Modeling of Electric Field Enhanced Tissue Dissociation

By
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B.S., Northeastern University, 2018

Thesis

Submitted in partial fulfillment of the requirements for the
Degree of Master of Science in Biomedical Engineering at Brown
University

PROVIDENCE, RHODE ISLAND

MAY 2024

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SYNOPSIS AND IMPACT OF THIS THESIS WORK

This thesis aims to elucidate the understanding of electric field effects on tissue dissociation. To achieve this goal, we designed and developed a comprehensive modeling approach that not only culminated in several technical insights but also contributed to the advancement of instrumentation design within the realm of single-cell genomics solutions. Exploring electric field-enhanced tissue dissociation introduces a novel perspective, seeking to bridge existing knowledge gaps.

To fulfill this aim, this research concentrated on building theoretical and experimental device design insights toward building future devices that are automated and easy to use by clinicians.

TECHNICAL BREAKTHROUGHS

This thesis introduces advancements in the understanding and application of electric fields within complex biological media. By developing and utilizing COMSOL modeling, this work predicts the interaction dynamics between electric fields and biological tissues incorporating critical variables such as electric field strength, tissue conductivity, and permittivity changes, providing a comprehensive framework for forecasting the impact of various electrical parameters on tissue dissociation processes. Additionally, a novel experimental setup has been designed, enabling the real-time monitoring and precise adjustment of electric fields through a control system for tissue dissociation. This capability significantly enhances the efficiency of tissue dissociation, optimizing it for more effective single-cell analysis.

Publication: S.Planchak, E.C. Welch, B. Phelps, **C. Tannock**, et al. "Fully-Automated Electrical Tissue Dissociation Device for Single-Cell Analysis," [Manuscript submitted for publication], IEEE Sensors Letters

INDUSTRY COLLABORATION

This thesis is the fruit of a collaboration effort with Revvity Life Sciences, made feasible through their financial support to the Tripathi Lab at Brown University, as well as through their invaluable scientific expertise and dialogue. This partnership has enabled me to adopt an industry-focused approach, where theoretical concepts could then be applied to the design and development of control systems for laboratory automation devices.

While pursuing this Master's in Biomedical Engineering, I have held a concurrent role at Revvity within their diagnostics division focused on their microfluidic product line. This dual engagement has provided me the opportunity to broaden my internal industry network at Revvity and deepen my comprehension of emerging technologies in the cell and gene therapy space.

MENTORING & DIVERSITY, EQUITY INCLUSION

Biomedical engineering at Brown provides an excellent platform to learn and be part of mentoring and diversity/inclusion activities. Through my work at Brown, I had opportunities to work with many undergraduate students on electrical board design concepts and scale-up for manufacturing. My contributions also came through mentoring Brown students last summer at our Revvity workplace at 3 Davol Square in downtown Providence.

ACKNOWLEDGEMENTS

The successful completion of this thesis could not have been achieved without the invaluable help and support from a multitude of incredible individuals.

First and foremost, I would like to extend my deepest gratitude to my advisor, Dr. Anubhav Tripathi, for his exceptional mentorship and for providing me with the resources essential for both my academic journey and my professional career. His innovative spirit and remarkable ability to foster collaboration have not only been instrumental to our research together but have also illuminated my path with groundbreaking insights at the intersection of life science and engineering. Dr. Tripathi's expertise, leadership, and open-minded approach to interdisciplinary research have been invaluable. I am deeply thankful for the breadth of wisdom and the spirit of innovation he has imparted to me.

I also extend my heartfelt gratitude to my colleagues, namely Duuluu Naranbat, Kathryn Whitehead, Sarah Planck, Khulan Unurbuyan, Khaliun Mayagmar, Sabrina Tolppi, and Jennifer Figueroa-Cruz. Working alongside these talented individuals has been a privilege. Their camaraderie, support, and shared inspiration have played a critical role in our joint efforts to push the boundaries of innovation in the biotechnology space. Every accomplishment we have celebrated is a testament to the power of collaboration within Triapthi's lab.

I would also like to thank Dr. Jacob Rosenstein and Dr. Edith Mathiowitz for their invaluable guidance and support as my thesis committee members. My academic journey has been greatly enriched by the knowledge gained from Dr. Rosenstein's courses in Bioelectronics and Mixed Signals, and Dr. Mathiowitz's expertise in Drug and Gene Delivery, both of which have significantly contributed to the depth and quality of my thesis work. Furthermore, I am very thankful to Dr. Marissa Gray for her exceptional leadership and support as the Director of the Biomedical Engineering Master's Program.

Lastly, but most importantly, I owe a profound thank you to my family and friends. Their belief in me and their words of encouragement as I've juggled a professional industry career with my graduate studies have been my foundation. Your love and support have kept me grounded, and for that, I am eternally thankful.

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Chapter 1

Background

1.1 The Significance of Tissue Dissociation in Cancer Research & Diagnostics

As per the Global Cancer Observatory's data in 2022, the global incidence of brain and central nervous system (CNS) cancers reached 321,731 cases, with 248,500 deaths reported worldwide. Within the United States alone, an average of over 12,000 cases of glioblastoma (GBM) are diagnosed annually. GMB, constituting 14.5% of all CNS tumors and 48.6% of malignant CNS tumors, stands out as one of the most formidable challenges in oncology. The median survival for GBM patients is a mere 15 months, with an estimated incidence ranging between 3.19 and 4.17 cases per 100,000 person-years. While GBM incidence typically rises with age, the pediatric population faces a lower estimated incidence of 0.85 cases per 100,000 individuals (Singla et al. 2021). Despite being recognized as the most aggressive primary brain tumor in adults, GBM poses significant diagnostic and therapeutic challenges.

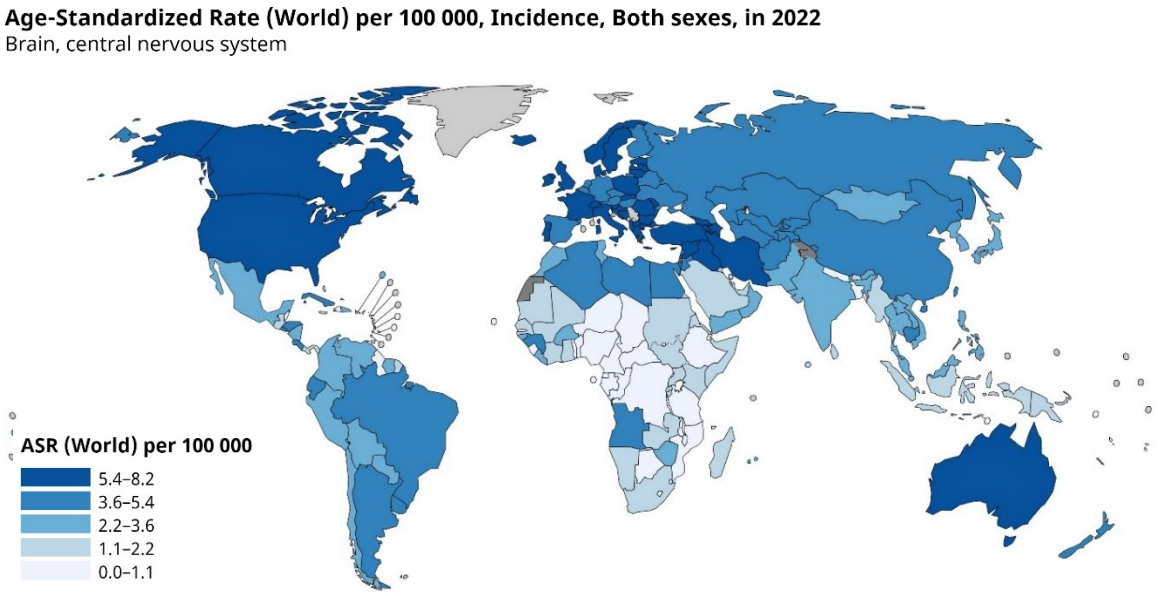

Figure 1: Global Distribution of Brain Tumor Cases

For example, recent advancements such as CAR-T cell therapy have demonstrated rapid regression of glioblastoma tumors during clinical trials at Mass General Hospital (Choi et al., 2024). While these therapies show promise in effectively managing GBM, the patient population experiencing substantial benefits remains fractional with the efficacy of these therapies varying significantly among patients. This variability suggests that different subpopulations within the tumors respond differently to the CAR-T therapies presenting a significant challenge in treatment standardization. A deeper understanding of the complex cellular heterogeneity within these tumors as subpopulations of cells can exhibit diverse characteristics responding differently to therapies is required.

To gain a deeper understanding of this heterogeneity, researchers rely on single-cell sequencing (SCS). However, conventional methods for dissociating tissue samples often comprise cell viability and introduce unwanted changes in gene expression. This is particularly critical within SCS analysis as such limitations can obscure vital information about the diverse cell populations within the tumor. Furthermore, conventional biopsy tissue dissociation methods are time-consuming and often inadequate where dissociation methods vary widely based on input material and tissue type. Thus, we explore the demonstrated efficacy of the *TissueToCellEz*, a technology developed by the Tripathi lab for advancing single-cell sequencing applications in glioblastoma research by applying electric fields across tissue-induced buffer medium.

