

Design and Development of an Electronic Cell Stimulation Chamber for Cell Culture.

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by

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Certificate

This is to certify that the work presented in the report entitled "***Design and Development of an Electronic Cell Stimulation Chamber for Cell Culture.***" by **Bhaskar Taye (224159002)** represents original work under the guidance of **Prof. Biman B. Mandal** and **Prof. Raghvendra Gupta**. This study has not been submitted elsewhere for a degree.

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Declaration

I do hereby declare that the matter embodied in the report entitled "***Design and Development of an Electronic Cell Stimulation Chamber for Cell Culture.***" is the result of an investigation carried out by me in the Jyoti & Bhupat Mehta School of Health Science & Technology, Indian Institute of Technology, Guwahati, India, under the guidance of **Prof. Biman B. Mandal** and **Dr. Raghvendra Gupta**. In keeping with the general practice of reporting scientific observations, due acknowledgment has been made wherever the work described is based on the findings of other investigators.

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Abstract

This Project focuses on the design, development, and optimization of an innovative electronic cell culture device aimed at revolutionising bioprocessing efficiency. Traditional cell culture techniques often face challenges related to manual intervention, inconsistent conditions, and limited scalability. The proposed electronic cell culture device integrates advanced sensors, actuators, and automated control systems to create a state-of-the-art platform for cell cultivation.

The primary objectives of this project include the design and fabrication of the electronic cell culture device, integration of real-time monitoring sensors for key parameters such as pH, temperature, and oxygen levels, and the implementation of automated control algorithms to maintain optimal culture conditions. The system aims to provide a user-friendly interface for researchers to easily program and monitor the cell culture process.

Furthermore, the project involves the optimization of the electronic cell culture device for various cell types and culture requirements. The efficiency and reliability of the system will be evaluated through extensive experimentation, comparing its performance against traditional cell culture methods. Additionally, the scalability of the device will be assessed to determine its suitability for large-scale bioprocessing applications.

The successful completion of this project will contribute to the advancement of cell culture technology, offering a cutting-edge solution for researchers and bioprocessing industries. The electronic cell culture device has the potential to enhance reproducibility, reduce manual labour, and improve overall efficiency in the production of biological products, thereby opening new avenues in the field of biotechnology.

1. Introduction

Bioprocessing and cell culture techniques play pivotal roles in the field of biotechnology, serving as fundamental tools for the production of therapeutic proteins, vaccines, and other biopharmaceuticals. However, conventional cell culture methods often face challenges related to manual handling, inconsistent environmental conditions, and limited scalability. To address these limitations, there is a growing demand for innovative technologies that can provide precise control, automation, and scalability in cell culture processes.

This project introduces the design, development, and optimization of an electronic cell culture device poised to revolutionise bioprocessing efficiency. The proposed system leverages cutting-edge technologies such as advanced sensors, actuators, and automated control algorithms to create a sophisticated platform for cell cultivation. By integrating real-time monitoring capabilities for key parameters such as pH, temperature, and oxygen levels, this electronic cell culture device aims to offer a robust and reliable solution for maintaining optimal culture conditions.

The motivation behind this project lies in the need for more efficient and reproducible cell culture methods, especially in the context of biopharmaceutical production. The limitations of traditional cell culture techniques underscore the importance of exploring innovative approaches to address the challenges associated with manual interventions and scalability issues. The electronic cell culture device envisioned in this project seeks to bridge these gaps, offering a user-friendly interface and the potential for seamless integration into various bioprocessing workflows.

As we embark on this research journey, the project aims not only to design and develop the electronic cell culture device but also to optimise its performance for different cell types and culture requirements. Through a comprehensive evaluation of the system's efficiency and scalability, we anticipate that the outcomes of this project will contribute significantly to advancing the state-of-the-art in cell culture technology and facilitating the production of high-quality biopharmaceuticals.

2. Literature Review

Various electronic stimulation chambers have been developed for cell culture applications. These chambers provide a controlled environment for the delivery of electrical stimulation to cells, which can be used to promote cell growth, differentiation, and function.

