Supplementary Information:

Selectively Cross-Linked Hydrogel-Based Cocktail Drug Delivery Micro-Chip for Colon Cancer Combinatorial Drug Screening using AI-CSR platform for Precision Medicine

Kiran Kaladharan[a], Chih-Hsuan Ouyang[a], Hsin-Yu Yang[a], and Fan-Gang Tseng[a,b,c,d*]

[a]Engineering and System Science, National Tsing Hua University, Hsinchu, Taiwan, R. O. C

[b]Institute of Nano Engineering and Micro Systems, National Tsing Hua University, Hsinchu, Taiwan.

[c]Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan.

[d]Research Center for Applied Sciences, Academia Sinica, Taipei, Taiwan, R. O. C.

*Corresponding author emails: Fan-Gang Tseng (fangang@ess.nthu.edu.tw)

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S1. Chip fabrication process

Polymethyl Methacrylate (PMMA) was selected as the substrate for crafting the channel mould. Initially, the substrate underwent precision cutting using a laser cutting machine, employing a 0.1mm knife with a cutting depth set at 0.05mm. Subsequently, the processed substrate was meticulously cleaned using alcohol to remove any residues. Following this, the substrate was immersed in an ultrasonic cleaner for a duration of 10 minutes to ensure the removal of any remaining particles on the surface. Turning our attention to the Polydimethylsiloxane (PDMS) mould, a meticulous procedure ensued. We employed a 10:1 ratio of PDMS A agent to PDMS B agent for the mould creation. The resulting chip, upon extraction from the mould, exhibited a thickness of approximately 2mm, a dimension determined to be optimal through rigorous testing and experimentation. This thickness ensures the chip's suitability for subsequent use in our experimental processes.

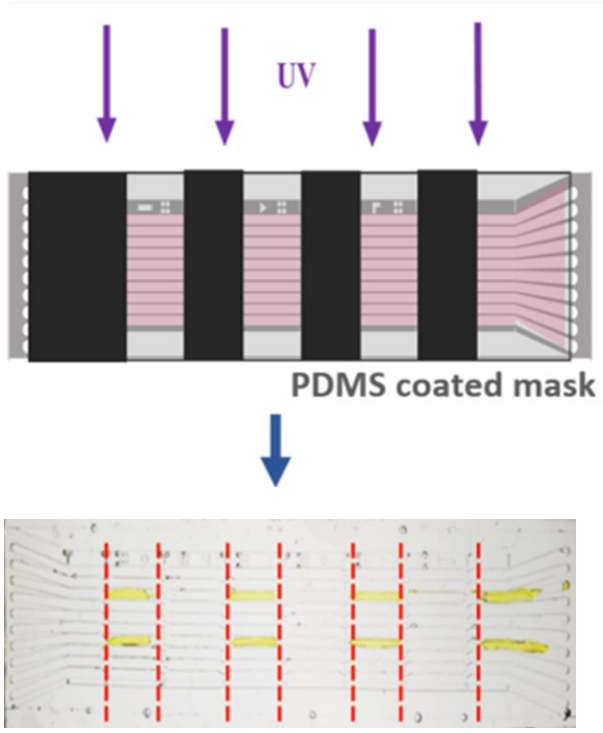

Fig. S1. Selective crosslinking test of the hydrogel by UV and photomask.