1.2 Challenges in Current Tissue Dissociation Methods

Tissue dissociation is critical in preparing single cells for various biological and medical research applications, including single-cell sequencing, cell culture establishment, and histological analysis. The main methods for tissue dissociation include mechanical, enzymatic, and a

combination of both, each with specific challenges. Mechanical methods can be limited by the types of tissues they can effectively process, often requiring user optimization to prevent undesirable alterations to transcriptomics. Enzymatic methods, while versatile, may affect cellular functions and surface markers, complicating downstream applications. Additionally, processing tissues under 25mg using either technology can be difficult, hindering research with small sample inputs. Another significant barrier is the high cost of existing instrumentation, often restricting accessibility for many labs. In contrast, the *TissueToCellEZ* technology provides a more cost-effective alternative, designed to handle various types and sizes of sample inputs. This system offers a mechanical stress-free and enzyme-free method for tissue dissociation, addressing the limitations of cost and sample processing inherent in traditional techniques.

1.3 Mechanical Tissue Dissociation

Mechanical tissue dissociation involves the physical breakdown of tissues into single cells or smaller cell clusters. This method has been utilized in various fields, such as regenerative medicine, cell transplantation, and RNA sequencing. While mechanical dissociation is fast and cost-effective, this method struggles to maintain the integrity of sensitive cells and can introduce physical stress that affects cell function (Lee et al., 2022). It often yields lower cell viability and can be inefficient for tough or fibrous tissues due to incomplete dissociation.

While mechanical dissociation techniques can vary, they often include fine mincing and filtration through cell strainers or the use of tools like scalpels, pipettes, or specialized devices that grind the tissue. Moreover, mechanical dissociation aims to provide an enzyme-free process for releasing single cells from tissue samples, offering an alternative to enzymatic methods (Wd et al., 2020).

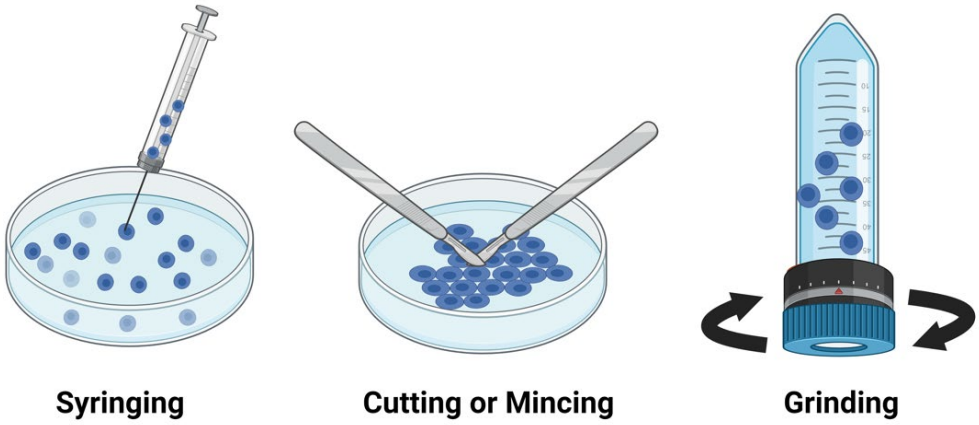

Figure 2: Mechanical Tissue Dissociation Techniques

Despite its conventional status, mechanical tissue dissociation remains crucial due to its ability to preserve surface markers and gene expression profiles, which enzymatic reactions may alter. As such, researchers continue to work innovatively to automate optimal shearing and cutting forces facilitating single-cell suspensions from solid tumors. These techniques, combined with real-time deformability cytometry and unsupervised data analysis, represent substantial advancements in the field (Soteriou et al., 2023).

1.4 Enzymatic Tissue Dissociation

In contrast, enzymatic tissue dissociation involves the use of enzymes to break down the extracellular matrix and disrupt cell-cell interactions, leading to the release of individual cells from tissue samples (Zhao et al., 2022). This technique is often employed due to its ability to efficiently isolate intact cell populations representing the composition of the original tissue. It is often considered superior to mechanical dissociation techniques as it leads to a higher yield of cells with less shear-force-associated damage affecting viability.

Enzymatic protocols involve using enzyme mixtures like collagenase, trypsin, or hyaluronidase to break down the tissue structures and cell adhesions, thus releasing individual cells for downstream analyses (Aronowitz et al., 2015). The process requires careful optimization to balance between complete dissociation and preserving cell health (Welch et al., 2021).

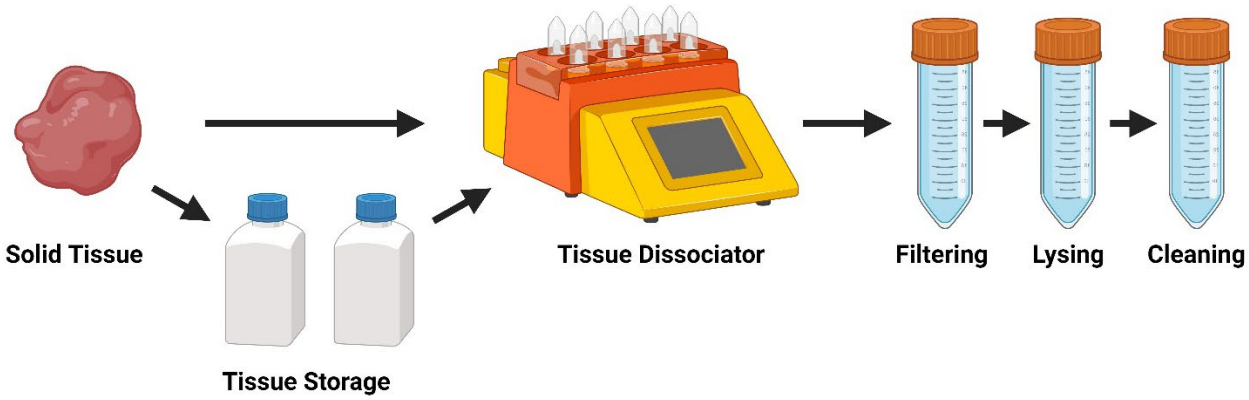

Figure 3: Enzymatic Tissue Dissociation Workflow

While enzymatic methods can be highly effective in obtaining single-cell suspensions, especially for dense or fibrous tissues, they can also alter cell surface proteins and affect cellular functions, which is a significant limitation for downstream applications that rely on surface marker detection or specific antibody recognition. This highlights a potential limitation of this technique showcasing the importance of selecting the most suitable dissociation technique based on the desired outcomes and experimental requirements.

1.5 Combinational Tissue Dissociation Methods

For efficient tissue dissociation, particularly with low amounts of input material for applications like single-cell RNA sequencing, leveraging both mechanical and enzymatic methods has started

to gain attention. This strategy addresses the limitations of each method when used in isolation—mechanical methods may not fully dissociate tissue, while enzymatic methods alone can affect cell viability and integrity. Studies have shown that combining both enzymatic and mechanical approaches can offer advantages in terms of preserving cell viability, minimizing transcriptional biases, and improving the overall quality of single-cell suspensions (O'Flanagan et al., 2019). This is due to the potential to mitigate stress-induced transcriptional artifacts with only gentle use of mechanical dissociation while enhancing the efficiency of cell dissociation processes to preserve surface markers and maintain integrity.

However, this method is not without its challenges. Limitations still arise from an inadequate amount of starting material, and these techniques can be both time-consuming and resource-intensive (Wiegleb et al., 2022). Additionally, the financial feasibility of sampling a larger number of cells to compensate for potential losses may pose challenges, particularly in research settings with limited budgets (Wu et al., 2018).

1.6 Programmable Electric Fields as a Novel Approach

The advent of single-cell analysis has driven the need for developing efficient, reliable methods to dissociate complex tissues into individual cells more consistently and reliably than traditional methods. Among these, electrical dissociation emerges as a novel, innovative technique, leveraging the intrinsic electrical properties within tissues and cells. This approach is distinguished by its minimal invasiveness and the precision with which electronics can manipulate biological samples.

Applying programming electric fields is at the core of this method, involving the careful application of varying voltage amplitudes, waveforms, and frequency of oscillation, among other parameters. It is hypothesized that this novel method will allow for fine-tuning conditions that break down tissue structures into single cells. By adjusting voltage amplitudes, the force exerted on charged particles within the cells can be controlled, enhancing the efficiency of tissue dissociation while avoiding cell damage. Frequency adjustments cater to the differential responses of cells, with lower frequencies better suited for larger particles and higher frequencies for affecting cellular and subcellular structures. The choice of waveform—be it sinusoidal, square, triangular, or sawtooth—may offer further refinement within dissociation processes, where each function offers opportunities to manipulate the electric field and how it interacts with tissue components.

