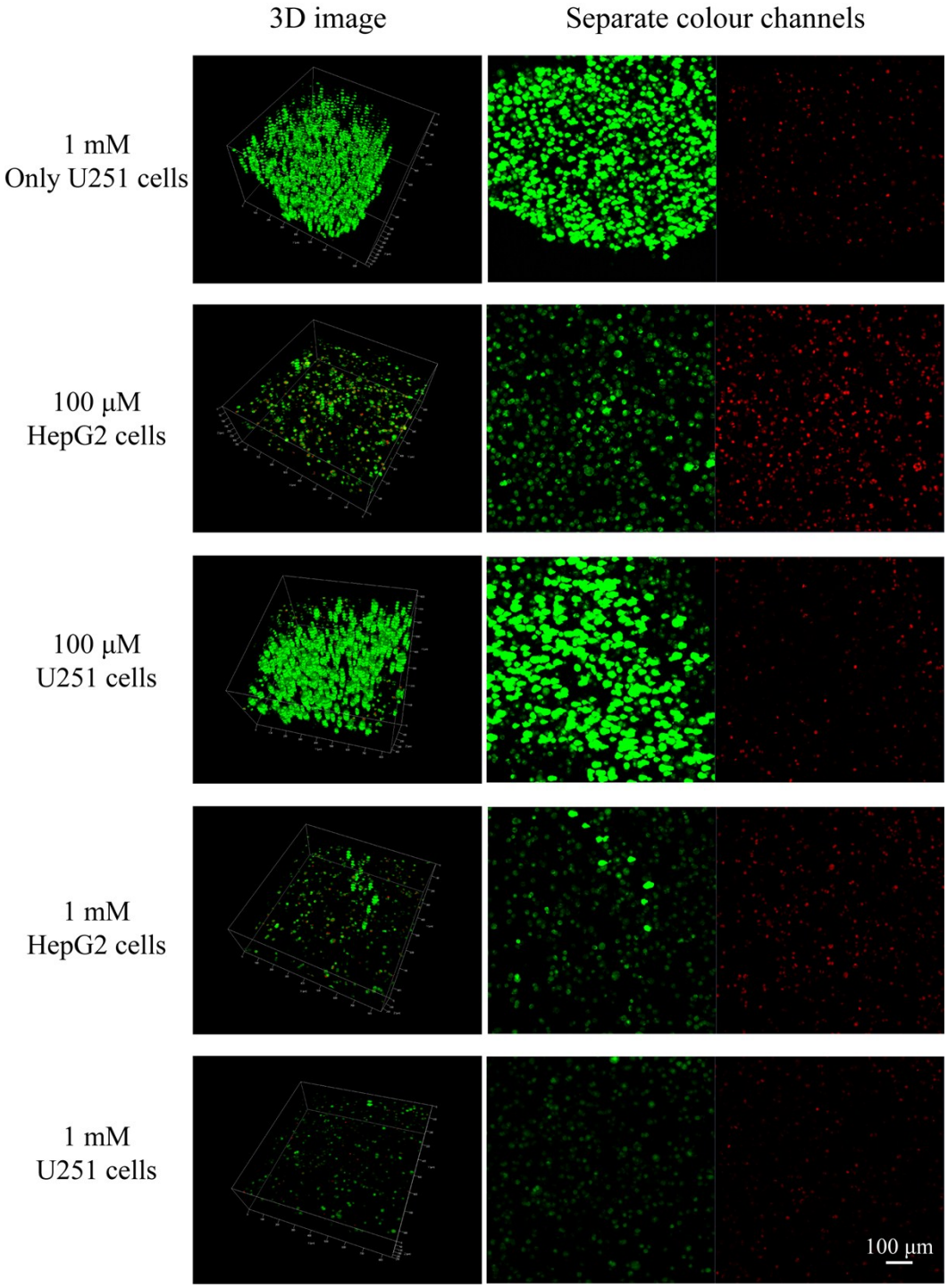

Fig. S9 Live/Dead staining assay of HepG2-U251 co-culture system with different tegafur concentrations, with 3D images and separate colour channels;

Diffusion equation

Here we adopted Fick's law of diffusion and used the software Comsol to simulate the diffusion situation.

In the Fick's law equations used here:

$$\frac{\partial c_j}{\partial t} + \nabla \cdot (-D_j \nabla c_j) + \mathbf{u} \cdot \nabla c_j = R_j$$

$$\mathbf{N}_j = -D_j \nabla c_j + \mathbf{u} c_j$$

The meaning of the parameters are: for species *j*, c_j is the concentration, *t* is time, D_j is diffusion coefficient, **u** is direction vector, R_j is diffusion flux, N_j is the molar flux.

And here the diffusion coefficient was set as 5×10^{-6} cm^2/s approximatively according to the molecular weight of tegafur. And the calculation was processed by the software.