A microfluidic system improves isolation for downstream culture of biliary exfoliated tumor cells from PTCD drainage

Shenbo Zhang [1], Jie Tang [2], Kui Zhao [1], Zhonghe Zhao [1], Zhengyu Jin [1], Zhiwei Wang [1], Rui Chen [2], Sihang Cheng [1]

Objective: This study aimed to validate the necessity and feasibility of isolating exfoliated biliary cells using a microfluidic system prior to downstream culture.
Methods: The inhibitory effect of bile on tumor cell proliferation was assessed using PANC-1 cells co-incubated with bile from four donors, followed by Cell Counting Kit-8 assay for cell viability. The isolation tefficiency of the microfluidic system was compared with conventional PBS washing using PANC-1-spiked bile samples. Patients undergoing percutaneous transhepatic cholangial drainage were prospectively enrolled. Exfoliated cells were isolated via the microfluidic system and subjected to either 2D culture or patient-derived organoid (PDO) development. To confirm the malignant origin of cultured cells, seven established PDOs were subcutaneously injected into NVSG immunodeficient mice (n = 3 per PDO). The xenografts were excised 6 weeks after transplantation for subsequent H&E staining and immunohistochemical analysis.
Results: Bile from three of four donors inhibited PANC-1 proliferation in a concentration-dependent manner, with marked inter-donor variability. The microfluidic system removed bile sludge more effectively than PBS washing, as indicated by a lower bilirubin remaining rate. In the 2D culture cohort (n = 105), the overall culture positivity rate was 59.0% (62/105). In the PDO cohort (n = 41), the overall culture positivity rate was 85.4% (35/41), of which four were positive but contaminated were labled failed. All seven transplanted PDOs successfully formed subcutaneous tumors in immunodeficient mice.Histopathological analysis supported the malignant origin of the subcutaneous tumors.
Conclusions: The microfluidic system effectively isolates exfoliated biliary cells by removing inhibitory bile components while maintaining cell viability. The established PDOs are confirmed to be of malignant origin, supporting the potential of this approach for downstream functional studies and personalized medicine applications.

Table 1. Baseline characteristics of the 2D and 3D culture groups.

Characteristics	2D culture (n=105)	PDO culture (n=41)
Age, mean ± SD (years)	63.2 ± 11.2	64.0 ± 9.3
Gender (male/female)	57/48	19/22
Reasons of jaundice		
Malignancy	100	41
Pancreatic cancer	59	27
Cholangiocarcinoma	21	7
Ampullary carcinoma	9	2
Metastasis	9	4
Gallbladder cancer	2	1
Benign	5	0
Positive culture (Yes/No)	62/43	35/6