Experimental study has demonstrated that electrical stimulation can facilitate the repair and regeneration of skin, bone, muscle, and nerve tissues. More knowledge of the fundamental mechanisms underlying electrical stimulation-based clinical treatments and enhanced tissue-engineered products through electro-bioreactor technologies has recently come from studies using electrical stimulation to influence cell behaviour related to proliferation, differentiation, and migration. Here, we describe a new tool for providing in vitro grown cells with direct current (DC) electrical stimulation (ES). Six tissue culture wells can each have a DC electrical current applied simultaneously in our streamlined electro-bioreactor. By overcoming earlier experimental duplicate constraints, the approach shortens the duration and expense of the experiment. [1]

When examining fundamental scientific and translational research concerns, cell culture is an extremely flexible instrument. The homogeneity and consequent reliability of the data produced by cell lines make them an advantageous tool for scientific investigation. The concepts guiding the establishment of a cell culture lab are presented in this paper, together with the protocols that guarantee the security of the cultivated cells and lab staff. Potential microbiological pollutants are also covered, along with early detection and prevention techniques. This chapter will provide an overview of common components and qualities of mammalian cell culture that help create an appropriate milieu for cell culture, since the choice of a specific cell line and circumstances for cell culture depends on the readout of the intended experiment. [2]

Recently, there have been proposals to monitor cell cultures and use cell bioreactors for various biological applications using three-dimensional printing technology. 3D printing technology can be used in tissue engineering to control tissue formation, which is essential to build tissue constructions with therapeutic value. Our research focuses on the technique of electrical impedance spectroscopy and examines 3D-printed sensors that have been utilised in biological laboratories for tissue engineering and cell culture applications. We also examine novel 3D-printed actuators that are employed in the highly significant field of tissue development and regenerative medicine to stimulate stem cell cultures.[3]

One innovative therapeutic strategy for the treatment of degenerative eye problems is electric stimulation (ES) of the cornea. The current method for delivering ES involves applying a mono-element electrode to the cornea's surface, which stimulates the eye evenly along the electrode site. There are reports that the position of the electrode and the location of the activated retinal region are somewhat correlated. Thus, we present here the creation of a sectioned surface electrode for human cornea specific electric stimulation. The suggested gadget has eighteen contact pads, a reference electrode, and sixteen separate microelectrodes.[4]

The development of skin-patchable and implantable energy storage materials for biometric information real-time monitoring, medical diagnosis and prognosis, and therapeutic applications is being driven by the ever-increasing demands on energy storage devices, which are a result of the rapid development of biomedical and information technologies. However, due to their special contact with human skin and tissue as well as biological dynamic settings, designing materials for energy storage is extremely difficult. The development of flexible skin patches and implantable energy storage devices is reviewed in this work, along with important concerns regarding electrode materials in terms of biological characteristics and energy storage performance. Comprehensive attention is given to the electrochemical and biological characteristics of the electrode materials, which include metals, carbon nanomaterials, metal oxides, biopolymers, and composites.[5]

In this work, a regulated DC variable power supply with a rating of (0–15)V and 5A is designed and implemented utilising solar PV and storage. The absence of the system's output being digitally presented was the gap identified by the literature review. Using a voltage regulator (LM33S), resistors, LEDs, capacitors, a 200 W solar PV, a 12 V/24 V20 A charge controller, and two 12 V, 8 Ah batteries, the study built and executed a DC variable power supply. The capacity of the voltage regulator, solar PV, charge controller, and battery ratings were designed during the design phase. A digital multimeter was used to conduct tests in order to ascertain the system's response. The system can power DC loads up to 75 W, according to the results obtained. Additionally, it was determined that the output voltage range of the constructed system is 1.21 V to 15.2 V. It was clear that DC power requirements within the design range could be satisfied for both basic experiments and further purposes.[6]

3. Research Gap

While significant strides have been made in the field of bioprocessing and cell culture, there exists a noticeable research gap concerning the integration of advanced electronic systems to enhance the efficiency and scalability of cell culture processes. Traditional methods often rely on manual interventions, leading to variations in culture conditions, and face challenges in maintaining consistent parameters crucial for optimal cell growth and product yield. The existing literature highlights the need for innovative solutions that can address these limitations and propel cell culture technology into a new era of automation and precision.

Current research in electronic cell culture devices has primarily focused on individual aspects such as sensor integration or automated control systems. However, there is a notable lack of comprehensive studies that holistically address the design, development, and optimization of an integrated electronic cell culture platform. Furthermore, existing systems may not be universally applicable, and their scalability to meet the demands of large-scale bioprocessing remains a subject of exploration.

Moreover, the research gap extends to the evaluation of electronic cell culture devices across a diverse range of cell types and culture requirements. Many studies have been limited to specific cell lines or conditions, hindering the generalizability of findings and their applicability to a broader spectrum of bioprocessing applications.