The application of programmable electric fields thus represents a sophisticated technique for tissue dissociation, enabling researchers to optimize the breakdown of complex tissue into viable single cells in a controlled, repeatable environment. This technology promises to revolutionize single-cell analysis, providing a versatile tool for advancing our understanding of cellular functions and interactions within tissues. The subsequent chapters of this work delve deeper into these concepts, thoroughly examining the dynamics of modeling electric field-induced tissue dissociation and its applications in refining traditional workflows from solid tissue to scRNASeq as shown below:

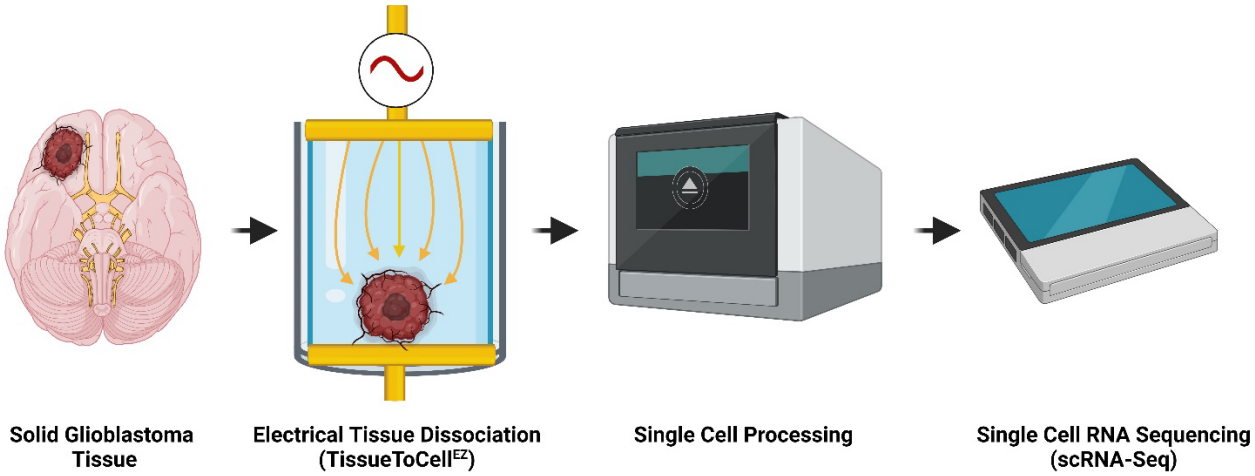

Figure 4: Programmable Electric Fields – Tissue Dissociation Workflow (not to scale)

To summarize, electric tissue dissociation has both technical and operational advantages. On the technical side, the participating cells constituting a heterogeneous tissue undergo dissociating mechanisms that may not alter cellular biology from the multi-omics perspective. From the operational perspective, the electric field-induced dissociation offers better automation capability and control based on tissue type and size.

Chapter 2

Dynamics of Electric Field-Induced Tissue Dissociation for Single-Cell Analysis

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This chapter focuses on developing a theoretical foundation for the manipulation of charged and non-charged cells within complex tissue structures. By discussing the fundamental equations and parameters of electric fields, it seeks to clarify the mechanisms of dissociation behind these innovative methods.

In the context of glioblastoma cells, which present a complex, multi-layered structure consisting of both charged and non-charged cells, leveraging both electrophoretic mobility and dielectrophoresis (DEP) principals can offer a comprehensive approach for effective dissociation. The integration of these techniques into our theoretical framework acknowledges the heterogeneity inherent in glioblastoma tissues and proposes ideas for promoting effective dissociation. The theory behind both phenomena is explored utilizing the following model as an experimental baseline:

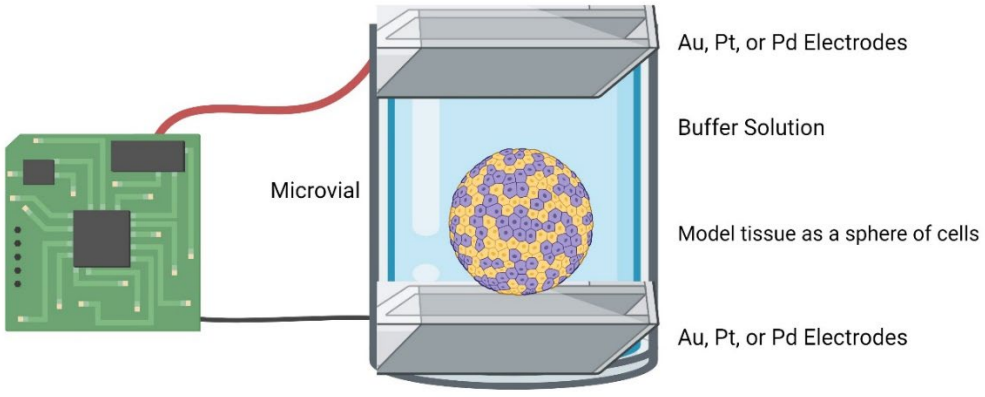


Figure 5: Model of Tissue Dissociation in Electric Fields (not to scale)

Before computational modeling, it is important to consider the tunable parameters within the experimental design and understand the tradeoffs of each parameter:

Electrodes: In bioelectronic applications, the choice of electrode material is critical where design considerations should evaluate the importance of conductivity, cost, durability, and bioinert properties. Copper is cost-effective but prone to corrosion, making it least suitable for tissue applications. Gold offers excellent biocompatibility and corrosion resistance but is expensive and soft, which may lead to deformation. Platinum provides unmatched durability and stability but at a high cost which is often deemed excessive. Lastly, palladium often strikes an optimal balance between affordability, corrosion resistance, and durability, making it a common choice in cost-sensitive yet demanding environments. For our studies, however, gold-plated electrodes were chosen for their commonplace in academic research, essential for evaluating reliable experimental outcomes. Approximate electrical conductivity values are provided below for reference:

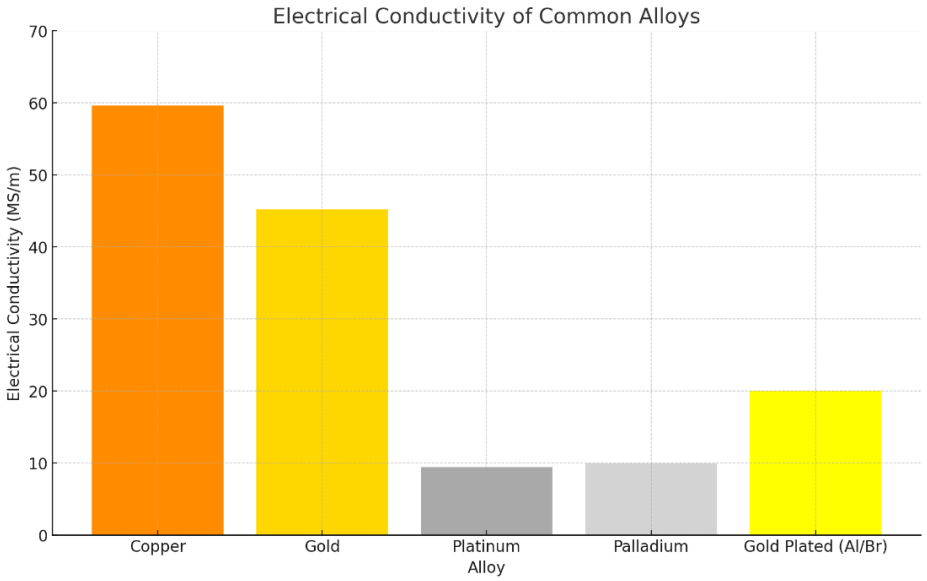


Figure 6: Conductivity of Common Alloy Types

Buffer Solution and Volume: The influence of buffer media on cellular viability is critical, particularly given the varying conductivity properties of different solutions. For instance, water with high salt/ion content has been observed to cause rapid sample loss due to bubbling or heating, attributable to its high conductivity. Conversely, a 300mM sucrose solution is used to mitigate osmotic stress on cells, aiming to balance conductivity while preserving cell integrity (Welch et al., 2022).

In pulsed or oscillating electric fields, where the current and voltage vary sinusoidally, it's essential to manage Joule heating to prevent damage to cellular structures. Joule heating can be calculated using the formula (1):

$$Q = I_{RMS}^2 * Z * t \quad (1)$$

Where:

- I_{RMS} is the current through the tissue
- Z is the electrical impedance of the tissue
- t is the total time over which the current is applied

Experimental testing is essential to determine the optimal voltage level that effectively applies electric fields without compromising cell viability due to Joule heating.

Applied Electric Field: The study of applied electric fields and their influence on tissue manipulation requires a nuanced understanding of several key variables, including the distance

between electrodes, waveform, and voltage amplitude. These elements are critical for tailoring electric field applications to achieve desired outcomes in tissue types and sizes.

Electrophoretic mobility measures how fast an ion or particle moves through a medium under the influence of an electric field, proportional to the particle's charge and inversely proportional to the medium's viscosity. It is a fundamental concept for understanding how cells can be separated from tissue in a buffer solution:

$$\mu_{ep} = \frac{v}{E} \tag{2}$$

Where:

- v is the velocity of ions or cells
- E is the average electric field strength

This property is crucial for the initial stages of dissociation, where the goal is to separate cells efficiently based on their electrophoretic differences.

Concurrently, dielectrophoresis (DEP) extends our capability to manipulate cells by inducing movement in both charged and non-charged cells through polarization effects in a non-uniform electric field. This not only broadens the applicability of electrical dissociation methods to a wider range of cell types found in glioblastoma tissues but is also presumed to enhance the effectiveness of cell separation and analysis.

By systematically exploring and applying the principles of electrophoretic mobility and DEP, we can shed light on a multi-faceted strategy for disentangling the intricate cell networks within

glioblastoma tissues. This approach holds the potential to revolutionize the field of single-cell analysis, offering insights into the optimization of electrical conditions to effectively manipulate both charged and non-charged cells in complex tissue structures.

