A method for preparing tumor tissues for ex vivo culture that retains T cells within live tumor fragments (LTFs) while preserving tissue viability and T cell function

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Poster 17

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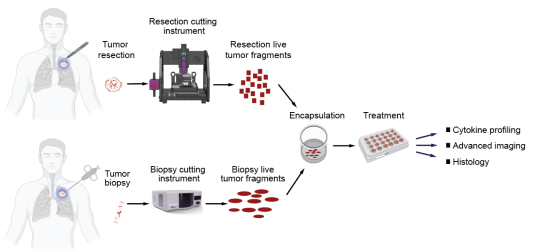
Introduction

- Characterization of response to immunotherapy in live tumor fragments (LTFs) ex vivo requires preservation of the tumor microenvironment (TME)
- Traditional tissue preparation methods disrupt the TME, limiting the translational value of these models
- To overcome this challenge, we have developed a method that incorporates automated cutting instruments designed to optimally fragment tumor resections and core needle biopsies (CNBs), and a proprietary hydrogel that supports the TME of LTFs in ex vivo culture
- This methodology is also amenable to longitudinal advanced imaging techniques such as dynamic optical coherence microscopy (dOCM)[1]
- Using this method, the Elephas Live Platform is optimized to assess response to immunotherapy in LTFs ex vivo[2]

Methods

Elephas Live Platform



Humanized patient derived xenograft (PDX): Tumors were derived by warm passaging PDX LTFs in the hind flank of NSG mice. When tumors reached ~50 mm³, mice were injected intravenously with 10-12 x 10⁶ human peripheral blood mononuclear cells.

Human tissue: Resected tumors were collected under a waiver of consent or informed patient consent and core needle biopsies were collected under informed patient consent. Tumor specimens were shipped overnight at ~4°C in media. Protocols for the collection of human specimens were approved by an institutional review board.

Tumor resections: Resections were embedded in 4.0% low gelling temperature agarose. Tumors were then cut into 300 µm x 300 µm x 300 µm resection LTFs using an automated cutting instrument. Resection LTFs were passed through a 200 µm filter and aliquoted into a 24-well plate at ~200 fragments/well and encapsulated in a commercial hydrogel (VitroGel®-3, TheWell Bioscience). After the hydrogel polymerized, 1 mL of culture media containing a treatment was added to each well. The plate was imaged on a digital microscope to calculate the tissue volume present in each well. Resection LTFs were then maintained at 37°C and 5% CO_2 throughout experimentation.

Core needle biopsies (CNBs): 12- to 20-gauge CNBs were embedded in agarose and cut at a 20° angle to create LTFs with a thickness of 300 µm using an automated cutting instrument. The biopsy LTFs were dispensed into a 24-well plate at a defined number based on the gauge of the CNB. LTFs were washed three times with Dulbecco's Phosphate Buffered Saline and encapsulated in 300 µL of a proprietary hydrogel. After hydrogel polymerization, 500 µL of media containing a treatment was added to each well with tissue. The plate was imaged on the digital microscope to calculate the tissue volume present in each well. Biopsy LTFs were maintained at 37°C throughout experimentation.

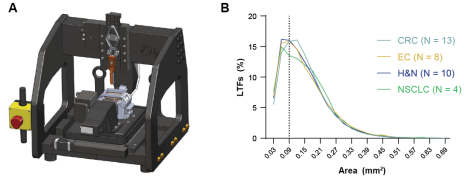
Culture and treatments: Following encapsulation, fragments were treated with drug or vehicle control. Negative control wells were treated with 50 µg/mL human IgG4 isotype control antibody immediately after plating. αCD3/αCD28 treatment was performed using 25 µL/mL of ImmunoCult™ Human CD3/CD28 T Cell Activator. αPD-1 treatment was performed using 50 µg/mL αPD-1 antibody.

Cytokine profiling: Conditioned media collected from individual culture wells at defined time points were assessed using the Human XL Cytokine Luminex Performance Assay (R&D Systems). The cytokine concentrations in each sample were interpolated from standard curves generated for each analyte. Using imaging software, the total tissue volume in each well was calculated to permit normalization of cytokine concentrations.