This research project aims to bridge these gaps by developing a unified electronic cell culture device that integrates advanced sensing, actuation, and control technologies. The optimization of this device for various cell types and the evaluation of its scalability will contribute valuable insights to the existing body of knowledge, providing a more holistic understanding of the potential of electronic systems in revolutionising cell culture processes. Through addressing these research gaps, this project aspires to pave the way for the adoption of electronic cell culture platforms in bioprocessing, ushering in a new era of efficiency and reproducibility in the production of biopharmaceuticals.

4. Proposed Objective

The primary objective of this research project is to design, develop, and implement an innovative Electronic Cell Stimulation Chamber (ECS Chamber) to enhance the precision and efficiency of in vitro cell culture studies. The focus will be on creating a versatile platform that integrates microcontroller-based automation, enabling precise control over the electronic stimulation parameters. The key goals of the project include:

- **System Design:** Develop a comprehensive design for the ECS Chamber, outlining the architecture, components, and subsystems required for automated electronic cell stimulation.
- **Microcontroller Integration:** Implement a robust microcontroller-based control system to regulate and modulate electronic stimulation parameters such as frequency, amplitude, and duration, providing a customizable and user-friendly interface.
- **Biocompatibility and Cell Viability:** Ensure the biocompatibility of the chamber materials and the stimulation methodology to maintain cell viability and physiological relevance during experiments.
- **Real-time Monitoring:** Incorporate real-time monitoring capabilities to observe cellular responses during stimulation, enabling researchers to analyse and adjust experimental conditions on-the-fly.
- **Customization and Flexibility:** Design the ECS Chamber with flexibility in mind, allowing researchers to easily customise stimulation profiles to mimic specific physiological conditions for diverse cell types and research objectives.
- **Data Logging and Analysis:** Implement a data logging system to record stimulation parameters and cellular responses over time. Develop analytical tools to process and interpret the collected data, aiding researchers in drawing meaningful conclusions from their experiments.
- **Validation and Optimization:** Validate the performance of the ECS Chamber through rigorous testing with various cell lines and culture conditions. Optimise the system based on experimental results to enhance its effectiveness and reliability.

By achieving these objectives, the project aims to contribute to the advancement of cell culture techniques, providing researchers with a sophisticated and automated tool for

conducting in-depth studies on cellular behaviour under controlled electronic stimulation conditions.

5. Methodology

- **Variable (Power supply)**

Components Required

Arduino Uno
LM317 Adjustable Voltage Regulator
N-Channel MOSFET
LCD Display (16x2)
Power Transistor with Heat Sink
Voltage Divider Resistors
Voltage regulator knob for Interface
Breadboard and jumper wires

Circuit Description:

Arduino Control: The Arduino generates a PWM signal to control the MOSFET, adjusting the output voltage. Analog inputs of the Arduino read the voltage across R1 for current sensing.

Voltage Regulation: LM317 is used as a variable voltage regulator. The voltage across R2 is adjusted through the potentiometer to set the desired output voltage.

Current Sensing: A current sense resistor (R1) is placed in series with the load. The voltage drop across R1 is measured by the Arduino to determine the output current.

LCD Display: The LCD displays the real-time values of voltage and current.

Control Interface: The voltage regulator allows the user to set and adjust the desired voltage.

Protection Diode: D1 provides protection against back EMF when the load is switched off.

Power Transistor: A power transistor is used to handle higher currents, and a heat sink is added for heat dissipation.

Arduino Uno code for the variable voltage supply.

```
#include <LiquidCrystal_I2C.h>

LiquidCrystal_I2C lcd(0x27, 16, 2);

const int pwmPin = 9;      // PWM pin connected to MOSFET gate
const int sensePin = A0;   // Analog input for current sensing
const int btnUp = 2;       // Button for increasing voltage
const int btnDown = 3;     // Button for decreasing voltage

float setVoltage = 0.0;    // Variable to store set voltage
float outputVoltage = 0.0; // Variable to store actual output voltage
float currentSense = 0.0; // Variable to store current sensing voltage
float current = 0.0;      // Variable to store calculated current

void setup() {
    pinMode(pwmPin, OUTPUT);
    pinMode(btnUp, INPUT_PULLUP);
    pinMode(btnDown, INPUT_PULLUP);

    lcd.begin(16, 2);
    lcd.print("Voltage: ");
}

void loop() {
    readButtons(); // Read user input
    setVoltage = map(analogRead(A1), 0, 1023, 0, 2400) / 100.0; // Set voltage based on
    analog input

    analogWrite(pwmPin, setVoltage / 24.0 * 255); // Adjust PWM duty cycle for voltage
    regulation
    delay(100);
}
```

```

outputVoltage = analogRead(A1) * 24.0 / 1023.0; // Read actual output voltage
currentSense = analogRead(sensePin) * 5.0 / 1023.0; // Read current sensing voltage
current = currentSense / 1.0; // Current calculation (adjust the value based on the
// current sense resistor)

displayValues(); // Update LCD with voltage and current values
}

void readButtons() {
    if (digitalRead(btnUp) == LOW && setVoltage < 24.0) {
        setVoltage += 0.1;
        delay(200);
    }

    if (digitalRead(btnDown) == LOW && setVoltage > 0.0) {
        setVoltage -= 0.1;
        delay(200);
    }
}

void displayValues() {
    led.setCursor(9, 0);
    led.print(" "); // Clear the previous voltage value
    led.setCursor(9, 0);
    led.print(outputVoltage, 2); // Display output voltage

    led.setCursor(0, 1);
    led.print("Curr: ");
    led.print(current, 2); // Display current
    led.print("A ");
}

