Electronic Supplementary Information

A high-throughput, open-space and reusable microfluidic chip for combinational drug screening on tumor spheroids

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Supporting material contents

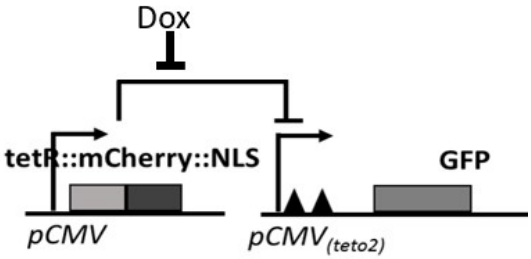

Figure S1. The schematic diagram of tetR-GFP expression circuit. The bacteria tet repressor (tetR) is fused with fluorescent protein mCherry, and nuclear localization sequence (NLS). It is under the control of a constitutive CMV promoter on the left. In the absence of Dox, tetR binds to the tetO site in the modified constitutive CMV promoter on the right, and represses the expression of GFP. With increasing in Dox concentration, increased amount of tetR is bound with Dox, which prevents it from binding to tetO, and gradually releases the repression on CMV promoter. GFP expression increases gradually.

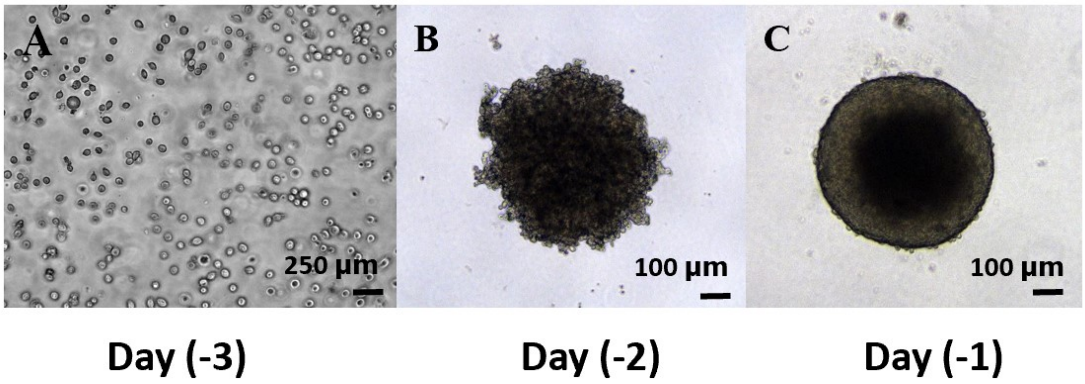

Figure S2. The images of the generation of a tumor spheroid from Day (-3) to Day (-1).

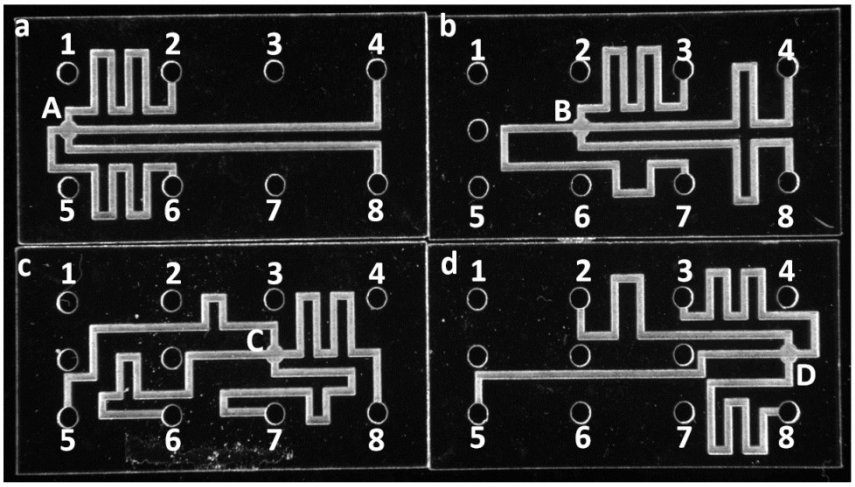

Figure S3. The photos of PMMA layers after laser engraving, which also demonstrate the designing of every PMMA layers of a 4-input DOE chip.