When applying an oscillating electric field across a tissue sample submerged in a buffer, charged cellular and subcellular components move according to their electrophoretic mobilities. This movement facilitates the dissociation of cells. The electric field strength in this thesis is defined as:

$$E_0(t) = \frac{V(t)}{d} = \frac{V_0 \sin(\omega t + \phi)}{d};\ E_{effective}(t) = \frac{E_0(t)}{k} = \frac{V_0 \sin(\omega t + \phi)}{d \cdot k} \qquad (3)$$

Where:

- E_0 is the applied electric field strength
- V_0 is the peak voltage amplitude across two electrodes
- ω is the angular frequency of the sinusoidal wave
- t is time
- d is the distance between two electrodes
- k the factor representing tissue and buffer medium permeability
- $E_{effective}$ is the effective electric field within the tissue or medium

This system highlights how both the physical setup through voltage, the distance between electrodes, and the medium's specific properties influence the electric field's effectiveness in tissue manipulation or cellular dissociation processes.

Tissue Type & Size: Tissue type and size will also significantly influence the setup, necessitating adjustments in how electric fields are applied—whether pulsed or oscillated—depending on the buffer-to-tissue ratio. Tissue size directly affects the displacement of buffer volume. In the subsequent sections, we model glioblastoma as a 1mm and 2.5mm radius sphere. In this model, the buffer constitutes 97.8% of the vial's volume, with the tissue making up only 2.2% for the 1mm sphere. This highlights that the buffer's conductivity is more critical than the tissue's conductivity. Conversely, a 2.5mm GBM sphere will occupy closer to 34% of the vial volume indicating tissue conductivity may become more important.

Furthermore, different tissue types respond uniquely to frequency changes, affecting both permittivity and conductivity differently. An accompanying table details the conductivity and permittivity values for various tissues, illustrating, for example, that glioblastoma and other brain white matter tissues exhibit lower permittivity compared to other common tissue types.

Table 1: Frequency-Dependent Classification of Tissue Types (IT'IS Foundation Database)

Sample Type	Frequency (*f*)	Permittivity (ε)	Conductivity (σ) (S/m)
Brain (White Matter)	1kHz	6.98E+4	6.26E-2
	100kHz	2.11E+3	8.18E-2
	1GHz	3.86E+1	6.22E-1
Liver	1kHz	8.57E+4	4.14E-2
	250MHz	5.52E+1	5.86E-1
	1GHz	4.64E+1	8.97E-1
Kidney	1kHz	2.13E+5	1.13E-1

	250MHz	7.36E+1	9.82E-1
	1GHz	5.79E+1	1.45E+0
Lung	1kHz	1.42E+5	7.95E-2
	250MHz	2.55E+1	3.46E-1
	1GHz	2.18E+1	4.74E-1

It has been found that electrical conductivity in tissues is primarily due to the movement of ions in the fluidic compartments of the tissue. At lower frequencies, ionic mobility is restricted by cell membranes leading to lower conductivity. As frequencies increases, the capacitive effects of cell membranes increase allowing ionic movement across tissue as shown in Figure 6.

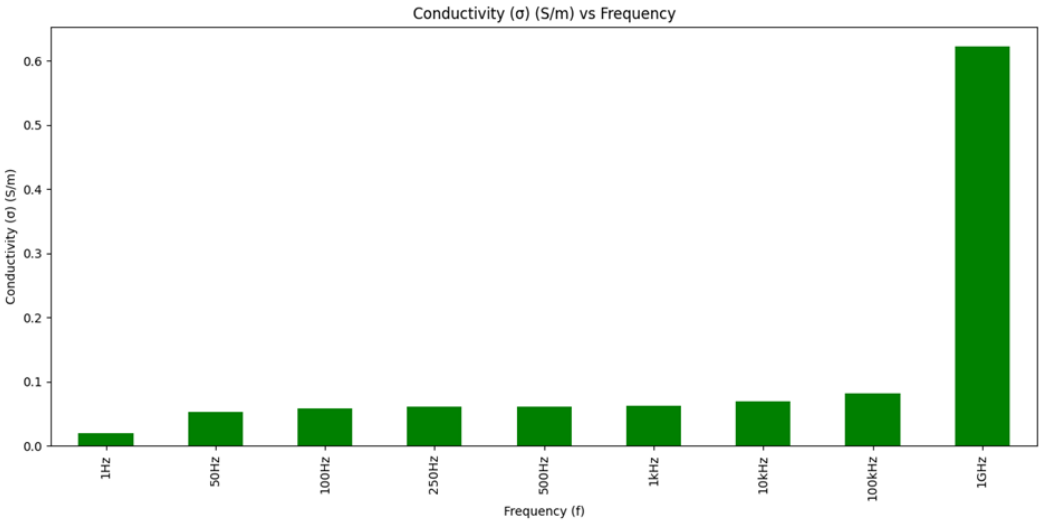


Figure 7: Conductivity vs Frequency for Brain (White Matter)

Likewise, frequency selection also affects the electrophoretic behavior of cellular components and the impedance of the medium. Lower frequencies favor the movement of larger particles, while higher frequencies can penetrate cell membranes more efficiently.

As tissue dissociation progresses, changes in tissue composition and buffer conductivity due to cell release likely alter the electrical properties of the system:

$$\frac{dE_{effective}}{dt} = f\big(V, d, k(t)\big) \tag{4}$$

This equation emphasizes the time-dependent nature of effective field strength, where $k(t)$ reflects changes in the medium's conductivity and permittivity over time due to tissue dissociation. As tissue stiffness and permeability break down as a function of the time of an applied electric field across a tissue we can hypothesize tunable adjustments of frequency can be utilized to control the conductivity and permittivity of softer tissue more prone to shearing. Figure 7 demonstrates the rapid fall off permittivity as a function of frequency.

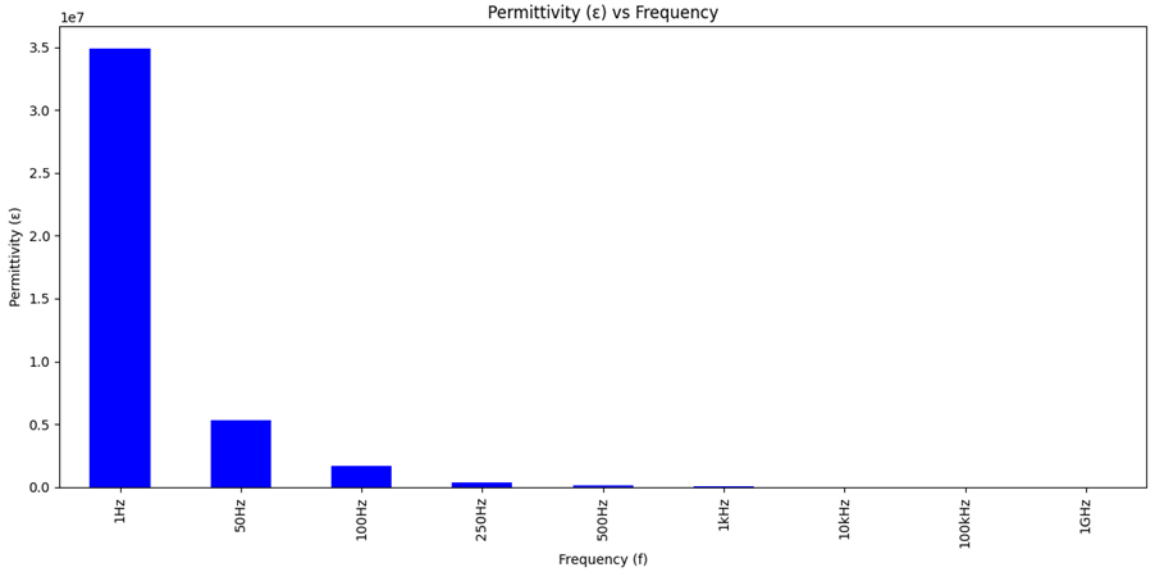


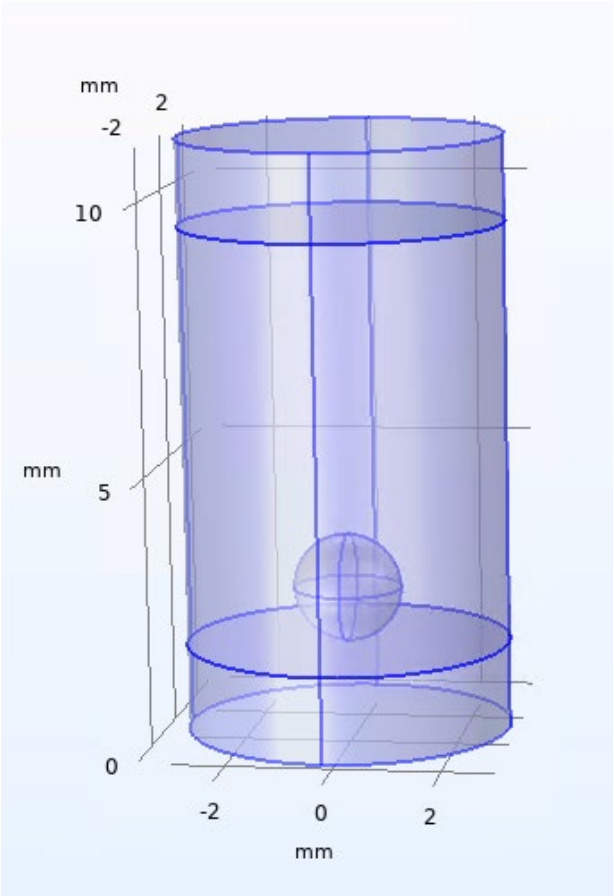
Figure 8: Permittivity vs Frequency for Brain (White Matter)

2.1 COMSOL Modeling

Understanding the interplay between electric fields and biological tissues is pivotal in the development of tissue dissociation methods aimed at single-cell analysis, particularly for applications like single-cell RNA sequencing (scRNA-seq). To this end, computational modeling serves as an essential tool for probing the intricacies of electrical stimulation in breaking down tissue matrices into single-cell suspensions. COMSOL Multiphysics, with its robust simulation capabilities, provides a platform for modeling the effects of various electrical parameters on tissues, thus enhancing our understanding, and guiding experimental designs. In the simulations, two foundational approaches are explored to analyze the behavior of electric fields and currents within biological tissues. The Electric Currents physics interface solves the current conservation equation based on Ohm's law, using the scalar electric potential as the dependent variable. Meanwhile, the Electrostatics interface applies Maxwell's equations, to model the electric field, also using scalar electric potential. These methods are instrumental in assessing how electric fields interact with biological tissues under various experimental conditions.

