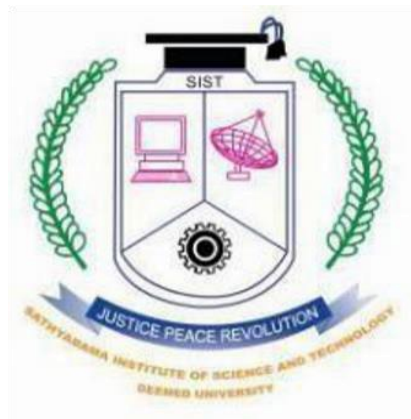


DESIGN OF ACOUSTIC LEVITATION EQUIPMENT FOR ORGAN CULTURE

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biomedical Engineering

By

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INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Shruthi L (40240034)**, **B Vijayalakshmi (40240043)** who have done the project work as a team and carried out the project entitled "**Design of Acoustic Levitation equipment for organ culture**" under our supervision from November 2023 to April 2024.

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We Shruthi L (40240034) and B Vijayalakshmi (40240043) hereby declare that the Project Report entitled "**DESIGN OF ACOUSTIC LEVITATION EQUIPMENT FOR ORGAN CULTURE**" done by us under the guidance of Dr. Krishnakumar S (Internal) and Mr. Carlton Ranjith Wilson Alphonse (External) at International Research Center is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biomedical Engineering.

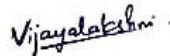
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ABSTRACT

Recent advancements in organoid research have aimed at overcoming the limitations posed by traditional methods of organoid cultivation, which often involve the use of complex scaffolds or gels. These traditional methods have been noted for introducing inconsistencies and reducing the reliability of experimental outcomes. A novel technique, acoustic levitation, presents a promising alternative by utilizing sound waves to facilitate the three-dimensional manipulation of cells without direct contact. This approach significantly enhances the precision in cell movement and negates the need for scaffolds or gels, thereby potentially improving the consistency and reproducibility of organoid experiments. Acoustic levitation also enables real-time observation of organoid growth and development, offering valuable insights into their behavior. The technique simulates a microgravity environment, useful for exploring cellular reactions in space-like conditions. The implementation involves the creation of a 3D model base equipped with ultrasonic transmitters on opposite sides, controlled by an Arduino Nano programmed to generate sound waves of sufficient intensity for levitation, powered by a 9V source. While the foundation for cell levitation has been successfully established, further research is required to optimize this method for cellular culturing. This advancement in organoid research methodology promises to simplify experimental procedures and make high-precision organoid studies more accessible to laboratories worldwide, potentially leading to significant breakthroughs in cellular biology and medicine.

Keywords: Acoustic levitation, Organoid, Three-Dimension, Piezo Ultrasonic Transmitters, Acoustofluidic, Sound Waves.

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CHAPTER 1

INTRODUCTION

Acoustic levitation is a technique where sound waves are used to make small objects float in the air (Andrade, *et.al.*, 2018). This works because the sound waves create areas of high and low pressure, pushing against gravity and holding the objects up. Depending on how the sound waves are directed, objects can float in different directions (Ashok, *et.al.*, 2020). Scientists use this technique in many fields like materials science, chemistry, and biology. For example, they can study how liquids and particles behave without gravity messing things up (Azar, *et.al.*, 2021). In chemistry, they can mix tiny amounts of liquids without needing containers. And in biology, they can study cells and tissues without touching them (Choi, *et.al.*, 2022).

Acoustic levitation is even used in space research to simulate what it's like in space. This helps scientists understand things like how fluids move around without gravity pulling them down (Corrò, *et.al.*, 2020). Plus, it's handy for delicate materials because it doesn't touch them. Scientists are always finding new ways to use acoustic levitation, from cool displays to tiny tools for manipulating really small stuff. It's a fascinating area of research that keeps getting better with time (de Souza, 2018).

