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DLM-GelMA/Tumor Slice Sandwich Structured Tumor on a Chip for Drug Efficacy Testing

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The in vitro recapitulation of tumor microenvironment is of great interest to preclinical screening of drugs. Compared with culture of cell lines, tumor organ slices can better preserve the complex tumor architecture and phenotypic activity of native cells, but are limited by their exposure to fluid shear and gradual degradation under perfusion culture. Here, we established a decellularized liver matrix (DLM)-GelMA “sandwich” structure and a perfusion-based microfluidic platform to support long-term culture of tumor slices with excellent structural integrity and cell viability over 7 days. The DLM-GelMA was able to secrete cytokines and growth factors while providing shear protection to the tumor slice via the sandwich structure, leading to the preservation of the tumor microenvironment where immune cells (CD3, CD8, CD68), tumor-associated fibroblasts (α-SMA), and extracellular matrix components (collagen I, fibronectin) were well maintained. Furthermore, this chip presented anti-tumor efficacy at cisplatin (20 μM) on tumor patients, demonstrating our platform’s efficacy to design patient-specific treatment regimens. Taken together, the successful development of this DLM-GelMA sandwich structure on the chip could faithfully reflect the tumor microenvironment and immune response, accelerating the screening process of drug molecules and providing insights for practical medicine.

Introduction

Globally, about 850,000 new instances of liver cancer are diagnosed every year, leading to widespread research in cancer treatment and targeted therapy1. Traditional animal models, while commonly used in pre-clinical research to establish the efficacy and safety of newly developed anti-cancer compounds or to facilitate precision medicine, are costly and time-consuming2. Thus, scientists developed the concept of organ-chips: the condensation of living cells or microtissues embedded within an in vitro microfluidic chip under perfusion culture to recapitulate key in vivo physio-pathological features of an organ for drug testing3. Specifically, by culturing cancer cells derived from patients within organ-on-chips and exposing the cells to different stimuli, scalable drug screening and preclinical tests of chemotherapy drugs can be achieved with reduced cost and time4. However, organ-on-chips technologies have shown limited capabilities in re-establishing the in vivo complexity of living tissues in terms of cellular components and organ-specific extracellular matrix (ECM), leading to dissatisfaction clinical outcomes5. In early studies, adherent cells were cultured directly in fluidic chambers, which do not reflect the native ECM of their in vivo counterparts6. Although fast advancement of hydrogel technology allows for encapsulation of various types of cells for 3D culture, this method merely encapsulates cells within an artificial matrix without mimicking the real architecture of native organs or re-assembling cell-cell interactions. More importantly, it is impractical to mimic the sophisticated cell ecosystem and homeostasis of living microtissues through a combination of cell lines alone7–9. On the other hand, culture of native microtissues such as tumors with high cell density and in vivo-esque ECM inevitably requires a constant media flow to meet the high demand for oxygen and nutrients to maintain structural integrity and local homeostasis10,11. This approach is also limited by the diffusion depth of dissolved oxygen and nutrients within the system without a functional vascular system. Direct tissue slices of tumors cultured on-chip by dynamic perfusion is thus proposed to overcome these challenges owing to its authentic histological and mechanical features, permeable tissue
thickness and straightforward processing for effective drug testing.12

Slice-on-chips contain a snapshot of an organ and preserve the three-dimensional structure of cells and essential cellular components such as immune cells, vascular cells, and stromal cells etc.13,14. Its low thickness also allows for efficient diffusion of molecules in and out of the tissue slice, enabling long-term perfusion culture of the system.7 Despite the obvious merits of slice-on-chips, a few limitations restrict the development of liver tumor-slice-on-chips owing to the difference in biophysical conditions between liver slices and their whole organ counterpart.15 Liver tumor slices were deprived of native biomechanical forces including fluid pressure, tension and solid stress compared with the in vivo microenvironment, as well as bulk mechanical support of the organ scaffold and protection by the peritoneum.16 Cells and tissues on the surface of liver slices are directly exposed to the fluid shear stress of the perfused medium.17 Combined with the lack of appropriate support of growth factors and signalling molecules from the host, these issues cause the harvested hepatocytes to quickly lose their differentiated features and maintain functions, and the tissue slice to undergo severe degradation.18,19 Additionally, the slicing process could induce significant trauma to tumor slices, causing a drop in cell viability on the initial days of culture,20 as well as a limitation of most perfusion cultures lasting only up to 3-5 days.21 This calls for an optimization of liver tumor slice-on-chips protocols to better preserve the stability of tissue slices and support long-term perfusion culture for pharmaceutical applications.