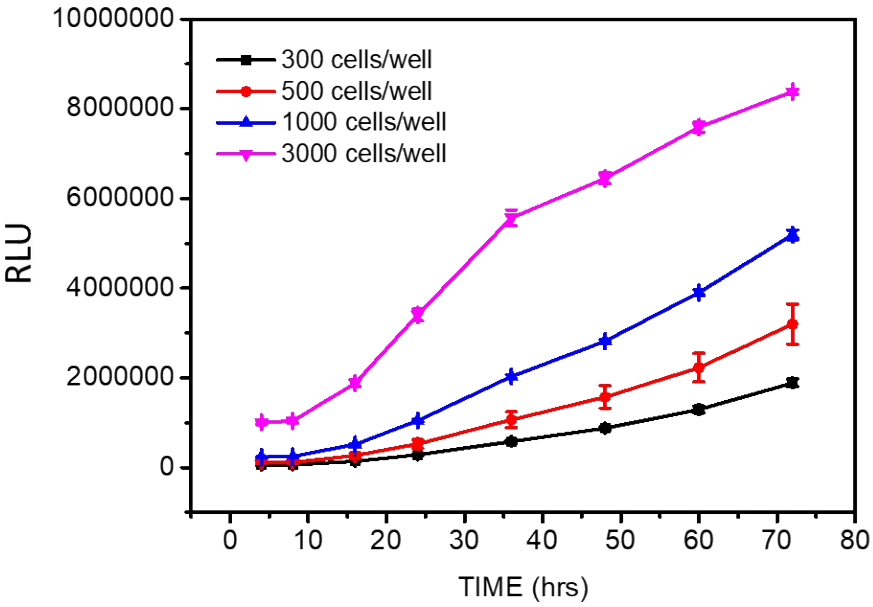

Fig. S2. Cell growth curve of HCT116 cells.

Table S1: Comparison EC50 values of ten drugs on HCT116 between drugs released from hydrogel and directly added to wells (cited from our group's previously published paper).

	EC 50 (released from hydrogel) (μM)	**EC 50 (directly added to well) (μM)** [1]
5-FU	9.02	8.56
Oxaliplatin	9.52	10.28
Gemcitabine	0.18	0.1832
Irinotecan	0.02	0.01
Folinic acid	Resistance	Resistance
Regorafenib	4.97	5.88
Lenvatinib	26.71	28.47
Bevacizumab	Resistance	Resistance
Cetuximab	Resistance	Resistance
Panitumumab	Resistance	Resistance

Table S2: Three levels of concentrations of all the ten drugs used for the cocktail drug screening experiments.

Drugs	Level (μM)		
	EC0 (0)	**EC10 (1)**	**EC20 (2)**
Regorafenib	0	2.53	3.45
Lenvatinib	0	2.78	6.56
5-FU	0	3.09	4.51
folinic acid	0	10	20
Bevacizumab	0	0.1	0.2
Cetuximab	0	0.1	0.2
Panitumumab	0	0.1	0.2
Oxaliplatin	0	0.405	1.34
Irinotecan	0	0.124	8.03
Gemicitabine	0	0.0124	0.0335

Table S3: Comparison between conventional method, other similar methods and Selectively Crosslinking Hydrogel Cocktail Chip

	Number of drugs combined at one time	Reaction time	Drug Timing Control	Drug-Dose Combination Optimization	Drug droplet evaporation
Conventional method[1]	Not applicable	~1.5 hours	Release all at once	AI can be integrated	Not applicable
UV-gel drug chip[2]	5 drugs	Not applicable	Can control the drug release rate	Feedback System Control (FSC) approach	Not applicable
Microfluidic-Enabled Print-to-Screen Platform[3]	up to 3 drugs	Not applicable	Drug array printed all at once	Require more extensive testing without any AI based optimization	Potential of droplet evaporation during and after the printing process
Plug-and-Play, Drug-on-Pillar Platform[4]	up to 3 drugs	Not applicable	Drug array printed all at once	A 1260-spot drug combination array to study drug effects	Potential of droplet evaporation during and after the printing process
Selectively crosslinking Hydrogel Cocktail Chip (This work)	up to 10 drugs	Not applicable	Can control the drug release rate by hydrogel concentration	AI-CSR platform to optimize drug-dose combinations across a 10-drug search space, enhancing precision and minimizing experimental iterations.	Not applicable

S2. References:

[1] Advanced Therapeutics - 2023 - Yang - In Vitro Study on AI-PRS Enabled Precision Cocktail Drugs Design for Treating Human.pdf n.d.

[2] Chen YT, Goudar VS, Wu RG, Hsieh HY, Yang CS, Chang HY, et al. A UV-sensitive hydrogel based combinatory drug delivery chip (UV gel-Drug Chip) for cancer cocktail drug screening. RSC Adv 2016;6:44425–34. https://doi.org/10.1039/c6ra01733a.

[3] Ding Y, Li J, Xiao W, Xiao K, Lee J, Bhardwaj U, et al. Microfluidic-Enabled Print-to-Screen Platform for High-Throughput Screening of Combinatorial Chemotherapy. Anal Chem 2015;87:10166–71. https://doi.org/10.1021/acs.analchem.5b00826.

[4] Quon G, Ajena Y, Lam KS, Pan T. screening implemented by microfluidic adaptive printing 2019;90:13969–77. https://doi.org/10.1021/acs.analchem.8b03456.A.