There are different types of acoustic levitation, each with its own way of working and uses. One type is single-axis acoustic levitation, where sound waves are sent along one line, usually up and down (Drost, and Clevers, 2017). This makes things float in a straight line and is good for simple tasks like moving things around or doing stuff without containers (Fatehullah, *et.al.*, 2016).

Another type is multi-axis acoustic levitation, where sound waves come from different directions (Garreta, *et.al.*, 2021). This lets things float in all directions,

giving more control over where they go. It's useful for more complicated jobs in science like studying materials or biology (Gunti, *et.al.*, 2021).

Then, there's resonant acoustic levitation, which relies on making things vibrate at just the right frequency to float (Hofer and Lutolf, 2021). This happens when the sound waves match the object's natural vibrations, making a pattern that holds it up (Lehmann, *et.al.*, 2019). On the flip side, non-resonant acoustic levitation doesn't need things to vibrate at all. It just uses the pressure from the sound waves to make stuff float, which is handy for lots of different objects (Li and Izpisua Belmonte, 2019).

There's also acoustic streaming levitation, which uses sound waves to push liquids around and hold things up (Li, *et.al.*, 2022). It's great for moving tiny particles or droplets in liquids, which helps with stuff like making materials or studying biology (McCauley and Wells, 2017). Aerosol-based acoustic levitation does a similar thing but with tiny particles in the air instead of liquids. This helps scientists understand how tiny bits in the air move around, which is important for things like the environment and weather (Novak, *et.al.*, 2020).

Acoustic holography levitation is another cool type that combines sound waves with holograms to move stuff in 3D (Osebor and Mordi, 2023). This lets scientists control where things go really precisely, which is useful for things like trapping tiny particles (Poletkin, 2020). And last but not least, acoustic tweezers use sound waves to pick up and move small things in liquids, like cells or particles. It's really helpful for biology and making tiny machines (Rossi, *et.al.*, 2018).

Organ culture, also known as organotypic culture, is a method used in science to study tissues and organs outside of the body they belong to (Sakalem, *et.al.*, 2021). This technique involves growing thin slices of tissue or whole organs in a special place where we can control the environment (Simian & Bissell, 2017). The goal is to make this environment similar to what the tissues experience inside a living body. Organ cultures help scientists understand how tissues work and what happens when things go wrong, like in diseases (Sundvik, *et.al.*, 2015).

By studying tissues in this way, researchers can learn about processes like cells growing, changing, and moving (Takahashi, 2019). They can also see how different types of cells in an organ interact with each other. Organ culture is used to study many organs and tissues such as skin, brain, liver, and kidneys (Tang, *et.al.*, 2022). Scientists can change the conditions in the culture to mimic specific situations like diseases or different environments (Xinaris, *et.al.*, 2015).

This method is versatile, meaning it can be used to study both normal tissue function and diseases. It's also helpful for testing new drugs or treatments to see how tissues respond (Xu, *et.al.*, 2018). Organ cultures have been used for a long time in science and have helped us understand how tissues grow and heal. They're like a window into the inner workings of our bodies, helping scientists find new ways to treat diseases and improve health (Zhou, *et.al.*, 2021).

Different methods and systems can be used to establish organ cultures. They may involve using special liquids or structures to support tissue growth (Choi, *et.al.*, 2022). Over time, organ culture techniques have gotten better thanks to advancements in technology and science. They're often used alongside other methods like imaging and molecular analysis to get a full picture of how tissues behave (Poletkin, 2020).

The different ways scientists grow tissues and organs outside of the body for research are discussed. One way is by using special cultures where tissues sit on a membrane with air on one side and liquid on the other (Hofer and Lutolf, 2021). This helps mimic how tissues grow in our bodies. Another method involves fully submerging tissues in liquid, providing a consistent environment for growth but might not allow as much air or nutrient flow (Sakalem, *et.al.*, 2021).

Another technique involves slicing tissues thinly and growing them on special platforms. This keeps the tissue structure intact and helps study how tissues work together (McCauley and Wells, 2017). Alternatively, scientists can grow tissues on 3D scaffolds or structures to better mimic their natural environment and interactions.