Here, we propose a decellularized-liver-matrix (DLM)-GelMA based tumor slice sandwich perfused microfluidic system for long-term culture and phenotype retention of liver tumor slices (Figure 1). Decellularized tissues retained their biophysical morphology, contained ample ECM, biochemical factors and proteins, and were widely used as tissue implants for biomedical purposes.22–24 However, the structural integrity and stability of tissue constructs based on a decellularized matrix are notably constrained by its low mechanical stability, degradation and deformation.25–27 It was noted that the incorporation of a decellularized matrix with GelMA significantly enhanced the mechanical properties.28 By mixing DLM with GelMA, a photocrosslinkable matrix capable of supporting hepatocyte survival and proliferation could be formed to sandwich and protect the liver tumor slice against direct fluid shear or contact stress on all sides, supplying the embedded cells with ECM components and native cytokines essential for survival. This enabled the long-term perfusion culture of liver slices as a sandwich microfluidic system with excellent mechanical stability, cell viability, proliferative activity, and immune cell phenotype retention over 7 days. Crucially, the DLM-GelMA sandwich structure well preserved the essential components of tumor ECM including fibronectin and collagen I over 7 days, and supported the growth of tumor-associated fibroblasts, enabling the active regulation of tumor microenvironment within the system. As proof-of-concept, tumor slices from liver cancer patients were harvested and cultured to assess the practicality and effectiveness of the sandwich microfluidic chip. The test with chemotherapy drug cisplatin effectively suppressed cancer growth within the DLM-GelMA liver slice, which reflected the clinical relevance of our proposed system. We envision that drug screening capabilities and accurate feedback of our system can aid in providing individualized therapy for patients.

Experimental Section
Decellularization of the rat livers
The decellularized liver scaffolds was prepared as previously described.22,21,29 Sprague-Dawley rats and their livers were used in this study with the approval of the Animal Ethics Committee of West China Hospital of Sichuan University (no. 20221205004). The livers were removed from the Sprague Dawley rats weighing 200-350 g. The decellularization protocol involved a series of steps: (1) perfusion with 300 mL PBS for 3 h, (2) perfusion with 1000 mL 0.1% SDS for 7 h, (3) perfusion with 300 mL 1% TritonX-100 for 1 h, (4) perfusion with 100 mL 60 U/mL DNase plus 6 U/mL RNase for 1 h, (5) perfusion with 500 mL PBS containing 2% penicillin-streptomycin and amphotericin B for 3 h. The liver was stored in a culture dish of 10 cm² surface area and preserved in the refrigerator at -80°C. All surgical instruments were sterilized by autoclave.

Characterization of the decellularized scaffolds of liver
The native liver tissues and DLM samples were treated with protease K at 56°C for 3 h, and extracted with the Tianan freeze-dried, embedded in paraffin, and sliced for H&E staining. The morphology of the native, decellularized whole liver was examined with Scanning Electron Microscopy (SEM, JSM-IT700HR, Tokyo, Japan). The samples were fixed, dehydrated, and treated with gold sputtering then observed by SEM.

The DNA from native liver tissues and DLM samples was treated with protease K at 56°C for 3 h, and extracted with the Tianan Genomic DNA Kit (Tiangen Biotechnology, Beijing, China) according to the manufacturer’s recommendations. DNA concentration of the freeze-dried native liver and DLM was quantified by a NanoDrop spectrophotometer (DeNovix, Wilmington, DE, USA).

Immunofluorescence staining was performed for both native liver tissues and DLM paraffin-embedded slices. Following the high-temperature repair of slices with citric acid (pH = 6), slice samples were incubated overnight with rabbit polyclonal collagen I, collagen IV, fibronectin, and laminin (Abcam, Cambridge, UK). The primary antibody-conjugated slices were incubated with Alexa Fluor® 594 (Abcam, Cambridge, UK)-labelled goat anti-rabbit antibody for 2 h. The nuclear staining was conducted with 4’,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Sigma-Aldrich, St. Louis, US). The images were obtained under the fluorescence microscope (Zeiss, Oberkochen, Germany).
Figure 1. The schematic diagram of the DLM-GelMA-based sandwich structure on the tumor slice-on-chips. Initially, the decellularized liver scaffold was acquired from a rat liver and combined with GelMA to form DLM-GelMA. The culture chamber comprised two layers of DLM-GelMA sandwiching the liver tumor slice of CDX (cell-derived xenograft) models or patients. Subsequently, the sandwich structure was perfused for predesignated periods to assess the performance of the sandwich configuration through immunostaining. Finally, drug testing would be carried out on the patient slices within the chip to monitor the efficacy of the treatment.

