Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic supplementary information

An engineered intra-tumoral heterogeneity with mimicry of accumulation of driver mutations of pancreatic cancers

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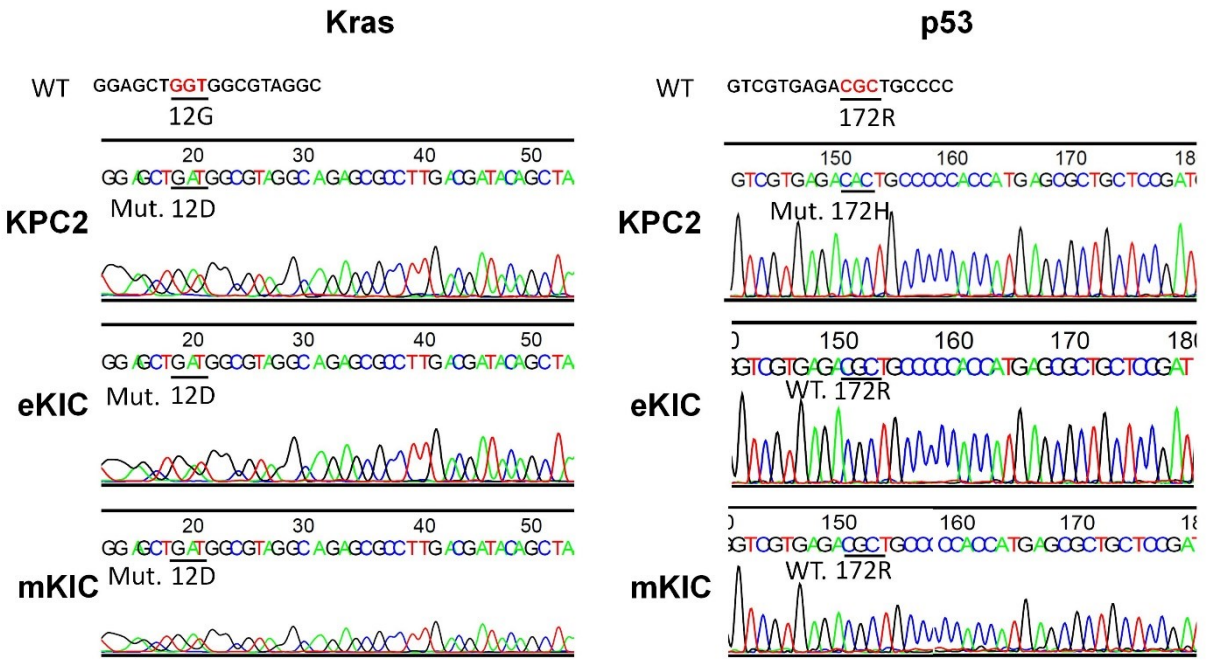

Fig. S1. RT-qPCR results of Kras and p53 sequencing. All three cell lines contain Kras G12D mutation. In p53, KPC2 cells contain the Trp53 mutation in R172H whereas the other cells have wild type Trp53.

Drug sensitivity assay

To evaluate the drug sensitivity, a viable cell growth assay was evaluated by measuring the area of cell nuclei stained by Hoechst 33342. The Hoechst 33342 stains cell nuclei in both live and dead cells. The dead cell detection was minimized by applying the 48-hour post culture period after gemcitabine treatment, where we assumed that the dead cell nuclei could be eliminated during the process. Apoptotic cells experience nuclei degradation. [1] We assumed that the detection of the degraded nuclei could be excluded after the post-culture process. Also, the nuclear area measurement minimizes the effect of DNA fragment detection compared with the count of nuclei. To verify the method, we performed the live/dead cell viability assay using both Hoechst 33342 (Hoechst) and Propidium Iodide (PI), where the dead cells are identified by PI fluorescence. For the live/dead cell viability assay, eKIC cells were cultured with 6mg/ml type I collagen in the simplified T-MOC device, composing only a layer of an interstitial channel with two side-lymphatic channels. The simplified T-MOC increases the medium perfusion by direct contact of the medium channel to the interstitial channel. The drug treatment procedure follows the equivalent timeline with the drug sensitivity assay described in the method section. We treated 20μM gemcitabine for 24 hours following by 48-hour preculture process. After another 48-hour post culture process, we stained the cells with Hoechst and PI. As a result, the nuclei of dead cells by gemcitabine treatment were mostly cleared out after 48-hour post culture as shown in the Fig.S2 A and B. Indeed, the nuclear area fraction of the live cells were not significantly different from the total nuclear area fraction of both live and dead cells. The nuclei of live cells were identified by subtracting Hoechst nuclear fluorescence to the dead cell nuclei's PI fluorescence. The results confirm that the cell survival determined by the normalized nuclear area is comparable to that by the standard live/dead cell viability assay.