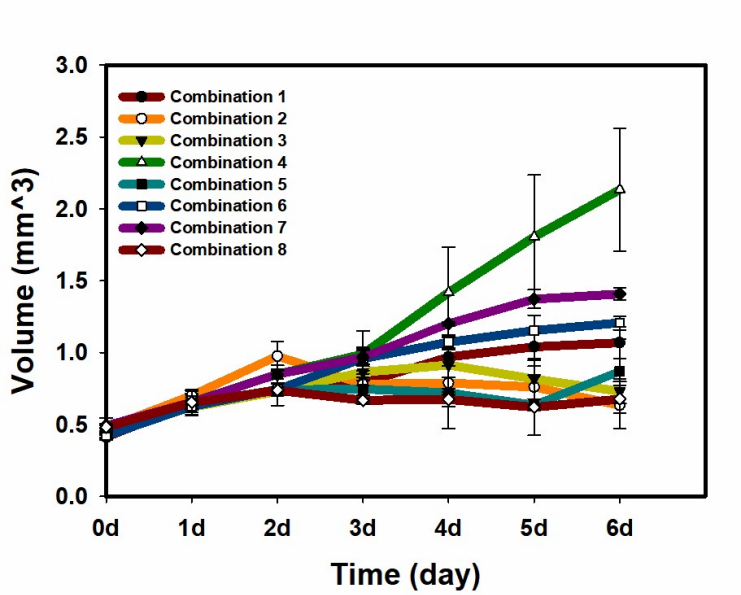

Figure S4. Tumor spheroids volumes under different factor combinations from Day 0 to Day 6.

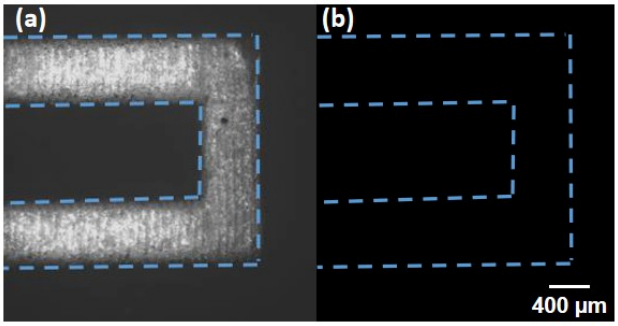

Figure S5. Images before (a) and after (b) washing the channel using water.

Table S1. The final experimental conditions for combinational drug screening. The bolded number represents add.

	L-glutamine (mM)	D-glucose (mM)	FBS (%)	Cisplatin (ug/mL)
Combination 1	1	5	2.5	0.0
Combination 2	**5**	5	2.5	**1.5**
Combination 3	1	**30**	2.5	**1.5**
Combination 4	**5**	**30**	2.5	0.0
Combination 5	1	5	**12.5**	**1.5**
Combination 6	**5**	5	**12.5**	0.0
Combination 7	1	**30**	**12.5**	0.0
Combination 8	**5**	**30**	**12.5**	**1.5**

Table S2. 4-input DOE designing tale from 'DOE simplified: practical tools for effective experimentation'(Mark J. Anderson and Patrick J. Whitecomb. Third edition). 1 represents add and 0 represents null.

Std	A	B	C	D
1	0	0	0	0
2	1	0	0	1
3	0	1	0	1
4	1	1	0	0
5	0	0	1	1
6	1	0	1	0
7	0	1	1	0
8	1	1	1	1

Table S3. The flow rate in the outlets of the DOE chip based on COMSOL simulation results.

Disperse layer	Outlet 1 (m/s)	Outlet 2 (m/s)	Outlet 3 (m/s)	Outlet 4 (m/s)	RSD (%)
A	0.0285	0.0282	0.0283	0.0281	0.017
B	0.0284	0.0282	0.0284	0.0279	0.023
C	0.0288	0.0282	0.0284	0.0287	0.027
D	0.0278	0.0297	0.0278	0.0279	0.093

Table S4. 7-input DOE designing tale from 'DOE simplified: practical tools for effective experimentation'(Mark J. Anderson and Patrick J. Whitecomb. Third edition). 1 represents add and 0 represents null.

Std	A	B	C	D	E	F	G
1	0	0	0	1	0	0	1
2	0	1	0	0	1	0	0
3	0	1	1	1	0	0	1
4	0	0	1	0	0	0	1
5	1	1	1	1	1	1	1
6	0	0	0	0	1	1	0
7	1	1	0	0	0	1	0
8	0	1	0	1	1	1	0
9	1	0	1	0	1	0	0
10	1	1	0	1	0	1	1
11	0	0	0	1	0	1	0
12	0	0	0	1	1	1	1
13	0	0	1	1	1	0	0
14	1	0	0	1	1	0	0
15	1	1	0	1	0	0	0
16	0	1	1	0	0	0	0
17	1	1	0	0	1	1	1
18	1	1	1	0	0	1	1
19	0	1	1	0	1	1	0
20	1	1	1	1	1	0	0
21	1	0	0	0	1	0	1
22	1	0	0	0	0	1	1
23	1	0	1	1	1	1	0
24	0	0	1	0	0	1	0
25	1	0	1	1	1	0	0
26	0	1	1	0	0	0	1
27	1	1	0	0	0	0	1
28	0	0	1	1	0	1	1
29	1	0	1	1	1	0	1
30	0	1	0	0	0	1	1

