Advanced Tissue-Engineered Constructs That Capture Intra-Tumoral Heterogeneity

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Background & Significance

Glioblastoma (GBM), one of the most common and most lethal types of brain cancer, occurs in ~6 of 100,000 people[1]. The median survival time is just 15 months after diagnosis, despite aggressive surgical resection of the primary tumor, chemotherapy, and radiation[2]. For patients over 45 years old, prognoses are exceptionally poor. Frequent appearance of treatment-resistant, recurrent tumors results in a 5-year survival rate of only 6-9%[3,4].

Significant barriers to identifying drugs with consistent *in vitro* to *in vivo* therapeutic responses exist due to the primary tumor's genetic heterogeneity and the inability of current experimental models to recapitulate this heterogeneity, resulting in aggressive invasion and therapeutic responses that cannot be predicted based on the GBM's initial histopathological classification[5-8]. While the brain's extracellular matrix (ECM) is a major contributor to treatment resistance and recurrence, the ECM is not accounted for in most *in vitro* models[9]. Furthermore, though *in vivo* models have the living host's microenvironment, significant disadvantages exist due to the cost, time, reproducibility, and complexity inherent to animal models.

Addressing GBM recurrence is a critical, unmet need that necessitates a new and innovative, but also precise and accurate, model for the testing of personalized medicine. An impactful experimental model that can accelerate progress in the field of GBM research would need to be:

1. Representative of the cancer's microenvironment
2. Able to preserve the primary tumor's genetic heterogeneity
3. Applicable for high-throughput screens to identify patient-specific treatments within a clinically actionable timeframe

Impact Statement

Our goal is to design a fast and reliable platform that keep cancer cells alive for drug testing after their removal from the patient with an extremely aggressive cancer such as GBM. To do this, we are working to create an environment for the cells, so that they feel as if they are still inside the patient and, as a result, will grow as if they are still inside the patient. This accuracy is critical, because it helps ensure a higher likelihood that drug responses seen in the laboratory are going to be what is seen later in the patient. The speed of how quickly we can test drugs is vital, because of how quickly recurrent tumors appear in these patients. Additionally, the number of how many laboratory copies we can make from each patient sample is essential, because we need to test many drugs simultaneously. An accurate and time-sensitive model GBM outside of the body, in a way that is also scalable for large quantities of drugs to be tested, has the potential to yield significant improvements in precision medicine, personalized treatment plans, and patient outcomes.

Methods & Hypothesis

Hyaluronic acid (HA) is an ECM component that is abundant in normal brain tissues, overexpressed in GBM, and is known to promote tumor growth, invasion, and therapeutic resistance[10-15]. Our laboratory has developed a 3D tissue-engineered model, where GBM cells are cultured in HA scaffolds (**Figure 1**) that approximate the brain's ECM[16]. HA content and stiffness of this gel can be independently modified to allow for optimal cell growth in 3D, as opposed to a 2D monolayer or in suspension culture. With a scaffold present that mimics the native ECM, cells can better display behaviors such as migration, which is essential to tumor invasion of healthy nearby tissues.

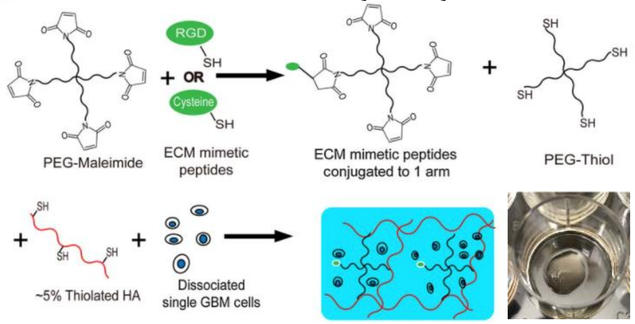

Figure 1. HA scaffold fabrication process[16,18]. HA is a polysaccharide that can be crosslinked to nearby HA strands to generate a gel-like substance.