**Mechanical testing**

The preparation process of the DLM-GelMA was as described in our previous study. The prepared DLM underwent lyophilization in liquid nitrogen and was then immersed in a solution containing 0.5 mg pepsin-0.1 M HCl, where it was agitated on an oscillator at 250 rpm for a duration of 10 h. Upon completion of the digestion process, the reaction was terminated by the addition of NaCl/10x PBS with pH adjustment to 7.5. After that, GelMA was combined with the resultant DLM solution at a 2:3 volume ratio. Subsequently, the DLM-GelMA solutions were exposed to UV light irradiation for 25 s. Young’s modulus was adopted to characterize the stiffness of the native liver, DLM, and DLM-GelMA. The mechanical characteristics of the samples were evaluated by a mechanical tester (EFL-MT5600, Suzhou, China), fitted with an adjustable mechanical characterization compression clamp. The samples experienced compression at a rate of 1 mm/min. Each mechanical characterization process was carried out at 37°C.

**Device fabrication and operation**

The master mold was designed using CorelDraw Graphics Suite (Corel, Canada) and fabricated with a CO2 laser cutting machine (Universal Laser System, USA) to cut the polymethylmethacrylate (PMMA) sheets. These sheets were then bonded using 50 μm-thick High-Strength Acrylic Adhesive 300LSE (3M Company, USA) and affixed inside petri dishes.

The polydimethylsiloxane (PDMS) prepolymer was homogeneously mixed in a 10:1 ratio of polymer solution and curing agent, followed by degassing in a vacuum for 5 min. Subsequently, PDMS was cast onto the master mold and left to cure at a temperature of 80°C for 1 h. After removing the PDMS layers from the master mold, it was peeled off to form the PDMS device.
the master mold, they were subjected to a cleansing process involving detergent and anhydrous ethanol. The prepared PMDS layer was clamped and fastened with the PMMA layer.

The prepared microfluidic chip was immersed and sanitized in a 75% (v/v) alcohol solution for 2 h. Subsequently, the microfluidic chip was positioned on a sterile bench (ThermoFisher, USA) and subject to ultraviolet radiation for 3 h. Finally, the chip was rinsed with PBS (Gibco, USA) containing 2% amphotericin and penicillin-streptomycin.

CDX models and clinical specimens
The 4-week-old nude mice were injected intravenously with HepG2 cells, following animal experimentation protocols described by West China Hospital of Sichuan University’s Animal Care Ethics Committee (no. 20221205004). Tumor volume was measured and recorded daily, and mice were sacrificed when the tumor volume was larger than 100 mm³. The liver cancer specimens were acquired from West China Hospital of Sichuan University. The project was approved by the Institutional Research Ethics Committee.

Tissue slice preparation
Tumor tissues of CDX models and cancer patients were rinsed and completely immersed in PBS containing 2% penicillin-streptomycin. The tumor tissues were then fixed in the horizontal plane and sliced with a vibratome Leica VT 1200S (Leica Microsystems) into 300 μm thick sections. Parameters were set according to the stiffness of the tumor. It was determined that 300-μm slices were the ideal thickness for liver tumors. All procedures were performed on ice.