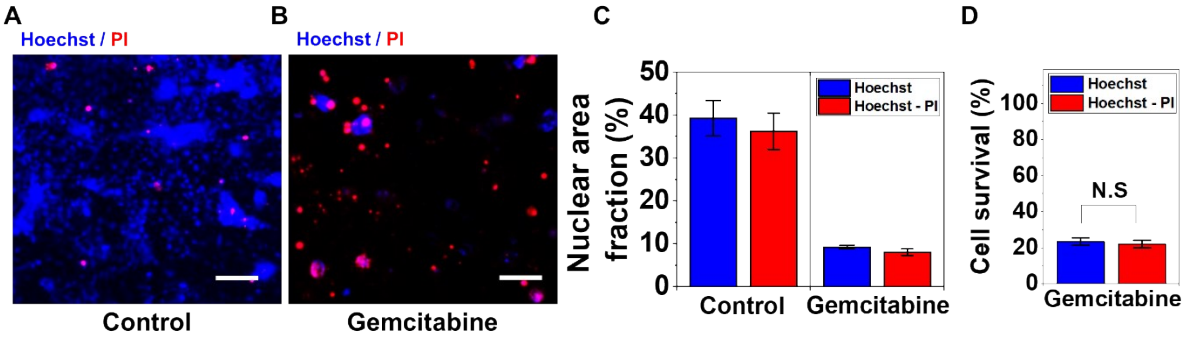

Fig. S2 Live/dead cell viability assay for eKIC cultured in simplified T-MOC. (A-B) Fluorescent micrograph of nuclei of both live (Hoechst, blue) and dead cells (PI, red) in (A) control and (B) 20μM gemcitabine treatment groups. Scale bar: 100μm (C) Nuclear area fraction of total nuclei of live and dead cells (Hoechst only) and live cells (Hoechst – PI). (D) Cellular response to gemcitabine evaluated by Hoechst only (blue) and standard live/dead cell staining (red). n≥3 Bar: mean ± S.E.M. N.S: not significance p >> 0.05 (Student t-test). The results showed that the quantified cell survival in this study is comparable to the standard method of live/dead cell assay.

Estimated Transvascular Permeability Values for iT-MOC Family of Devices

We previously reported accumulation of nanoparticles (NP) in iT-MOC family of devices for various NP sizes and membrane pore sizes. [2] The contribution of microvascular endothelial cells (MVEC) to the membrane functional barrier was also investigated. As further characterization of transport around iT-MOC, we provide estimates for transvascular permeability realized within the devices based on data presented in our earlier work[2] and Kedem-Katachansky formulation [3,4] governing the transvascular solute transport.

During device operation, solutes composed of molecular or nanoparticle drug particles that are perfused through the capillary channel permeate the membrane and accumulate within the interstitial channel overtime. At the early phases of perfusion, it has been observed that accumulation of the solutes within the interstitial channel is confined to a region directly underneath the capillary channel. [2] The rate of drug accumulation in the interstitial channel within that time frame is then given by:

$$K_{av}V\frac{dC}{dt} = J_s A \tag{1}$$

where C is the average drug concentration within the interstitial channel, K_{av} is the available volume fraction, A is the area of the membrane forming the bottom wall of the capillary channel, V is the volume of the region within the interstitial channel directly underneath the capillary channel.

The solute flux across the membrane, J_s is given by Kedem-Katachansky formulation as:

$$J_s = J_v f_s \overline{C}_s + P(C_v - C) \tag{2}$$

where P is the microvascular permeability. f_s is microvascular retardation coefficient, C_v is the solute concentration within the capillary channel, and $\overline{C}_s$ is the logarithmic mean difference of the solute concentrations within the capillary and interstitial channels.

Considering the early time points where C is small compared to C_v, the drug concentration within the interstitial channel can be solved from Equations (1) and (2) as a function of time as follows:

$$\frac{C}{C_v} = \left(1 + \frac{h_s}{P}\right)\left(1 - e^{-\frac{P}{K_{av}H}t}\right) \quad (3)$$

Just as permeability, P, represents the significance of diffusive solute transport across the membrane, $h_s = J_v f_s$ indicates the contribution of convective solute transport. H is the thickness of the initial accumulation region taken as the full thickness of the interstitial channel.

The transport parameters P and h_s were estimated by fitting Equation (3) to the first 3 hours of accumulation profiles[2] using a least squares curve fitting algorithm in MATLAB. The estimated parameters are reported in Table 1.

Table 1. Estimated Transvascular Transport Parameters for iT-MOC family of devices using

Model System	NP Diameter (nm)	Membrane Pore Size (nm)	P (m/s)	h_s (m/s)
iT-MOC	100	100*	-	-
	100	400	4.5×10^{-8}	4.0×10^{-7}
	100	1000	3.0×10^{-8}	2.9×10^{-7}
	100	1000 + MVEC	7.0×10^{-10}	7.0×10^{-8}
	200	400	6.0×10^{-11}	3.3×10^{-9}
	500	400	3.2×10^{-12}	$< 10^{-16}$
Human colon adenocarcinoma xenograft[5]	120	N/A	2.0×10^{-10}	N/A

* No accumulation was observed.

References

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