These HA biomaterial scaffolds:

1.Are affordable, easily fabricated, and modifiable to match physiological properties of the tumor microenvironment

2.Have been shown to preserve some pathological features that are lost in other culture methods

3.Have erlotinib (**Figure 2**) and temozolomide resistance kinetics that resemble those in murine xenografts[16,17]

We expand upon previous findings by examining the heterogeneity captured by different GBM models derived from tissues immediately after resection. Our overall hypothesis is that HA scaffolds can, more accurately than the best *in vitro* and *in vivo* models, maintain cell populations that are representative of the primary tumor.

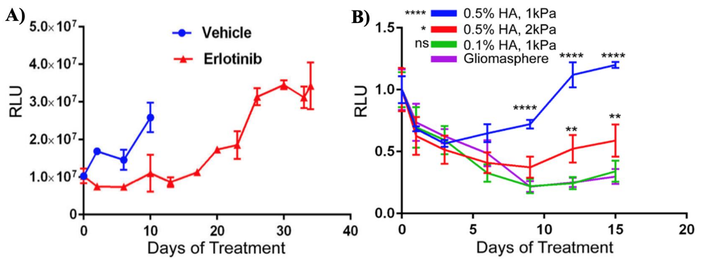

Figure 2. Example of drug resistance tracking through time in xenografts (A) and *in vitro* (B) after erlotinib treatment by measuring relative luminescence units (RLU) of GBM cells that constitutively express a luciferase reporter[16].

Outcome Measures & Goals

Our goals are to create a single-cell RNA sequencing (scRNAseq) database of primary tumors and their experimental model derivatives, then identify *in vitro* and *in vivo* models that best represent a primary tumor's cellular makeup across various patient lines from different GBM subtypes. A schematic of how each model will be set up from de-identified patient samples and the tissues that will be analyzed can be seen in **Figure 3**.

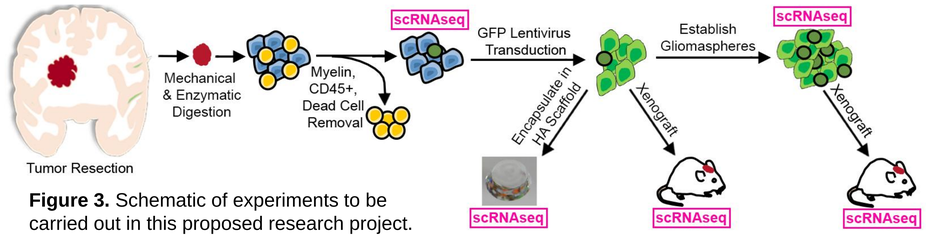

Figure 3. Schematic of experiments to be carried out in this proposed research project.

scRNAseq (**Figure 4**) is a powerful tool that quantifies gene expression levels, by sequencing the mRNA, unique to each individual cells in a sample. Generation of cell-specific gene expression matrices allows for bioinformatical identification of the different cell types that comprise a tissue.

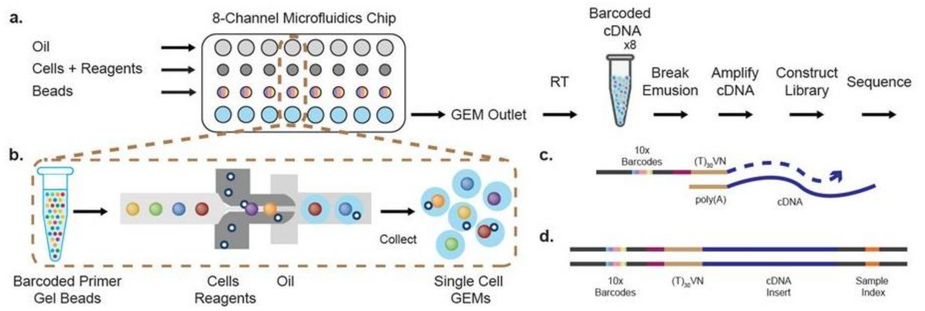

Figure 4. Schematic of scRNAseq library preparation[19].

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