Tumor tissue slice culture in Microfluidic chip
After the sequential assembly of the chip components, the DLM-GelMA based microfluidic chip was prepared. 30 μL of DLM-GelMA solution was added to the chip microwell, followed by exposure to a 405 nm UV light source for 25 s to cure. Subsequently, the tumor slices were placed onto the cured layer of DLM-GelMA and covered with an additional 30 μL of DLM-GelMA solution, which was then cured by UV exposure, forming a sandwich structure of tumor slice between two DLM-GelMA layers. In contrast, the non-sandwich group did not contain any DLM-GelMA while all other conditions were kept consistent with the sandwich group. The microwells were then covered with a cork. DMEM containing 10% FBS and 1% amphotericin was added to the chip microwell, followed by exposure to a 3% DLM-GelMA solution, which was then cured by UV exposure, forming a sandwich structure of tumor slice between two DLM-GelMA layers. In contrast, the non-sandwich group did not contain any DLM-GelMA while all other conditions were kept consistent with the sandwich group. The microwells were then covered with a cork. DMEM containing 10% FBS and 1% penicillin-streptomycin was used for perfusion culture at 2 μL/min, and the chip was placed in a 37°C incubator with 5% CO₂ throughout the experiments.

Cell Counting Kit-8 (CCK-8) assay
The viability of the tissue slices was evaluated on days 4 and 7 using the CCK-8 assay. 10 μL of CCK-8 solution and 90 μL of DMEM were mixed and added to each well of the 96-well plate, then tissue slices were transferred to the above solution. The plate was maintained at 37°C in an incubator with 5% CO₂. After removing the slices from the plates, the plates were placed in a microplate scanner. (Multiskan GO; Thermo Fisher Scientific, Inc.). Each sample was analysed for absorbance at 450 nm.

Treatment of the patient liver tumor tissue slices in microfluidic chip
Cisplatin was added to the perfusion culture medium at different concentrations (0, 10, 20 μM). The drug-loaded medium was then perfused into the system when the slices on the chip had been cultured with normal perfusion medium for 48 h. The tumor slices were removed from the chip after perfusing for 72 h, fixed with 4% paraformaldehyde, and embedded in paraffin for subsequent immunostaining.

Immunohistochemical (IHC) / immunofluorescence (IF) staining of CDX models and the patient tumor tissue slices
Immunohistochemical (IHC) staining The slices were incubated with primary antibodies Ki-67, CD3, CD8, CD68 (Abcam, Cambridge, UK) overnight. The tissue slices were then counterstained with hematoxylin (Sigma-Aldrich, St. Louis, US).

Immunofluorescence (IF) staining The slices were incubated with primary antibodies alpha-fetoprotein (AFP), cleaved caspase-3, α-SMA, fibronectin, and collagen I (Abcam, Cambridge, UK) overnight. Then, the slices were incubated with Alexa Fluor® 594 (Abcam, Cambridge, UK)-labelled goat anti-rabbit antibody for 2 h. Nuclear staining was performed with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). All samples were imaged under a fluorescence microscope (Zeiss, Oberkochen, Germany).

Statistical analysis
Statistical analysis of the data obtained in this paper was performed using the one-way analysis of variance (ANOVA) method. P < 0.05 was considered to indicate a statistically significant difference. Three repeats were performed for all experiments unless otherwise specified.

Results
In this study, the DLM was successfully produced through chemical perfusion. During the decellularization process, the liver tissue exhibited a progressive loss of its initial red coloration, transitioning to a white appearance, and ultimately achieving transparency (Figure 2A). The decellularization procedure was confirmed with H&E staining, SEM, DNA quantification, and immunofluorescence staining. The histological analysis of the decellularized liver specimen demonstrated pink coloration, indicative of the absence of cellular nuclear or components. The decellularization process resulted in a fibrous and mesh-like structure of the liver tissue, as confirmed by SEM (Figure 2B). The preservation of ECM components was confirmed by immunostaining for collagen I, collagen IV, fibronectin, and laminin, which remained present throughout the decellularization process. Furthermore, the absence of DAPI staining indicated minimal cellular remnants within the acellular scaffolds, suggesting the efficacy of the decellularization process (Figure 2C). Quantitative analysis of DNA content revealed that the total DNA in the native liver and DLM was 3856.0 ± 98.48 ng/mg and 7.3 ± 1.9 ng/mg, respectively, indicating the removal of more than 99% of the DNA following decellularization. The decellularized sample exhibited a DNA quantity of less than 50 ng/mg dry weight, as determined by the employed procedures, thereby satisfying the established decellularization criteria (Figure 2D). Collectively,
our findings showed that liver cells were effectively removed during the decellularization process, while vital ECM proteins were retained within the DLM.