```

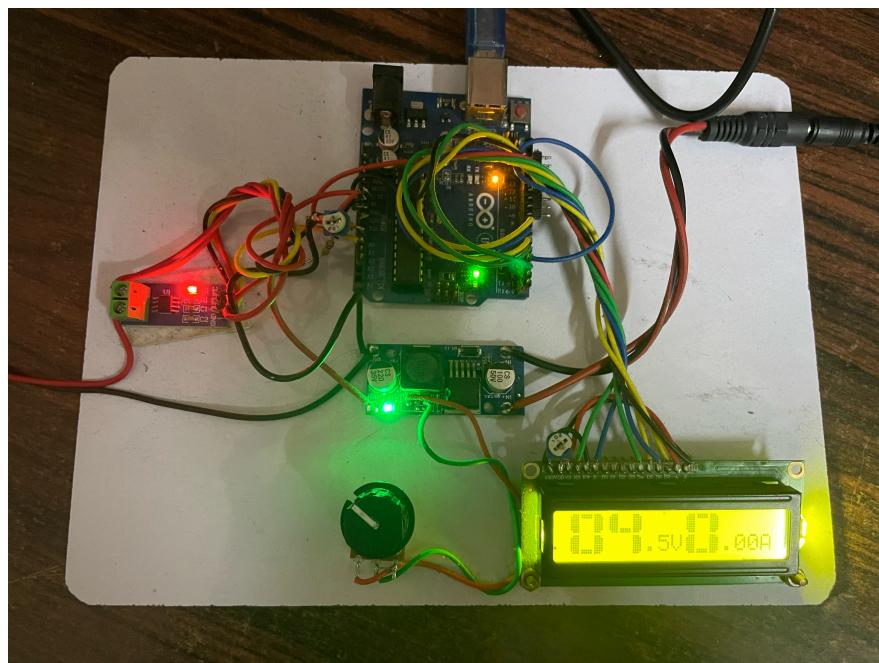


Fig. 1 - The variable power supply module.

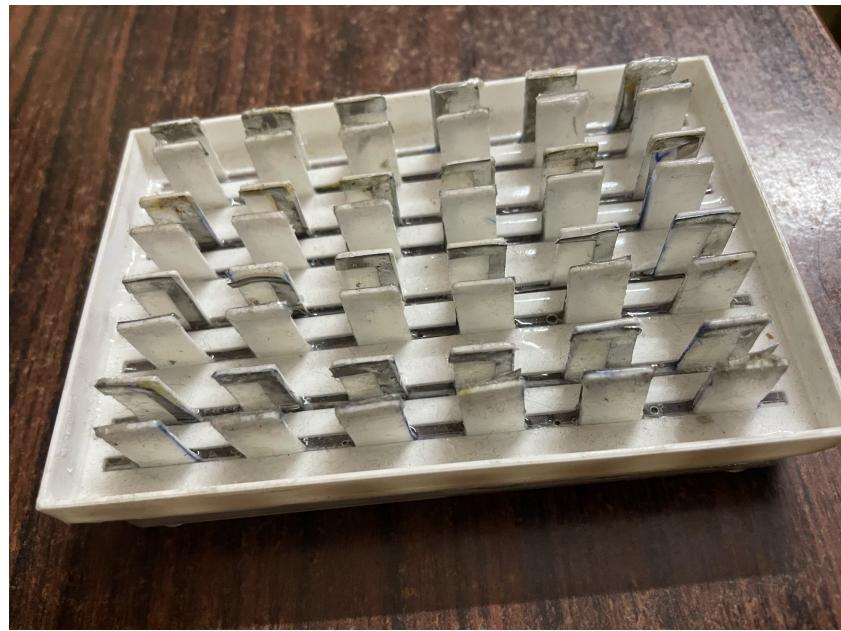


Fig. 2 - Cell culture plates embedded with stainless steel electrodes.