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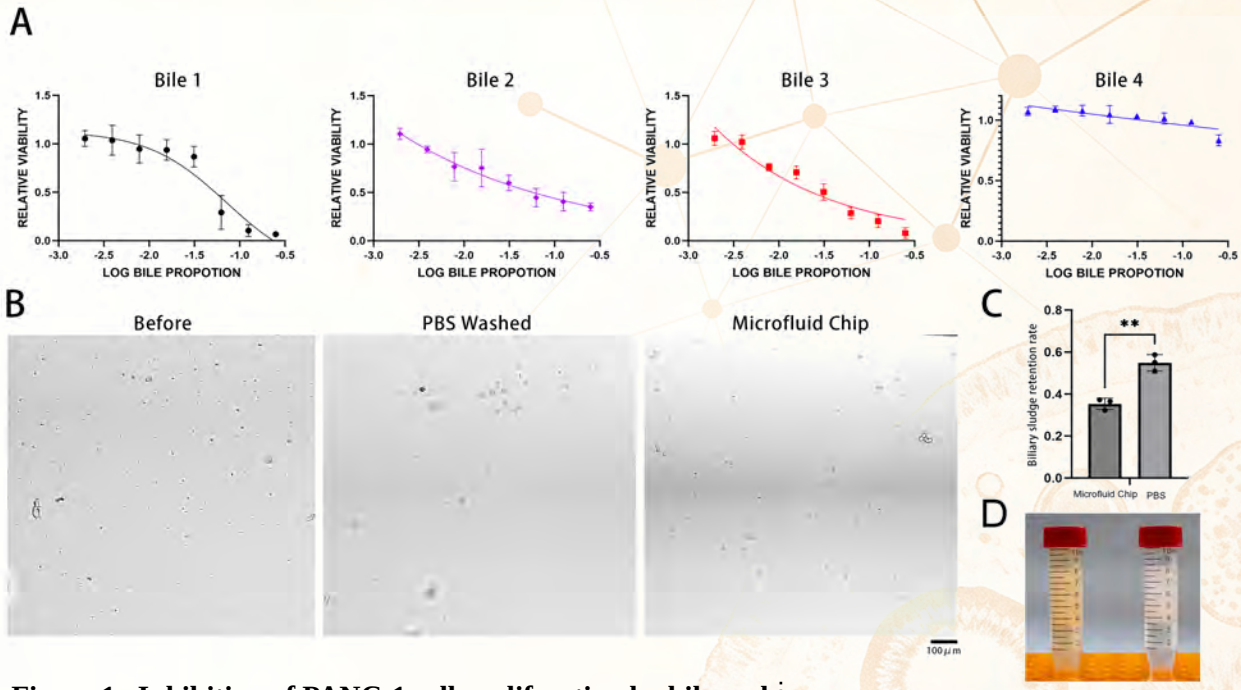


Figure 1 . Inhibition of PANC-1 cell proliferation by bile and isolation efficiency of the microfluidic system for exfoliated cells.
(A) Log10 dose-response curves of bile samples from four different donors. **(B)** Representative microscopic images of a bile sample before PBS washing, after PBS washing, and after processing with the microfluidic system. **(C)** Comparison of the relative bilirubin remaining rate (RRR) between the microfluidic group and the PBS wash group. **(D)** PBS-washed bile sample (left) and microfluidic system-processed bile sample (right).

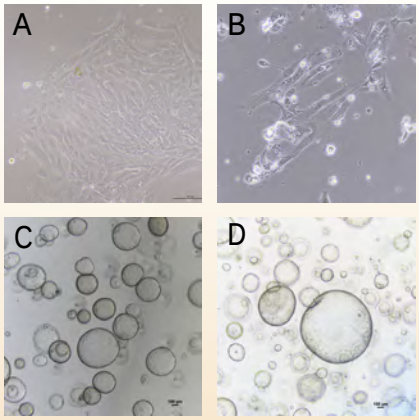


Figure 2 Representative cases of 2D culture and PDOs.
(A, B) Images of 2D-cultured isolated cells from patients with pancreatic cancer **(A)** and cholangiocarcinoma **(B)** on day 14. **(C, D)** Images of PDOs from patients with pancreatic cancer **(C)** and metastatic renal carcinoma **(D)** on day 14.

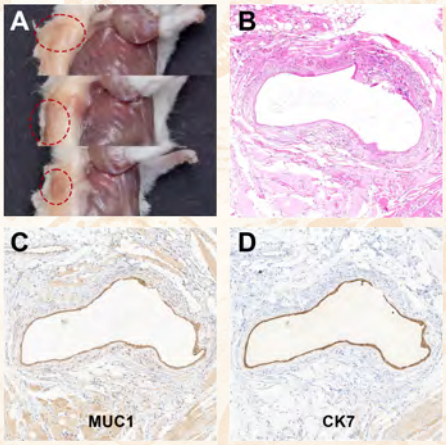


Figure 3 Representative case of xenograft tumor from a patient of pancreatic cancer.
(A) Macroscopic images of xenograft tumors (the subcutaneous tumors are indicated by red dashed ellipses). **(B-D)** Representative microscopic images showing H&E staining **(B)** and immunohistochemistry for MUC1 **(C)** and CK7 **(D)**.

Acknowledgments
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