The purpose of DLM-GelMA was to offer a biomimetic extracellular microenvironment that comprised of biological structures and chemical microenvironments to promote liver functions and improve cell growth, as well as to provide biomechanical support to preserve the natural structure of the liver. Hence, DLM-GelMA was mechanically characterized by measuring Young’s modulus. The findings revealed that DLM-GelMA hydrogels exhibited Young’s modulus of 5.69 kPa, indicating no significant difference in the native liver while pure DLM displayed a significantly decreased stiffness compared to DLM-GelMA (Figure 2E). The DLM integrated with GelMA significantly raised the toughness of the pure DLM to match the native liver, which suggested that DLM-GelMA was capable of providing mechanical support equivalent to native liver tissues. For subsequent experiments, the sandwich structure group was prepared by embedding the tumor slice between the top and bottom layer of DLM-GelMA, while the non-sandwich structure group referred to the non-treated tumor slice.

The microfluidic chip was developed to culture these tissue slices and conduct multiple assays (Figure 3A). The chip is composed of a three-layer structure of PDMS and PMMA. In particular, the top PMMA layer of the chip possesses a thickness of 5 mm and features three circular holes of 10 mm in diameter. Similarly, the middle PDMS layer also has three circular holes that align with the holes on the top layer and includes a flow channel with dimensions of 70 mm in length, 10 mm in width, and 1 mm in depth. The bottom PMMA chip contained no features and sandwiched the middle PDMS layer with the top PMMA layer to support perfusion in the flow channel. The tissue slices are directly placed into the chamber of the chip through the circular holes before being sealed with the cork. This chip has one inlet and outlet where the medium can be continuously injected to culture the tissue slices. It is worth noting that the tissue chamber was created taller than the sandwich structure to prevent contact between the slice and the top PMMA layer. The tumor slices were put into microfluidic chips for culture, and the flow rate of the medium was maintained at 2 μL/min.

Figure 3. (A) The (i) layer-by-layer and (ii) assembled schematic diagram of the chip (upper PMMA layer with holes, middle PDMS/PMMA layer of microchannel array, bottom plain PMMA layer for support) and (iii) photograph of the assembled chip. (B) The result of H&E staining, nuclear staining (DAPI), Ki-67, AFP immunostaining for the sandwich structure, and the non-sandwich group on days 4 and 7 culture. (C, D) Quantitative expression of Ki-67, AFP from (B). Scale bar: 200 μm. n = 3, Aug ± SE, **p < 0.01, ***p < 0.001, ****p < 0.0001.

To assess the DLM-GelMA sandwich structure’s ability to support tumor slices of CDX model with growth factors and biophysical cues such as stiffness and shear stress, and the overall feasibility of incorporating the sandwich structure within a microfluidic chip for long-term perfusion culture, the chips were perfused for 7 days for sandwich groups and non-sandwich groups, and characterized in terms of tumor slices’ histological features and viability. Analysis of the images obtained from H&E and DAPI staining of the sandwich group indicated that the cell density of the tumor slices remained consistent on both days 4 and 7, while the non-sandwich group displayed a gradual reduction in cell density (Figure 3B). The immunohistochemistry analysis of Ki-67, a marker of cellular proliferation, demonstrated that the sandwich group exhibited significantly higher expression levels compared to the other group on both days 4 and 7. Additionally, the expression of alpha-fetoprotein (AFP), a liver cancer-specific protein, was found to be obviously higher in the sandwich group than in the group that did not have sandwihes on days 4 and 7 (Figure 3C, D). Meanwhile, Live/dead assay was used to assess patient liver tumor tissue slices’ viability over the 7-day culture period. The cell viability of the slices was found...
to be maintained at a level exceeding 75% when cultured on a sandwich structure, whereas the non-sandwich culturing method resulted in cell viability of less than 20% after 7 days (Figure 4A, B). These findings suggested that the sandwich substrate provided better preservation of cell viability in comparison to non-sandwich culturing over the course of the experiment. Similarly, the CCK-8 expression of the sandwich group was approximately three times higher than that of the non-sandwich group on day 4 and two times higher on day 7 as illustrated in Figure 4C, showing vastly superior metabolic activity of cells cultured in a sandwich group. Importantly, the metabolic activity of cells in the sandwich group displayed no significant decrease between two time points, indicating that the continued support from the DLM-GelMA sandwich structure could well preserve the survival and proliferation of cells. These results showed that the DLM-GelMA sandwich group was able to better preserve the survival, proliferation and phenotype of tumor slices compared to direct exposure to perfusion medium of the non-sandwich group.