Code S1. The code used to calculate the *p*-values of Friedman's test.

```
DOE=importdata('DOE.csv');% 4-inlet DOE design, shown in Table S2
OS=importdata('day1_live_3d.xlsx');
OSR=zeros(8,3);
for i0=1:3
    [temp,j1]=sort(OS(:,i0),'descend');
    OSR(j1,i0)=[1:8]';% ranking within each experiments
end
% analyze single factors
% find combinations with or without single factors
l1=zeros(4,4,'uint8'); % DOE with L-glutamine, D-glucose, FBS, or Cisplatin
l0=zeros(4,4,'uint8'); % DOE without L-glutamine, D-glucose, FBS, or Cisplatin
for i1=1:4
    l1(:,i1)=find(DOE(:,i1)==1);
    l0(:,i1)=find(DOE(:,i1)==0);
end
% Friedman's statistic test single factor contributions
rs1=zeros(12,4);
rs0=zeros(12,4);
for i1=1:4
    % pool together ranking orders of three exps
    rs1(:,i1)=[OSR(l1(:,i1),1);OSR(l1(:,i1),2);OSR(l1(:,i1),3)];
    % all results with single factor
    rs0(:,i1)=[OSR(l0(:,i1),1);OSR(l0(:,i1),2);OSR(l0(:,i1),3)];
    % all results without single factor
    % Freidman test
    pFOS1(i1)=friedman([rs1(:,i1) rs0(:,i1)],3);
end
% analyze factor-factor interactions
% find combinations with or without a pair of factors
l21=zeros(2,6,'uint8'); % DOE with L-glutamine^D-glucose L-glutamine^FBS L-glutamine^Cisplatin .. FBS^Cisplatin
l20=zeros(2,6,'uint8'); % DOE with ~(L-glutamine^D-glucose) ~(L-glutamine^FBS) ~(L-glutamine^Cisplatin) .. ~(FBS^Cisplatin)
j0=0;
for i1=1:3
    for i2=i1+1:4
        j0=j0+1;
        j1=find((DOE(:,i1)==1 & DOE(:,i2)==1)==1); % DOE with L-glutamine^D-glucose L-glutamine^FBS L-glutamine^Cisplatin .. FBS^Cisplatin
        l21(1:length(j1),j0)=j1;
        j2=find((DOE(:,i1)==0 & DOE(:,i2)==0)==1); % DOE with ~(L-glutamine^D-glucose) ~(L-glutamine^FBS) ~(L-glutamine^Cisplatin) .. ~(FBS^Cisplatin)
        l20(1:length(j2),j0)=j2;
```

```
    end
end
% Friedman's statistic test factor-pair contributions
rs21=zeros(6,6);
rs20=zeros(6,6);
for i1=1:6
    % pool together ranking orders of three exps
    rs21(:,i1)=[OSR(l21(:,i1),1);OSR(l21(:,i1),2);OSR(l21(:,i1),3)];
    rs20(:,i1)=[OSR(l20(:,i1),1);OSR(l20(:,i1),2);OSR(l20(:,i1),3)];
    % Freidman test
    pFOS2(i1)=friedman([rs21(:,i1) rs20(:,i1)],3,);
end
% analyze factor-factor interactions
% find combinations with or without a pair of factors
l31=zeros(2,1,'uint8'); % DOE with      D-glucose^Cisplatin    l30=zeros(2,1,'uint8'); % DOE with (~D-glucose)^Cisplatin
j0=0;
for i1=2
    for i2=4
        j0=j0+1;
        j1=find((DOE(:,i1)==1 & DOE(:,i2)==1)==1); % DOE with       D-glucose^Cisplatin
        l31(1:length(j1),j0)=j1;
        j2=find((DOE(:,i1)==0 & DOE(:,i2)==1)==1); % DOE with    (~D-glucose)^Cisplatin
        l30(1:length(j2),j0)=j2;
    end
end
% Friedman's statistic test factor-pair contributions
rs31=zeros(6,1);
rs30=zeros(6,1);
for i1=1
    % pool together ranking orders of three exps
    rs31(:,i1)=[OSR(l31(:,i1),1);OSR(l31(:,i1),2);OSR(l31(:,i1),3)];
    rs30(:,i1)=[OSR(l30(:,i1),1);OSR(l30(:,i1),2);OSR(l30(:,i1),3)];
    % Freidman test
    pFOS3(i1)=friedman([rs31(:,i1) rs30(:,i1)],3);
end
```