The focus of this section is to simulate the effects of electric fields on a 1mm radius glioblastoma tissue sphere, emphasizing the dynamics of tissue breakdown for efficient single-cell recovery. By varying voltage, permittivity, and conductivity—and observing their changes across different frequencies—we aim to optimize conditions that favor the dissociation of the tissue into viable single cells. These parameters are critical as they influence the tissue's response to electric stimuli, affecting the efficiency of cell separation necessary for downstream scRNA-seq analysis.

The simulation employs a spherical model of a cellular matrix within a 6mm diameter and 11mm long vial. Gold-plated electrodes, selected for their optimal electrical properties and biocompatibility, are seated on the top and bottom of this setup, each at a 1.6mm thickness. A 2mm gap sits between them in which the tissue biopsy and sucrose water buffer are situated mimicking the physiological environment and providing a medium for electric transmission while also supporting the electrical properties required for effective tissue dissociation. This configuration modeled below ensures a focused and uniform electric field is applied across the tissue:



Electrode(s):
Material: Gold (Au)
Electrical Conductivity (S/m): 4.1E7
Relative Permittivity: 6.9
Relative Permeability: 1
Coefficient of Thermal Expansion (1/K): 14.2E−6
Heat Capacity at Constant Pressure (J/(kg·K)): 129
Density (kg/m^3): 19300
Thermal Conductivity (W/(m·K)): 317
Young's Modulus (Pa): 78E9
Poisson's Ratio: 0.44

Tissue:
Material: Glioblastoma - Brain (White Matter)
Electrical Conductivity (S/m): Function of frequency.
Relative Permittivity: Function of frequency.
Relative Permeability: 1
Coefficient of Thermal Expansion (1/K): N/A
Heat Capacity at Constant Pressure (J/(kg·K)): 3600
Density (kg/m^3): 1041
Thermal Conductivity (W/(m·K)): 0.5
Young's Modulus (Pa): 750 (average)
Poisson's Ratio: N/A

Buffer:
Material: Sucrose Water
Electrical Conductivity (S/m): 5.5E-3
Relative Permittivity: 80
Relative Permeability: 1
Coefficient of Thermal Expansion (1/K): 2.07E-4
Heat Capacity at Constant Pressure (J/(kg·K)): 4184
Density (kg/m^3): 1000
Thermal Conductivity (W/(m·K)): 0.6
Young's Modulus (Pa): N/A
Poisson's Ratio: N/A

Figure 9: COMSOL Modeling Setup of Brain Tissue

Through these simulations, we explored how different electric field strengths, frequencies, and tissue properties affect the breakdown of cellular matrices into single cells. This approach not only helps in understanding the fundamental aspects of tissue-electric field interactions but also aids in refining the protocols for tissue dissociation tailored for scRNA-seq applications. The insights gained here are crucial for developing more effective and gentle dissociation methods that preserve cell integrity and functionality, vital for accurate downstream genetic analysis.

2.2 Impact of Electric Field Variability on Baseline Tissue

In the initial stages of our COMSOL Multiphysics simulations, direct current (DC) voltages were varied to establish a baseline understanding of how the electric field within a tissue biopsy and buffer-encapsulated system changes in response to different electrical potentials. The voltage at the top electrode was adjusted from 20V to -20V, while keeping the permittivity and conductivity of the model constant at the known values for white matter brain tissue.

Table 2: Baseline Tissue Modeling Conditions under DC Voltage

Parameter	Value
Tissue Radius	1mm
Relative Permittivity	3.49e7
Electrical Conductivity (σ)	2.01e-2 (S/m)
Electric Potential (Top Electrode)	[20V,10V,1V,-1V,-10V,-20V]

As expected, applying a positive voltage potential to the top electrode causes electric fields to travel in a downward direction towards the ground reference electrode. Negatively charged cells are expected to migrate upwards, towards the positive pole, due to electrophoretic forces.

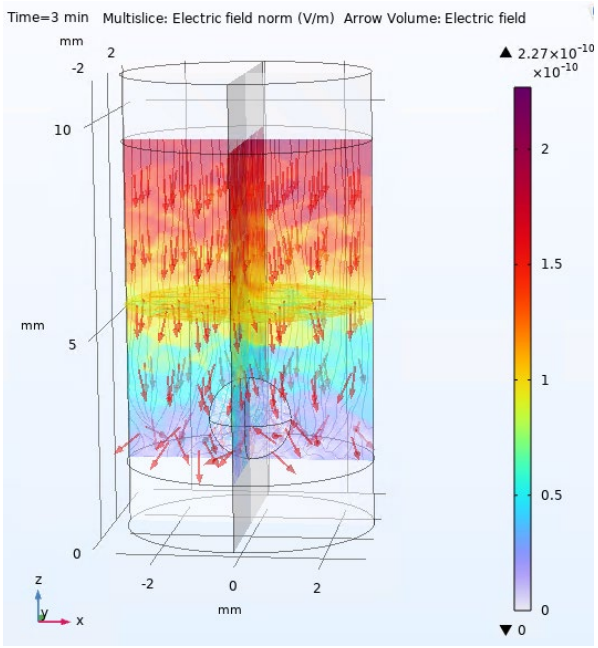


Figure 10: Electric Field (V/m) at 10V

Conversely, when a negative voltage potential was applied to the top electrode, the direction of electric fields was reversed represented by the change in vector fields. These observations are consistent with the principles of electrophoresis, where charged particles move in a fluid under the influence of a uniform electric field. It is also anticipated that increasing electric field strengths by increasing DC voltage will lead to a decrease in cellular adhesion, potentially facilitating easier dissociation of cells from the matrix. This aspect is particularly important as it will enhance the efficiency of cell separation needed for successful scRNA-seq applications, ensuring higher viability and integrity of the extracted cells.

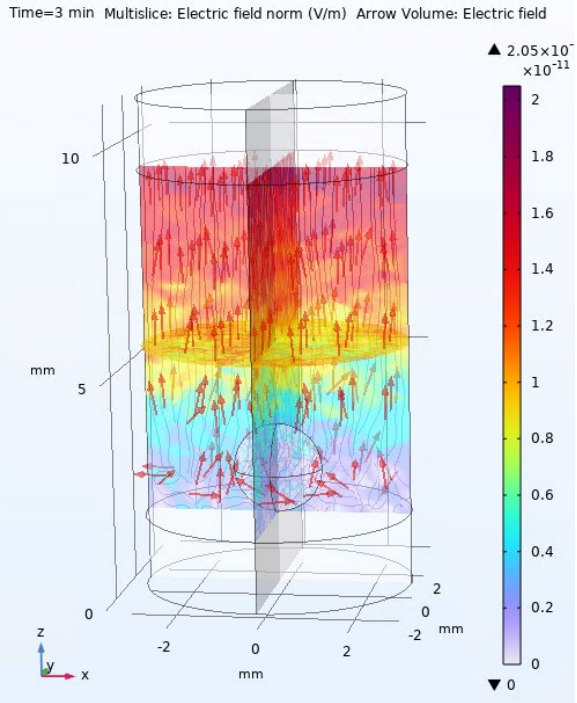


Figure 11: Electric Field (V/m) at -1V

2.3 Influence of Permittivity Variations on Electric Field Behavior

Permittivity (ε) is a fundamental property of the tissue material and quantifies the capacitance of a material to polarize in response to an external electric field. This parameter is pivotal in deciphering the interactions between electric fields and various biological tissues that exhibit diverse permittivities.

Table 3: Tissue Parameters with High Permittivity

Parameter	Value
Tissue Radius	1 mm
Relative permittivity	3.49 e7
Electrical Conductivity (σ)	2.01 e-2 (S/m)
Electric Potential (Top Electrode)	10 V

When the tissue's permittivity is set to a high value (ε = 3.49e7), the observed uniform downward movement of electric field vectors from the top electrode towards the tissue and ground reference electrode can be attributed to the high polarizability of the tissue. With higher permittivity, the tissue can store more electric charge, leading to significant polarization. This polarization effect aligns the dipoles within the tissue in such a way that they tend to reinforce the external electric field, promoting a more directed and uniform field distribution. The tissue acts as a good dielectric medium, allowing the electric field to maintain its integrity and directionality as it passes through, essentially guiding the field lines straight toward the ground electrode.