Figure 5. (A, C) The tumor slices from the CDX model and patients were cultured for 7 days in sandwich slice-on-chips. Scale bar: 100 μm. (B) Quantification of the slices in (A) viability during the culture period. (C) CCK-8 results of the sandwich and non-sandwich group after culture for day 4 and 7. n = 3, Avg ± SE. ***p < 0.0001. ns: no statistical significance.

To demonstrate the practicality of supporting perfusion culture of tumor slices from different specimens, fresh samples were obtained from the CDX model and tumor patients immediately after surgical resection. The slices were subsequently prepared and cultured in a sandwich structure within the chip. Images of the slices were captured on day 1, 3, 5, and 7 during the culture period (Figure 5A, C). The surface area of CDX model slices was measured and calculated to be 40.4 mm² to 39.8 mm², whereas patient samples ranged from 44.2 mm² to 42.6 mm² on day 1 and 7, resulting in a tissue preservation ratio of over 95%. During the culture period, no visible shrinkage or wrinkling of the liver slices was recorded. The results showed minimal changes in both surface area and gross morphology for both the CDX model and the tumor patient groups (Figure 5B, D), indicating that the DLM-GelMA sandwich structure was viable for preserving both mouse and human liver slices.

The tumor microenvironment encompasses immune and stromal cells alongside tumor cells, which collectively influence the migration and proliferation of tumor tissue, and was widely researched for its clinical potential for cancer immunotherapy using the native immune system. However, evaluation of immunotherapy effects on living organisms had proved difficult. Our system was hypothesized to provide a means to visualize the treatment efficacy of immunotherapy on human cancer owing to its optical transparency and accessibility to normally deep-within-tissue cell microenvironments. To assess the viability of immune cells within our system, the following cell markers were examined with immunohistochemistry staining using markers of T cells (CD3, CD8) and macrophages (CD68) (Figure 6A). There was no discernible difference in the proportion of immune cells between day 1 and 7 (Figure 6B), showing a sustained survival of both immune cell types within the sandwich structure. Additionally, to evaluate the expression levels of tumor-associated fibroblasts (α-SMA), and ECM components (such as collagen I, fibronectin, etc.), tissue slices cultured in DLM-GelMA sandwiches were subjected to immunofluorescence staining after day 1 and 7 (Figure 6C). The levels of fibronectin and collagen I, key components of the ECM in tumors, remained relatively stable on day 1 and 7 compared to the baseline levels. Similarly, the expression of α-SMA, which serves as a marker for tumor fibroblasts, exhibited no noteworthy alterations. This finding implies that the DLM-GelMA could sufficiently maintain the stability of the tumor microenvironment (Figure 6D). Based on the above results, it can be inferred that the tumor slices cultured under the sandwich model condition effectively retained a relatively intact tumor microenvironment.
The objective of this study was to assess the viability of utilizing the DLM-GelMA sandwich structure as a drug screening platform through the development of in vitro drug testing on tumor slices of the patients. To examine the response of the tumor slices in this culture model, varying concentrations of cisplatin (0, 10, 20 μM) were perfused through the chip. The resulting tumor slices were then removed from the DLM-GelMA sandwich structure after 72 h for paraffin-embedded slicing. The results of H&E and DAPI staining revealed that the number of cells displayed a negative correlation with the response to cisplatin. Similarly, as drug concentration increased, a decrease in Ki-67 was observed, signifying that tumor cell proliferation was inhibited (Figure 7A). Meanwhile, caspase staining was also carried out to assess the cell viability of tumor slices after cisplatin treatment. The caspase family played a crucial role in mediating apoptosis, with caspase-3 serving as the principal executor molecule involved in numerous apoptosis signaling pathways. Caspase-3 staining was conducted at a drug concentration of 0 μM to assess the presence of apoptotic cells in the slices and indicate the sustained viability of the cells following a period of culture. The findings depicted in Figure 7C indicated that the fluorescence signal of caspase-3 in cisplatin-treated tumor slices increased in a dosage-dependent manner, with the highest expression observed at a cisplatin concentration of 20 μM. As tumors of different patients may have varied drug resistance and receptiveness to chemotherapy, the drug screening capabilities and accurate feedback of our system can aid in providing individualized therapy for patients.