Some cultures involve constantly flowing liquid through the tissues to provide nutrients and remove waste air (Andrade, *et.al.*, 2018). These are good for studying processes like blood vessel growth or how drugs are broken down. Microfluidic devices, like tiny organs-on-a-chip, are also used to recreate organ functions in the lab, offering precise control over conditions (Dupuis, *et.al.*, 2022).

Co-culture systems involve growing different cell types together to recreate complex tissues (Jeger-Madiot, *et.al.*, 2021). This helps understand how tissues develop and respond to things like diseases. Scientists also use imaging techniques to watch tissues grow and change over time, giving insights into their behavior (Rabiet, *et.al.*, 2022).

Advanced methods include using bioengineering to create functional tissues for transplantation or regenerative medicine (van der Vaart and Clever, 2021). These techniques vary depending on the tissue and what researchers want to learn. Making sure the culture conditions are just right is crucial for keeping tissues healthy and functional (Lecoq, *et.al.*, 2022). And, of course, ethics play a big role when using human or animal tissues for research.

There are a few ways we can use it to make tissue culture better. One way is by floating tiny pieces of tissue in a liquid using sound waves (Cai, *et.al.*, 2020). This lets scientists move the tissue around really precisely, which helps them see it from all angles.

Another way is by using acoustic levitation. By making tissue float in a special way, scientists can see how it grows and changes without gravity pulling it down (Ao, *et.al.*, 2021). This is useful for understanding things like how tissues develop and heal. Acoustic levitation also lets scientists handle tissue without touching it. This means they don't need any supports or structures to hold the tissue up, which can be better for its growth (Li, *et.al.*, 2020).

Additionally, it can be combined with tiny channels filled with liquid to make a really controlled environment for growing tissue. Using acoustic levitation can also help scientists study lots of tissue samples at once. By floating them all together, they can do many experiments at the same time and see how different things affect tissue growth and health (Miao, *et.al.*, 2023).

CHAPTER 2

LITERATURE SURVEY

Self-organization and culture of Mesenchymal Stem Cell spheroids in acoustic levitation. The review discusses reliable use of 3D cellular models still requires standardized protocols with well-controlled and reproducible parameters. To address this challenge, a robust and scaffold-free approach is proposed, which relies on multi-trap acoustic levitation. Various biological characterizations showed that the cells inside the spheroids were viable, maintained the expression of their cell surface markers, and had a higher differentiation capacity compared to standard 2D culture conditions. These results open the path to long-time cell culture in acoustic levitation of cell sheets or spheroids for any type of cells (Jeger-Madiot, *et.al.*, 2021).

Towards concentric topology in organoids: Structuration and culture of brain-derived spheroids in a Bulk Acoustic Wave resonator. This review paper on organoid research, has shown a surge in the development of innovative tools and methodologies aimed at precisely influencing the self-organization and differentiation processes. This trend is driven by an increasing need for reproducible cultivation and analysis of organoids. In this context, the introduction of a novel approach employing acoustic levitation for the cultivation of brain organoids derived from both primary neurons and glioblastomas obtained from patients. This method is designed to establish a concentric topology within the organoids, mimicking the architectural intricacies observed in vivo (Dupuis, *et.al.*, 2022).

Culture of hepatic and stem cell spheroids in acoustic levitation induces different self-organization dynamics. The review discusses about Drug-induced liver injuries (DILI) that account for over half of acute liver failures, posing significant challenges for public health and drug development. Current drug testing relies primarily on animal models and two-dimensional (2D) human cell cultures, each with inherent

limitations. Recognizing the need for improved human-based toxicity tests, a shift toward enhancing functionality through three-dimensional (3D) cultures, including organoids and organs-on-chip, is underway. Leveraging acoustofluidics, which manipulates single cells acoustically, can aid in forming multicellular spheroids and organoids. Our microfluidics chip demonstrates the cultivation of viable hepatic cell spheroids for extended periods, undergoing various treatments via continuous medium flux. This robust technique enables self-organization of cells, resulting in standardized spheroid formation within the microchip (Rabiet, *et.al.*, 2022).

