NOVEL HIGH-THROUGHPUT SCREENING SYSTEM FOR CANCER THERAPY WITH SIMULTANEOUS COMBINATION TREATMENTS

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ABSTRACT

In this paper, we report the development of a microfluidic high-throughput screening system for high-throughput screening of drug molecule interactions on cancer cell death. A key feature is that 64 pair-wise concentrations (between eight concentrations of two drugs) are generated using a traditional diffusive microfluidic mixer and a time-dependent variable concentration mixer. Using this novel high-throughput combination array, we generated pair-wise combinations of the cancer drug sensitizer doxorubicin and the cytokine TRAIL, and used it for on-chip screening against PC3 prostate cancer cells. Our results identify a concentration range where doxorubicin and TRAIL are synergistic and demonstrates the utility of the system as a high-throughput anti-cancer drug screening tool.

KEYWORDS: Combination treatment, high-throughput screening, TRAIL, Doxorubicin, prostate cancer

INTRODUCTION

Traditional single-chemosensitizer discovery approaches, although moderately successful, are inherently low-throughput and do not allow for comparison of interactions between drugs or sensitizing agents. Combination therapies have emerged as powerful alternatives to single-agent therapies since they enhance cell death by overcoming multiple resistance mechanisms in cancer cells. Most of the combination treatments explored employ an ‘ad-hoc approach’ for the discovery of potential sensitizing agents in which a single agent is investigated for its ability to sensitize cancer cells at a few discrete concentrations. This low-throughput approach limits the rate of discovery of novel candidates that are effective against cancer cells, as dose-dependent interactions can be missed. To overcome these limitations, we have developed a high-throughput platform for generating combinations of chemotherapeutic drugs at user-defined concentrations, and applied it to the identification of combinations that lead to higher prostate cancer cell death.

EXPERIMENTAL

The concept underlying the simultaneous combinational generator is described in Figure 1(a). The device is fabricated in two layers - a pneumatic layer for controlling cell chambers and a second layer for generating the different concentrations of chemical molecule using diffusive-mixers. A concentration gradient of the drug A is generated by a traditional diffusive-mixer. Drug B is mixed with buffer to generate a desired concentration and is introduced into the two streams used for generating the concentration range of drug A (i.e., upstream of the diffusive mixer). Since each row of cell culturing chambers can be individually controlled using the pneumatic channel, this enables a specific set of combination (range of concentrations of A with a single concentration of B) to be introduced into each row of cell culture chambers. By varying flow rate of B and its coupled flow rate of buffer, a different concentration of B can be generated, which leads to a second set of pair-wise concentrations of A and B in the second row of chambers. The operations of the syringe pumps and the pneumatic channel are controlled by a programmable LabView interface for controlling the flow rate (and hence, concentration) of Drug B (Figure 1(b)).

![Figure 1: (a) Concept of combinatorial pair-wise concentration generation, (b) Experimental setup for pair-wise concentration generation. A gradient of drug A is generated by diffusive mixing in a christmas tree gradient mixer using Syringe A. Concentration range of drug B is formed by mixing with drug A at different flow-rate combinations](image)
Figure 2 demonstrates a simulated pair-wise combination treatment scheme using color dyes. First, a syringe pump was used to introduce Drug A solution and buffer into a diffusive mixer to generate “the horizontal gradient”. This is done with all cell culture chamber valves closed. After the gradient had stabilized, valve-arrays corresponding to each row were opened for 10 sec to capture the generated gradient concentration solution. Then, the cell culture chambers were filled with violet dye, and the blue/yellow color gradient was re-introduced through the diffusive mixer inlet (Figure 2a). Next, violet dye diluted with buffer was added to the blue/yellow color gradient and introduced into a specific row of chambers (Figure 2b). Other rows of chambers were similarly populated (Figure 2c). These processes were automatically controlled and operated in LabView. Figure 2c shows a micrograph of a completed simultaneous gradient array. Figure 2d represents wash out of the regions surrounding the cell culture chambers with orange dye to simulate the media refreshing process after cell seeding. The fidelity of the system in keeping the two colors separate is evident from the lack of diffusion and mixing after 5 h between the dyes present inside and outside the cell culture chamber.

In a typical experiment, PC3 prostate cancer cells were seeded into the micro cell chambers through the diffusive-mixer inlets. Media in the chambers was automatically refreshed every 3 hours for 1 day prior to the experiment. Then each row of eight micro cell chambers was refreshed with pair-wise drug combination generated as described above and captured by pneumatic controlled chambers. This process was repeated in-order every 3 hours. Since each chamber is completely and exposed to only a small amount of liquid, shear stress effects due to flow are minimized.

Figure 3: Combination of sensitizer doxorubicin and TRAIL treated PC3 cells.
RESULTS AND DISCUSSION
Representative sequential combination treatment results from generating pair-wise combinations of doxorubicin (0 – 3 µM) and TRAIL (0 – 10 ng/mL) are shown in Figure 3. There is little reduction in cell viability with doxorubicin alone (orange) or TRAIL alone (green) after 24 h whereas exposure to combinations of doxorubicin and TRAIL leads to much higher cell death than the corresponding controls (red).

Figure 4: Simultaneous combination treatment with doxorubicin and TRAIL for 36 hours

The number of cells remaining in each chamber was quantified. Figure 4 shows the same data plotted as a function of doxorubicin or TRAIL concentration. The data show no reduction in cell viability upon exposure to only TRAIL or doxorubicin (black squares). With increasing concentrations of the combination, an increase in PC3 cell death is observed. Since doxorubicin alone led to more cell death than TRAIL alone, further studies are required to minimize side-effects and identify the optimal concentration of the sensitizer to be used.

CONCLUSION
We have developed a microfluidic high-throughput screening system for identifying effective anti-cancer drug combinations from 64 user-defined pair-wise combinations of drug mixtures. The applicability of this system for identifying optimal sensitizer and drug combinations for PC3 prostate cancer cells was shown. We envision this system to be useful for identifying novel concentration-dependent interactions between different drug molecules.

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REFERENCES

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