Histology: Resection LTFs were fixed in 10% phosphate buffered formalin and transferred to 70% alcohol before being pre-embedded in a 2% agarose solution in a custom-designed 3D printed mold. Biopsy LTFs were fixed and directly transferred in their culture encapsulated condition into 70% alcohol. Resection and biopsy LTFs were paraffin embedded in blocks and sectioned at 5-µm thickness. Sections were mounted to slides using a tissue flotation water bath. Slides were stained with hematoxylin and eosin (H&E) or processed for immunohistochemistry (IHC) and immunofluorescence using Ventana Medical Systems Discovery Ultra Autostainer.

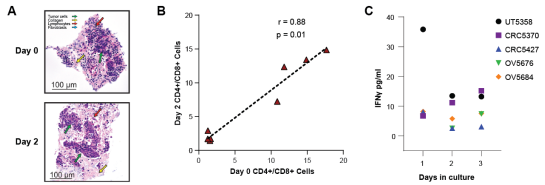
Optical coherence microscopy: A custom designed optical coherence microscopy (OCM) system[1], was fitted with a 15X lens (0.80 NA) and used to acquire time resolved, volumetric dOCM data for viability analyses. Images were acquired every 24 hours for the same tumor specimen over 3 days of culture. To obtain the dOCM data, the time series of each cross section was first registered to the center frame. For each pixel in the time series of volumetric OCM intensity I(x, y, z, t), fast Fourier transform was performed to obtain the power spectrum P(x, y, z, f).

1 A specialized instrument for cutting tumor resections produces LTFs of expected size



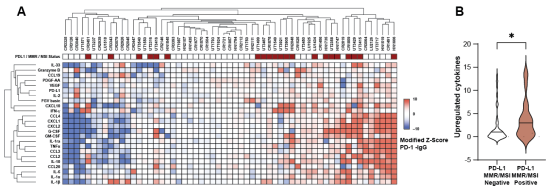
A A specialized cutting instrument was used to fragment human tumor resections to create LTFs at user specified dimensions. **B** LTFs were prepared from tumor resections by cutting at 300 µm (length) x 300 µm (width) x 300 µm (depth) dimensions. Surface areas (mm²) from one face of LTFs were measured using a digital camera and image analysis software developed internally. Frequency distributions of surface areas from a single face of LTFs for colorectal (CRC), endometrial (EC), head and neck (H&N) and lung (NSCLC) tumors are reported. The dotted line represents expected surface area for a single face of a 300 µm x 300 µm x 300 µm fragment.

2 Histological features, CD4+/CD8+ ratio and T cell function of LTFs are preserved over 2 days of ex vivo culture



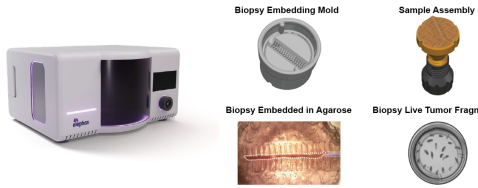
A H&E-stained sections of head and neck LTFs showing conservation of tumor cells (green arrow), collagen fibers (yellow arrow) and lymphocytes (red arrow) at Day 0 and Day 2 of culture. **B** Correlation plot of CD4+ to CD8+ cell ratios on Day 0 vs. Day 2 of culture as assessed by IHC (N = 8 human tumors: colorectal, n=1; bladder, n=1; head and neck, n=2; ovarian, n=3; pancreatic, n=1) r and p values pertain to Spearman correlation analysis. **C** Changes in LTF T cell function over 3 days in culture as assessed by IFNγ production following 24 hours of stimulation with αCD3/αCD28. Data are reported for 5 unique human tumors (UT, uterine, CRC, colorectal, OV, ovarian).

3 Unsupervised hierarchical clustering groups PD-L1+ / dMMR / MSI-H samples amongst those w/ greatest cytokine upregulation following ICI treatment



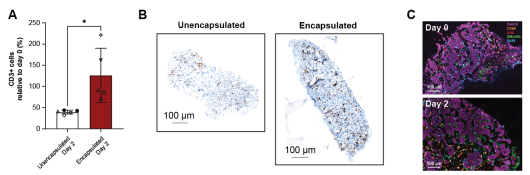
A Unsupervised hierarchical clustering of cytokine profiles from patient tumor resections using modified z-scores of the difference in cytokine concentrations between the ICI and IgG treated groups. To account for variability across wells, replicate wells for each treatment group were run when sufficient tissue was available (n=3 for 48 specimens, n=2 for 6 specimens and n=1 for 5 specimens). Positive modified z scores (capped at 10) are depicted in shades of red and negative modified Z-scores (capped at -10) are depicted in shades of blue. Samples with Z-scores of 0 are depicted in white. PD-L1+, dMMR, and MSI-H specimens are annotated with dark red boxes in the upper track. PD-L1-, pMMR, and MSS specimens are annotated in white. N=59 specimens. **B** The number of upregulated cytokines for individual specimens, defined by a modified Z-score ±5, is significantly higher in the PD-L1+/dMMR/MSI-H cohort compared to the PD-L1-/pMMR/MSS cohort. * p<0.05

