Modeling tumor immunity with a two-tissue microfluidic recirculating co-culture system

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Tumors communicate with nearby lymph nodes through lymphatic channels and the blood vasculature, and this two-way exchange of antigens and secreted signals is thought to be critical to tumor progression or control. However, it is challenging to study two-organ interactions in vivo due to complex connections and practical constraints on sampling. Furthermore, existing bioengineered models of tumor immunity typically focus on the tumor compartment and exclude events in the downstream lymph node, such as immunosuppression.

To address this issue, we developed and validated a novel microfluidic platform to test the impact of continuous cross-talk between live tissue slices of murine tumor and lymph node (Fig. 1A-C). Tissue slices were supported on semi-permeable membranes and integrated into a single three-layer PDMS chip. The device transported media cyclically between the slices, using peristaltic pumps to provide recirculating flow. First, we demonstrated protein release and capture from one slice to another, using protein-soaked agarose samples to model the tissue (Fig. 1D). Tissue viability was maintained for 24 hr on the chip.

Next, we tested the ability of the device to model tumor-immune interactions. Tumor cells (4T1 breast cancer line) were injected into mice, and after 7 days the tumor, lymph nodes, and mammary fat pads were collected. Lymph nodes were tested for their ability to secrete the cytokine interferon-γ (IFN-γ) after polyclonal T cell stimulation, either immediately after tissue harvest or after co-culture with tumor samples on the microfluidic chip. Interestingly, lymph node tissues cultured for 24 hr together with tumor tissue exhibited reduced IFN-γ secretion compared to tissues cultured with healthy fat pad, consistent with immunosuppression observed in vivo (Fig. 1E). Thus, the chip has the potential to model complex tumor-lymph node interactions in an experimentally accessible format.

**Figure 1.** A microfluidic chip to co-culture tumor and immune tissues under continuous recirculating flow. Schematics of (A) top view and (B) cross-section of a single well. Flow passed through each tissue on its way to the next well. (C) Photo of assembled chip, with labeled culture wells (1, 2) and peristaltic pumps (P1, P2). (D) Slices of agarose were loaded with neutravidin-rhodamine (1) or biotinylated beads (2), and their fluorescent intensities were measured before and after on-chip co-culture for 24 hr, revealing transfer of the protein between slices.

(E) Lymph nodes (non-tumor-draining) were co-cultured with tumor or healthy fat pad tissue on chip for 24-hr, then tested for IFN-γ secretion after stimulation with anti-CD3, revealing suppression after co-culture with tumor slices.

In conclusion, we describe a novel microfluidic system to support two-way cross-talk between live tissue samples. The chip successfully modeled one aspect of tumor-induced lymph node immunosuppression. Looking ahead, this chip may provide a powerful platform to investigate mechanisms of immunosuppression and test the effects of anti-tumor immunotherapies ex vivo.