Fig. 3 - Normal cell culture plates

6. Result.

In our recent experiments, we successfully constructed a Variable Power Supply and an Electronic Cell Stimulation chamber to investigate the differential effects of electronic stimulation on cell culture compared to traditional, non-stimulated conditions. The Variable Power Supply facilitated precise control over voltage levels, allowing us to modulate the electrical stimulation administered to the cells within the ECS chamber. Concurrently, we maintained a control group of cells cultured conventionally, providing a baseline for comparison.

The next phase of our research will involve a deeper analysis of the underlying molecular mechanisms, functional outcomes, and the potential translational implications of these observed differences. Through this work, we aim to contribute valuable insights to the evolving field of cell culture technology and electronic cell stimulation, with implications for diverse applications in biotechnology and medical research.

7. Conclusion

In conclusion, the successful construction of the Variable Power Supply and Electronic Cell Stimulation chamber has set the stage for a compelling exploration into the impact of

electronic stimulation on cell culture. As we move forward, the planned data analysis promises to unveil key insights into the differences between ECS and normal cell culture conditions. The initial observations, encompassing variations in cellular morphology and proliferation rates, hint at the potential influence of electronic stimulation on fundamental cellular behaviours.

The comparative study, pitting ECS-exposed cells against their conventionally cultured counterparts, holds the promise of revealing nuanced responses at the molecular and functional levels. The meticulous data analysis, incorporating gene expression studies, protein profiling, and statistical validation, is poised to provide a comprehensive understanding of the cellular dynamics influenced by electronic stimulation.

8. Future Work

The Electronic Cell Stimulation Chamber for cell culture represents a cutting-edge technology with a wide range of applications and potential for future advancements. As researchers continue to explore the intricate mechanisms of cellular behaviour and the impact of electrical stimulation on various cell types, several avenues of future work emerge. Here, we discuss potential directions for future research and development related to the Electronic Cell Stimulation Chamber.

1. Closed-Loop Control Systems:

Implementing closed-loop control systems within the Electronic Cell Stimulation Chamber could enable real-time adjustments based on cellular responses. Integrating sensors that monitor cell behaviour and dynamically adapting stimulation parameters could lead to more accurate and adaptive experiments, allowing for a deeper understanding of cellular dynamics.

2. Integration with Microfluidics:

As technology progresses, integrating the Electronic Cell Stimulation Chamber with microfluidic systems could open new possibilities. Combining electronic cell stimulation with precise fluid control can simulate more realistic *in vivo* microenvironments. This could

enhance studies on cell migration, tissue development, and response to dynamic biochemical cues.

3. Multi-Channel Stimulation:

Expanding the Electronic Cell Stimulation Chamber to support multi-channel stimulation would enable researchers to study the interactions between different cell types or analyse the impact of varied stimulation patterns on a single cell type. This could be particularly valuable in fields such as neuroscience, where intricate cell signalling networks play a crucial role.

5. Wireless Connectivity and Remote Monitoring:

Developing the Electronic Cell Stimulation Chamber with wireless connectivity features could facilitate remote monitoring and control. Researchers could observe and adjust experiments from a distance, allowing for increased flexibility in experimental design and reducing the need for constant physical presence in the laboratory.

6. Artificial Intelligence (AI) Integration:

Implementing AI algorithms for data analysis and pattern recognition could enhance the capabilities of the Electronic Cell Stimulation Chamber. Machine learning models could identify subtle cellular responses, correlate data across multiple experiments, and provide valuable insights into complex cellular behaviours under electronic stimulation.

7. Advanced Biocompatible Materials:

Continued research into biocompatible materials for the construction of the Electronic Cell Stimulation Chamber could lead to innovations in cell culture technology. Materials that closely mimic physiological conditions, support long-term cell viability, and minimise the impact of electrical stimulation on cellular health would be instrumental in advancing the field.

8. High-Throughput Screening Applications:

Adapting the Electronic Cell Stimulation Chamber for high-throughput screening could accelerate drug discovery and personalised medicine research. Creating a platform that allows simultaneous stimulation and monitoring of multiple cell cultures could streamline the identification of compounds with specific cellular responses.

9. Collaboration with Bioinformatics:

Collaborating with bioinformatics experts could enable the integration of Electronic Cell Stimulation Chamber data with large-scale omics datasets. Analysing the molecular and genetic changes in cells subjected to electronic stimulation could provide a holistic understanding of the underlying mechanisms and help identify potential biomarkers.

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