1 An automated intrument for cutting core needle biopsies (CNBs) produces LTFs for ex vivo culture



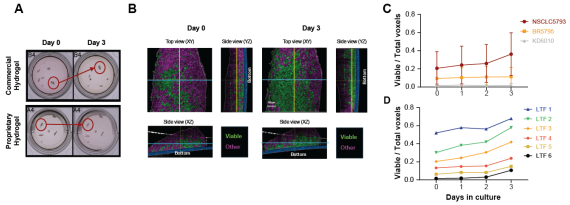
A proprietary cutting instrument was designed to cut CNBs into LTFs. Individual CNBs suspended in buffer are poured into a biopsy embedding mold. Openings at the bottom of a trough in the embedding mold allow for the buffer to drain out while positioning the biopsy horizontally at the bottom of the mold. Low melting point agarose is then added to the mold. A holder (depicted in yellow) is inserted into the mold while the agarose is still in liquid form, allowing for the agarose to permeate the holder. As the agarose solidifies, it embeds the biopsy and connects with the holder as a single moving piece. When the holder is removed from the mold, a sample assembly is formed such that the biopsy and agarose are retained on the top of the holder. The sample assembly is then transferred to the cutting instrument to cut oblong slices. The image of the biopsy embedded in agarose is from a 20 gauge NSCLC core needle biopsy.

2 Encapsulation of biopsy LTFs in a proprietary hydrogel helps to preserve the tumor microenvironment during ex vivo culture



A Biopsy LTFs prepared from humanized NSCLC PDX tumors were cultured for 2 days either unencapsulated or encapsulated in a proprietary hydrogel. A significant decrease in the number of CD3+ cells, normalized to tissue area, is seen in unencapsulated compared to encapsulated biopsy LTFs (each symbol represents 1 tumor, *p<0.05). **B** CD3-stained IHC sections of representative NSCLC humanized PDX biopsy LTFs from unencapsulated (left) and encapsulated (right) tissues after 2 days of ex vivo culture. **C** Multiplex immunofluorescence-labeled fragments from a human liver cancer core needle biopsy show the presence of tumor cells (PanCK), macrophages (CD68), T Cells (CD3), stroma/fibroblasts (SMActin) and cell nuclei (DAPI) at Day 0 and Day 2 in culture.

3 Encapsulation of biopsy LTFs in a proprietary hydrogel provides positional stability enabling longitudinal advanced imaging



A The spatial stability of biopsy LTFs over 2 days of culture when encapsulated in hydrogel. Circles highlight regions of hydrogel where LTF stability is compromised with commercial hydrogel encapsulation but not with encapsulation in a proprietary hydrogel. **B** Dynamic optical coherence microscopy of a human NSCLC biopsy LTF on days 0 and 3 in culture. Voxels positive for metabolic activity, indicating tissue viability, are false-colored in green and voxels negative for metabolic activity are false-colored in purple. **C** The ratios of viable voxels to total voxels for 3 human tumors showing maintenance of viability over 3 days in culture. (NSCLC, lung; BR, breast; KD, kidney). **D** The ratio of viable voxels to total voxels for 6 LTFs from NSCLC5793, highlighting the heterogeneity in viability across LTFs from the same biopsy while also demonstrating maintenance of viability over 3 days of ex vivo culture for each individual LTF.

Conclusions

- Here we present an optimized method designed to preserve the native TME in LTFs during ex vivo culture
- Automated cutting instruments precisely fragment tumor resections and CNBs while preserving tissue architecture
- Encapsulation of LTFs in a proprietary hydrogel, supports viability, retains histological features, and sequesters functional T cells during ex vivo culture
- LTFs remain stable in position, enabling longitudinal advanced imaging techniques such as dOCM
- Deployment of this methodology enables the Elephas Live Platform to accelerate the development of personalized cancer treatments and improve the prediction of patient response to immunotherapy

References

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Abhijeet Prasad
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