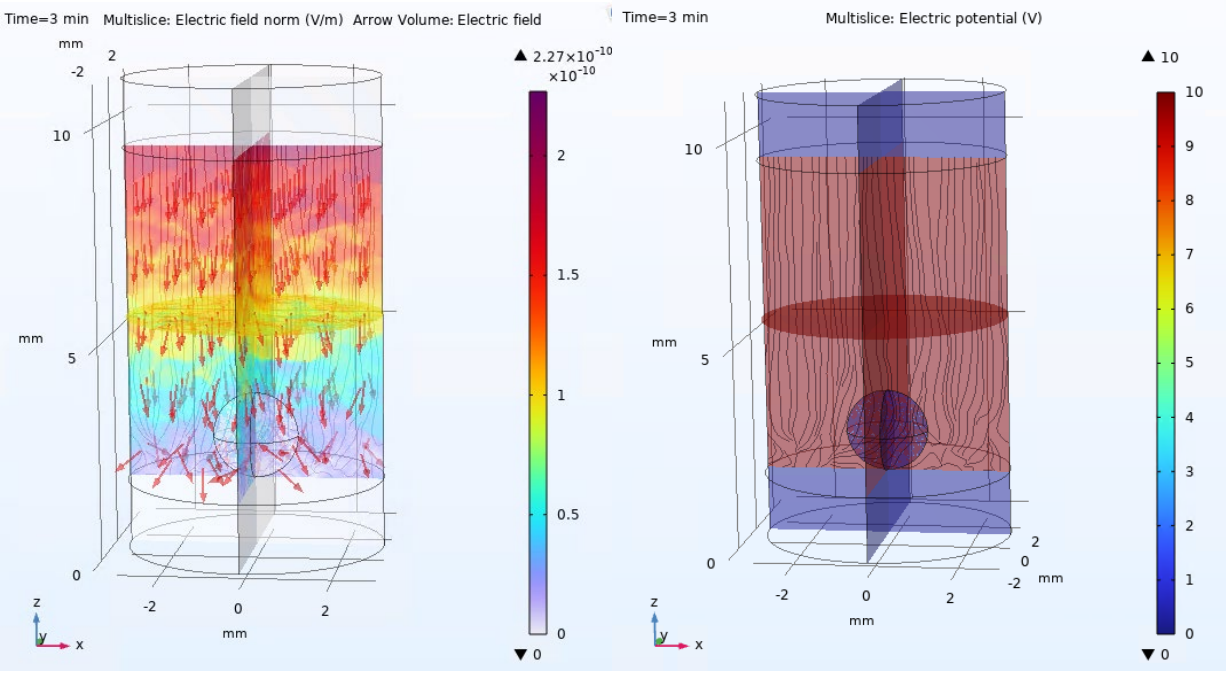


Figure 12: Electric Field Vectors for High Permittivity

Conversely, when the permittivity of the tissue is reduced by a magnitude to 3.49e6, the behavior of the electric field vectors changes dramatically. The lower permittivity indicates that the tissue's

ability to polarize in response to the electric field is diminished. This reduction in polarization leads to less internal field cancellation and a lower ability to guide and shape the electric field. As a result, the electric field experiences less uniformity and directionality. The vectors acting stochastically within the buffer signify that multiple interactions and complex behaviors are taking place, such as:

- **Increased Electric Field Dispersion**: Due to the reduced guiding effect of the tissue, the electric field spreads out more erratically.
- **Reflection and Refraction**: Changes in field direction can occur at interfaces where there are discontinuities in permittivity, leading to phenomena similar to light reflecting and refracting at material boundaries.
- **Enhanced Sensitivity to Geometric and Material Inhomogeneities**: Any slight variations in the geometry of the setup or inhomogeneities in the buffer or tissue can have more pronounced effects, leading to the observed non-uniform behavior of the field vectors.

Table 4: Tissue Parameters with Low Permittivity

Parameter	Value
Tissue Radius	1 mm
Relative permittivity	3.49 e6
Electrical Conductivity (σ)	2.01 e-2 (S/m)
Electric Potential (top electrode)	10 V

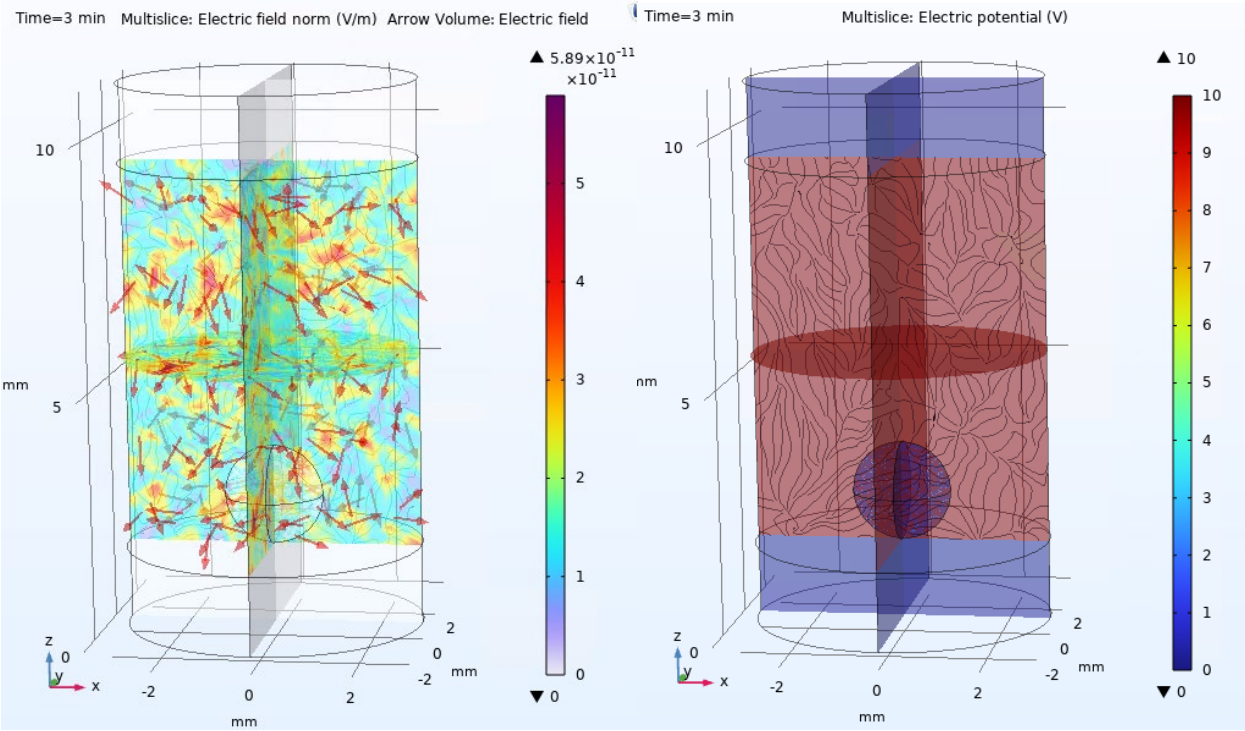


Figure 13: Electric Field Conditions under Low Permittivity

In a complete analysis of the COMSOL simulations concerning electric field interactions with biological tissues, it is hypothesized that scenarios with lower tissue permittivity will be more effective for tissue dissociation into single cells. This inference is predicated upon the observation of less uniform electric field vectors associated with lower relative permittivity values, though we must consider the possibility that these patterns could be influenced by simulation noise or computational artifacts, necessitating additional verification. The less uniform distribution of the electric field is likely to induce more dynamic and varied interactions within the tissue matrix, potentially leading to increased mechanical stresses and localized disruptions. Such conditions are conducive to breaking down cellular and extracellular connections, thereby enhancing the dissociation process. Contrarily, the higher permittivity scenario, which shows a more uniform and directed electric field, might not provide the same level of mechanical perturbation necessary for

effective tissue breakdown. Thus, the lower permittivity setting, by fostering a more chaotic electric field environment, is believed to generate multiple points of tissue disruption, thereby facilitating a more efficient dissociation into single cells.

In summary, while it is essential to acknowledge the impact of permittivity, it is also inherently frequency-dependant. The analysis of electric field behaviors in tissues with varying permittivities—specifically ε = 3.49e7 at 1 Hz for brain white matter and ε = 3.49e6 at 65 Hz—highlights this influence. The disparity in permittivity values corresponds to significant differences in the electric field distribution within the tissue, as observed in the simulations. This variability in electric field behavior supports the hypothesis that lower permittivity at higher frequencies facilitates more effective tissue dissociation a conclusion that is corroborated by findings in which tissue dissociation was effectively achieved using a 10V/cm square wave at 1kHz across parallel plate electrodes (Planchak et al., 2024).

2.4 Impact of Conductivity Variations on System Model

It may be unexpected that variations in conductivity, ranging from the $2.01e^{-1}$ S/m to $2.01e^{-3}$ S/m, do not significantly alter the behavior of the applied electric field within the system. An explanation for this observation may be considered as follows:

1.) *Electric field distribution*: The distribution of the electric field within a medium is primarily determined by the system's geometry, the configuration of the electrodes, and the applied voltage. Even if the tissue's conductivity changes, if it remains relatively minor compared to other system components, the overall electric field pattern is unlikely to show substantial changes. Factors such as buffer volume, electrode properties, and field strength

play more crucial roles in influencing the dissociation process although this could change based on increasing tissue size.

