A sustainable material for capturing and killing circulating tumor cells

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Statement of Purpose: Circulating tumor cells are the key source of cancer metastasis that is the major reason for cancer death[1]. Thus, it is important to develop safe and efficient methods to remove them out of the circulation. In this study, we developed a multifunctional material with the capability of capturing and killing cells by using a bilayer hydrogel **(Fig. 1A)**. The bottom layer hydrogel was used for sustained drug release; the top one was used to capture cancer cells via aptamer-mediated cell recognition. We demonstrated the concept by evaluating the functionalities of the hydrogel in cell capture, drug delivery and cell killing.

Methods: The bilayer polyacrylamide hydrogel was prepared on a silanized glass slide. DNA aptamer (sgc8c)[2] that specifically bind CCRF-CEM cell was conjugated on the top hydrogel layer. DNA duplexes that were used to control the release of Dox were chemically incorporated into in the bottom hydrogel layer.

The hydrogels were incubated with 40 µL of DPBS containing 5 mM $MgCl_2$ and Doxorubicin (500 µM) at 4 °C for 24 h to load Dox. To examine Dox release from the hydrogel, the Dox-loaded hydrogels were incubated in 500 µL of DPBS with a shaking speed of 70 rpm at 37°C. At predetermined time points, the supernatants were collected and replaced with 500 µL of fresh DPBS. The collected supernatants were measured using a Tecan F200 Pro Micro-plate reader (Tecan US Inc., San Jose, CA) at 592 nm.

To study the interactions between CCRF-CEM cells and the affinity hydrogel, the hydrogel incubated with 500 µL of cell suspension (3×10^5 cells in the cell culture medium supplemented with 5 mM $MgCl_2$) in a 48-well plate at 37 °C for 1 h. The 48-well plate was gently shaken at 70 rpm to remove unbound cells

Quantitative analysis of the cell viability was performed using a CellTiter MTS cell assay kit. In brief, CCRF-CEM cells were collected from the hydrogel surface, seeded into 96-well plate with a population of 2.5×10^4 cells in 100 µL of cell culture medium and cultured in CO_2 incubator for 48 h. The CellTiter reagent (20 µL) was added to each well and incubated with the cells at 37 °C for 3 h. The absorbance at 490 nm was measured using the Tecan Micro-plate reader.

Results: The aptamer-functionalized hydrogel was able to capture target cells specifically (**Fig. 1B**). Approximately 1,200 CCRF-CEM cells per mm^2 were observed on the aptamer functionalized hydrogel whereas only ~10 K299 cells (control) per mm^2 were observed on the hydrogel.

The controlled release data show that Dox was released from dsDNA–functionalized hydrogels in a sustained manner. Moreover, the release of Dox from the dsDNA-functionalized hydrogel exhibited a pseudo-linear release profile after 4 h. The concentration of Dox in the release media was close to or higher than 1 µM during the entire 144 h test and was above 2 µM within the first 48 h (**Fig. 1C**).

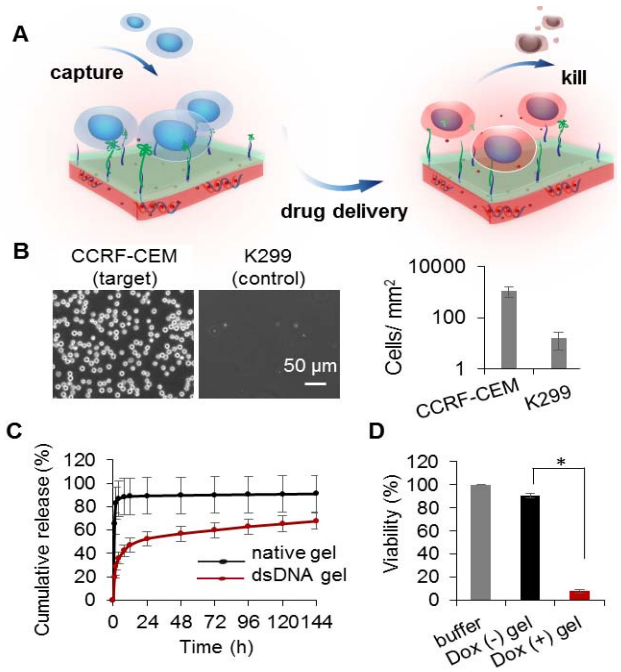

Figure 1. (A) Conceptual illustration of cell capture and killing. (B) Examination of cell capture specificity. (C) Comparison between the double stranded (dsDNA)-functionalized hydrogel and the native control hydrogel in sustained Dox release (n=3). (D) Analysis of cell viability with the MTS assay. * P<0.001; n=3.

The quantitative analysis of cell viability showed that the viability of the cells captured onto the multifunctional hydrogel was decreased to less than 10% in comparison to those in the control groups (**Fig. 1D**).

Conclusions: The results show that the multifunctional hydrogel could capture target cells specifically and release drugs locally. More than 90% of the captured cells were killed after their short residency on the hydrogel. Therefore, this study has demonstrated a promising capture-and-kill concept for destroying tumor cells in the circulation.

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Reference:

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