DLM-GelMA served as both a barrier for shear protection and bioreservoir of native proteins and growth factors for liver tumor slices in our system. The experimental results demonstrated the long-term culture potential of DLM-GelMA tumor slice sandwich structure and the preservation of cell phenotypes. In another study on perfusion culture of skin cancer biopsy samples at 2 μL/min, the tumor slices secreted approximately double the amount of secreted lactate dehydrogenase (LDH), a marker of tissue injury, on days 6 and 7 of the culture process. This showed that the long-term perfusion exposure to liquid shear stress greatly inhibited the sustained perfusion culture of tissue slices, while our system was able to maintain well over 75% cell viability as well as no decrease in metabolic activity compared with the fresh tissue samples. Compared with previously reported work, the designed platform exhibits better cell viability as presented (Table 1). Meanwhile, studies had investigated the potential of creating smaller tumor slices in the form of microdissected tissues (MDTs) (500 μm in diameter) to enhance the nutrient exchange and lower hypoxia in cultured cells. The improved diffusion successfully increased the survival rates and Ki-67 expression in MDTs compared with slices cultured in both perfusion and traditional culture, but sacrificed tumor morphology, tissue features and phenotypic evolution. Additionally, their increased surface-area-to-volume ratio conferred unfavorable properties for enduring liquid shear, greatly limiting MDT use in the perfusion culture, while our system was able to preserve the Ki-67 expression and viability of the perfusion cultured tumor slices over 7 days using DLM-GelMA sandwich structure. Chakrabarty et al. used Mebiol hydrogel to affix tumor slices onto their microfluidic chip, which served similar purposes of shear protection and nutrient exchange. In their study, they were able to demonstrate reduced DNA damage in their slice-on-chips by S3BP1 immunostaining.
compared with the ex vivo slices, as well as a reduction in activated immune pathways in their RNA-sequencing results. However, a considerable amount (11.34% after 7-day culture) was still observed. We hypothesized that the DNA damage stemmed from the lack of native biological factors from the hydrogel structure, which consisted only poly (N-isopropylacrylamide) and poly (ethylene glycol), which did not recapitulate the tumor microenvironment biochemically. Moreover, Mebiol gel exhibited a storage modulus of under 1 kPa at 37°C, which hardly resembled the stiffness of native liver or tumor tissues. This disparity may have caused the cells to undergo phenotypic changes differently from native tissues. For future work in our study, a thorough investigation into the biological pathways in tumor cells, stromal cells, and immune cells within the tumor microenvironment would be conducted to systematically elucidate the mechanism of DNA damage prevention and its relationship with the native tissue resemblance of our DLM-GelMA tumor slice sandwich structure and perfusion-based microfluidic platform. This will further justify our system as an accurate, reliable and reproducible drug screening platform for cancer therapy.

### Table 1. Characteristics of slice-on-chips involved in the published paper.

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### Conclusion

This study sets out to establish an innovative “sandwich” tumor slice culture method using a microfluidic chip. GelMA and DLM were utilized to create a biomimetic extracellular microenvironment. The “sandwich” method was devised to wrap a liver tumor slice between two layers of DLM-GelMA, which closely resembles the geometry in vivo. By using a microfluidic chip, we created a dynamic microenvironment enabling efficient material flow and exchange. In addition, this combination provides biomechanical support to maintain the natural structure of the tumor slice. Our approach greatly increased cell survival in tumor sections compared with traditional methods of tissue section culture and maintained a relatively intact immune microenvironment to validate the utility of the anti-cancer model drug (cisplatin). This study successfully demonstrated the effectiveness of a microfluidic-based perfusion culture system in replicating the in vivo environmental signals to mimic the in vitro tumor microenvironment, which can be used as an oncology drug screening platform to provide personalized therapeutic options for cancer patients. It is expected that sandwich technology will facilitate and enhance the implementation of on-slice modeling soon.

### Author Contributions

W. H. and H. B. performed experiments and performed the paper writing. S. W. and X. Z. performed the chip design. H. J., D. W. and R. Y. performed the silicon wafer fabrication. Q. S., Q. L. and J. L. provide suggestions on data analysis. X. Z., C. S., Q. D., L. W. and G. W revised the paper writing.

### Conflicts of interest

There are no conflicts to declare.

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### Notes and references