2.) *Electrode-Tissue Interface:* In the studied model, spherical glioblastoma tissue is placed directly on the conductive bottom electrode. Under these conditions, variations in tissue conductivity might not significantly affect the field strength experienced by the tissue, especially since the gold electrode's conductivity of 4.1E7 S/m dominates, effectively overshadowing the tissue's lower conductivity.

In conclusion, the conductivity of the tissue does not play a primary role in influencing the electric field in this setup. Therefore, it is not a major factor in inducing stresses or electrical phenomena, such as electroporation or dielectrophoresis, that could disrupt cell-to-cell adhesions and the extracellular matrix in this model.

2.5 Influence of Tissue Size on Electric Field Interactions

The variations in electric field distribution within tissue samples of different sizes, as observed in COMSOL simulations, illustrate a notable influence of geometric and boundary conditions on the electric field uniformity. Specifically, in the smaller 1mm radius tissue model, there is a pronounced decrease in electric field intensity near the tissue, particularly at the location closest to the bottom electrode. This is largely due to the increased relative impact of the boundaries in

smaller samples, where the proximity to the electrodes alters the field's behavior more significantly.

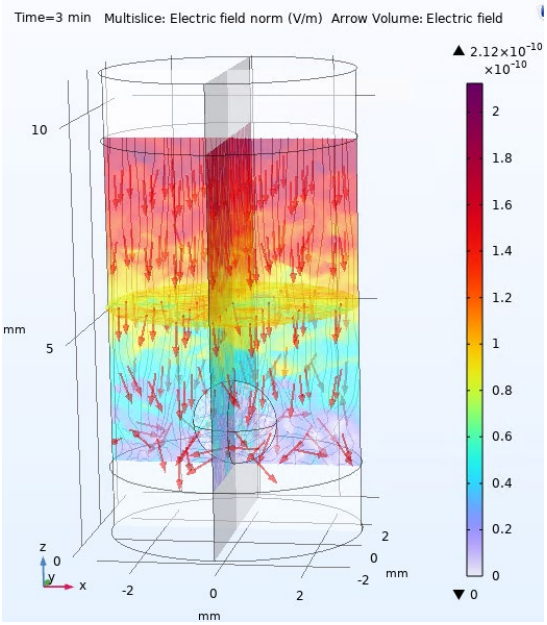


Figure 14: 1mm Radius Tissue

On the other hand, when the tissue size increases to a 2.5mm radius, the material properties of the tissue—such as conductivity and permittivity—start to play a more dominant role, accounting for approximately 34% of the system's behavior. In this larger model, the electric field interacts more intensely and evenly with the tissue properties, facilitated by both electrodes making contact with

the sample. This setup represents a non-ideal aspect where adjustments in electrode spacing are necessary to optimize electric field effectiveness across the tissue.

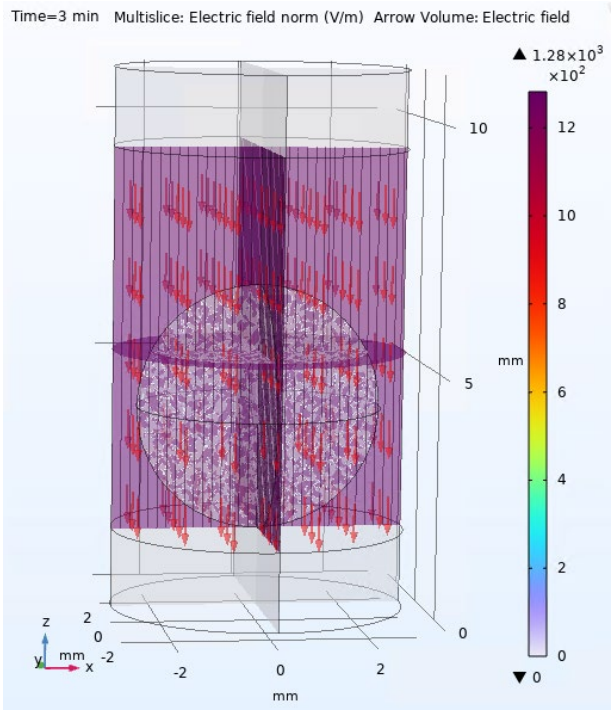


Figure 15: 2.5mm Radius Tissue

Chapter 3

Control System for Tissue Dissociation Device

3.1 Introduction:

Building on the detailed examination of electric field modeling and tissue dissociation for single-cell extraction discussed in previous chapters, this chapter aims to explore the design and development of a comprehensive control system. Implemented via the schematic and layout design of a printed circuit board (PCB), the system is engineered to apply electric fields to tissue biopsies within a buffer solution, moving it closer to practical, real-world applications. The mixed-signal electronic design approach was carefully chosen to allow for system flexibility, offering key advantages such as programmable control of complex waveforms enabling rapid testing and refinement of parameters such as amplitude, frequency, waveform, and configurable DC offset for iterative experimentation to determine optimal tissue dissociation efficiency.

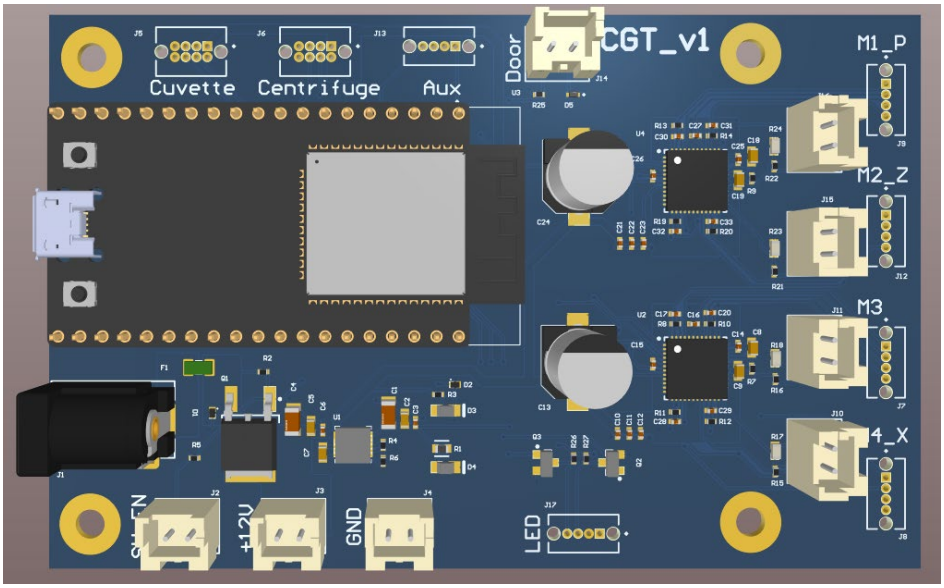


Figure 16: PCB Layout for Tissue Dissociation Device

This approach is further substantiated by the research findings detailed in a recent paper under review: Planchak et al. (2024) outlining the development of a fully automated electric tissue dissociation device.

While the focus remains on the design of oscillating electric fields for tissue dissociation, the PCB also incorporates power regulators, stepper motor drivers, and various other on-board sensors for full system control. These design considerations are incorporated not only to advance this work toward a commercially viable solution but also to enable repeatable results when automating the downstream processes such as purification and centrifugation before the resuspension of single cells for extraction. A schematic depicting the different components of the device is shown below:

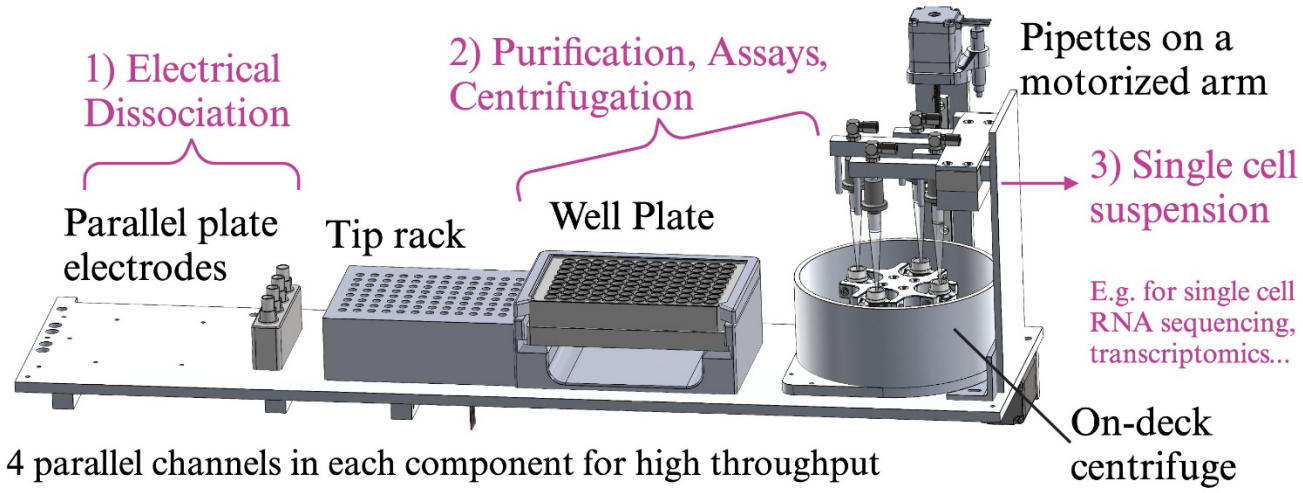


Figure 17: Automated Tissue Dissociation Device

The core components for device connectivity and control of the platform are highlighted in the table below:

3.2 Core Electrical Components:

Microcontroller	At the heart of the system is a small-footprint ESP32 microcontroller chosen for its robust processing capabilities and versatile I/O options. It controls the system functionality include waveform generation, motor driver control, and system monitoring.
Power Supply Regulation	The system incorporates a series of LDO power regulators that deliver multiple voltage outputs – plus 12 volts, minus 12 volts, plus 5 volts, and 3.3 volts. These power levels are crucial to drive motors and oscillate electric fields within the desired ranges for the tissue dissociation process.
Motor Control	Two Trinamic TMC5072 motor controllers are integrated to drive up to four stepper motors. Each motor is also equipped with limit switches for precise homing. Motors are utilized through the system for liquid transfers, pipetting, and on-deck centrifugation.
Safety and Monitoring	Dedicated door detection sensors and LED drivers are included within the design to ensure safety during normal operation, avoid pinch hazards, and provide visual status indicators for enhancing the user interaction with the platform.
Connectivity and Control	Additional hooks are in place to power and control additional subsystems that may be required during the biological tissue dissociation process. Isothermal and/or thermal cycling along with other sample processing needs may facilitate improved dissociation. Additionally, Bluetooth and Wi-Fi connectivity are also onboard the design for data processing and post-run analysis.
Waveform Generator	At the system's core is a waveform generator comprising a digital-to-analog converter and a 4-stage amplifier design allowing for configurability of oscillating electric fields along with current sensing capabilities to better quantify the dissociation process.

3.3 Oscillating Electric Fields:

To create a configurable waveform generator for testing and experimenting with different electrical field oscillations, frequencies, and amplitudes, a digital-to-analog converter with four subsequent op-amp stages is implemented for programmable signal conditioning:

Table 5: Waveform Characterstics

Waveforms	Sinusoidal, Square, Triangular, Sawtooth
Frequencies	1Hz – 20kHz
Amplitude	10V, 20V (peak-to-peak)

In this setup, a dual output 12-bit digital-to-analog converter (MCP4922) is connected to the ESP32 microcontroller which facilitates the generating waveforms within a 0-5V range. The first output, Channel A of the integrated circuit is dedicated to waveform generation, while the second output, Channel B manages the DC offset, facilitating simple adjustment of the baseline voltage of the waveform and offset any noise introduced into the system.

To enhance stability and minimize output noise, a high-precision voltage reference (REF6025) is connected to both reference voltage pins on the MCP4922. This connection is critical for maintaining the integrity of the waveform throughout the tissue dissociation process.

For optimal signal conditioning the output from the MCP4922 passes through a rail-to-rail operational amplifier (OPA196). This amplifier is configured as a second-order low-pass Butterworth filter with a cutoff frequency of 20 kHz. The filter's role is to smooth out the step-like transitions produced by the analog converter and to remove high-frequency noise preventing undesired tissue responses.

Given the MCP4922's maximum output limitation of 5V peak-to-peak, and the requirement for a waveform amplitude extending from -10V to 10V, the system incorporates a differential amplifier setup with an OPA2134 op-amp. This setup features a gain stage set to Av=2.49, designed to amplify the signal to a 20V peak-to-peak. Additionally, the differential amplifier setup acts as a subtractor circuit that utilizes the secondary Channel B output from the MCP4922 to adjust the DC offset, thereby ensuring control over the waveform's baseline voltage.

Electrode Interface and Tissue Exposure

The amplified signal is then applied to a gold-plated electrode, chosen for its excellent electrical conductivity and bioinert stability. The electrode interfaces with the buffer solution containing the biopsy tissue, where the oscillated electric fields facilitate the dissociation process into single cells.

Bioimpedance Spectroscopy

Bioimpedance spectroscopy is a valuable technique used to measure the impedance of biological tissues over a range of frequencies, providing valuable data on cellular and tissue response when exposed to electric fields. This method is particularly effective for assessing tissue composition and structural changes.

To monitor and measure the interaction of electrical signals with biological tissue samples, an INA821 precision amplifier is configured with a 1kΩ shunt resistor, allowing for detailed measurement of electrical absorption and impedance of the tissue. This configuration essentially provides sensing feedback to the control system, vital for monitoring how the electric field affects the buffer and tissue matrix. Such analysis might be the key to determining the optimal electrical

conditions required to permeabilize tissues effectively without causing irreversible alterations (Trainito, et al., 2015). The signal is then passed through a low-impedance buffer, which ensures the voltage signal pertinent to the tissue dissociation process is accurately relayed to the ADC pin on the ESP32. This step prevents potential damage or data corruption from impedance mismatches, safeguarding the system. A high-level overview of the circuit is provided below for reference:

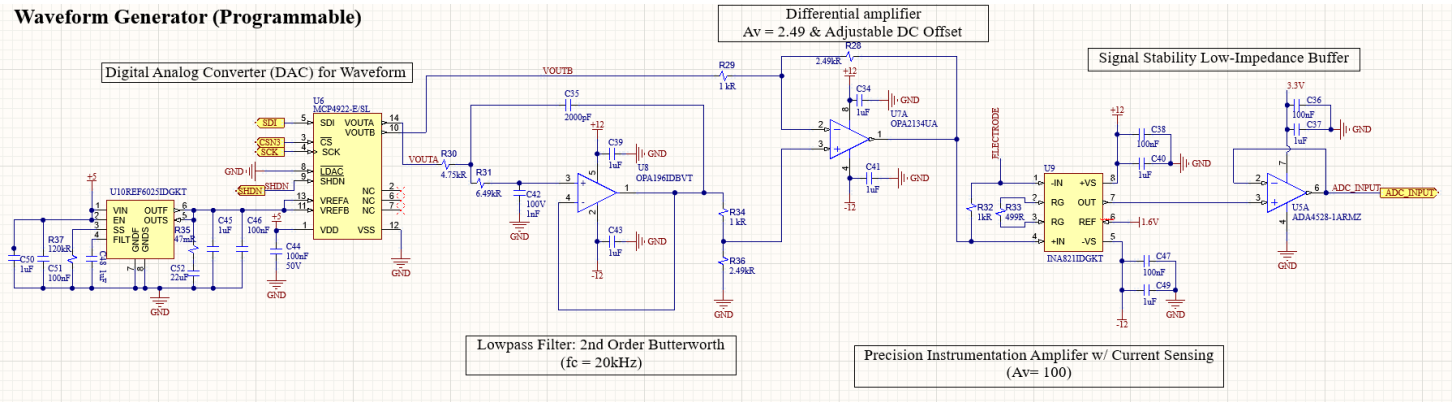


Figure 18: Schematic Layout for Programmable Waveform Generator

Finally, consideration was given to a purely analog design for waveform generation using resistor-capacitor (RC) and inductor-capacitor (LC) oscillators as an alternative to a mixed-signal electronics solution. This approach offers potential advantages such as reduced complexity, improved signal fidelity, and enhanced real-time responsiveness. The continuous nature of analog signals facilitates smooth waveform generation, eliminating quantization errors and sampling artifacts. This can be particularly beneficial in applications that demand high signal integrity and precision.

As experimental understanding of tissue dissociation progresses, refining the design with more specific parameters will be important. However, given the current need to optimize parameters and

adjust functionality dynamically, a digitally programmable design was chosen for its superior flexibility.

Chapter 4

Conclusions and Future Directions

This study enhances the comprehension of tissue dissociation using electric fields, establishing a solid foundation for future exploration of novel tissue dissociation methods. It outlines key experimental parameters and their impacts, offering a foundational insight into how tissues respond to electric fields. The forthcoming phase will focus on implementing the control systems designed in this thesis to refine test criteria aiming for consistently high dissociation rates to achieve 90% ± 5% cell viability for 1mm tissue samples consistently. Future endeavors will also require optimizing simulation parameters in COMSOL, such as tissue size, buffer volume, electrode material, and electrode spacing to optimize cell viability outcomes and improve method efficacy. Additionally, further comprehension of the dynamic changes in permittivity and conductivity in response to variations in frequency, field strength, and exposure time is necessary before expanding the studies to additional sample types such as liver, kidney, and lung.

Future Applications:

The methodologies developed for modeling electric fields in tissue dissociation have broad applicability across the biomedical sciences. For instance, the use of oscillating high-voltage electric fields in capillary electrophoresis could herald transformative advances in biomolecular separation techniques. This area of research, particularly promising for its potential to streamline diagnostic and research workflows, is being actively explored through collaborations with industry leaders like Revvity. Additionally, the application of both DC and AC electric fields extends beyond tissue dissociation into other sample preparation techniques. For example, the application

of electric fields to dried blood spots for DNA extraction represents a novel approach that significantly enhances the recovery of genetic material, demonstrating its utility in genetic analysis and forensic applications (Machado et al., 2019). Moreover, the electrical strategies discussed herein hold tremendous potential for revolutionizing drug delivery and gene therapy, where precise control over molecular transport is essential. By continuing to innovate and apply these electrical field techniques, we can significantly enhance therapeutic and diagnostic capabilities within the biomedical sector.

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