Novel acoustofluidic approach using a Bulk Acoustic Wave resonator to control the topology and culture conditions of brain-derived spheroids. The author's review inquired into the importance of organoids as potent tools for investigating developmental stages, highlighting their capacity to autonomously self-assemble and mimic tissue properties. Simultaneously, advanced techniques like bio-printing and microfluidic organ-on-a-chip systems provide increased precision in controlling tissue topology and the chemical environment. Particularly noteworthy is the growing interest in the scaffold-free technique of acoustic levitation, which allows for the structuring of organoids while facilitating self-organization (Dupuis, *et.al.*, 2022).

Airway organoids as models of human disease. The author's review discusses current developments in the pulmonary organoid field, highlighting the potential and limitations of current models and the author describes that studies developing and applying organoid technology have greatly increased in volume and visibility over the past decade. Organoids are three-dimensional structures that are established from pluripotent stem cells (PSCs) or adult tissue stem cells (ASCs). They consist of organ-specific cell types that self-organize through cell sorting and spatially restricted lineage commitment to generate architectural and functional characteristics of the tissue of interest. The field of respiratory development and disease has been particularly productive in this regard. Starting from human cells (PSCs or ASCs), models of the two segments of the lung, the airways and the

alveoli, can be built. Such organoids allow the study of development, physiology, and disease and thus bridge the gap between animal models and clinical studies (van der Vaart and Clever, 2021).

Study of the influence of microgravity on brain-derived spheroids in acoustic levitation. Introduced is a scaffold-free approach, wherein multi-node acoustic levitation is employed to organize and cultivate organoids, facilitating the creation of large cell aggregates in a contactless, label-free manner with enhanced cell-cell interactions. The advantage of controlling a dynamic mechanical force field around growing cell aggregates is offered by acoustic levitation, facilitated by Acoustic Radiation Force (ARF). This technology allows for the observation of electrical activity in both 2D neuron layers and 3D neuronal spheroids. The experiment analysis contributes to a better understanding of the impact of gravity on neuronal activity. Preliminary results indicate that gravity variations, ranging from hypergravity (2g) to microgravity (0 g), influence neuronal activity (Lecoq, *et.al.*, 2022).

Trapping cell spheroids and organoids using digital acoustofluidics. The review discusses a digital acoustofluidic method aimed at revolutionizing the precise manipulation of cell spheroids and organoids for tissue engineering and regenerative medicine. With the utilization of a C-shaped integrated digital transducer (IDT), localized vibrations are applied to create a trapping node, effectively immobilizing cell spheroids within a diameter range of 20 μm to 300 μm . Demonstrating remarkable dexterity, precise control over the interaction of spheroid cells and organoids is achieved by our digital acoustofluidic device, showcasing excellent biocompatibility. The broad applications of this technology encompass tissue engineering, regenerative medicine, and fundamental cell biology research (Cai, *et.al.*, 2020).

Building programmable multicompartment artificial cells incorporating remotely activated protein channels using microfluidics and acoustic levitation. The review discusses the development of a droplet laboratory platform to engineer complex emulsion-based, multicompartment artificial cells, using microfluidics and acoustic levitation. Intracellular compartments are functional units that support the metabolism within living cells, through spatiotemporal regulation of chemical reactions and biological processes. The assembly of droplet networks is three-dimensionally patterned with fluidic input configurations determining droplet contents and connectivity, whilst acoustic manipulation can be harnessed to reconfigure the droplet network in situ. This offers possibilities beyond one-time chemically mediated activation to provide repeated, non-contact, control of membrane protein function (Li, *et.al.*, 2022).

Controllable fusion of human brain organoids using acoustofluidics. The author's review discusses a passive acoustofluidic method for standardizing fused organoid models, addressing challenges of heterogeneity and variable reproducibility. Using a hexagonal acoustofluidic device, dynamic acoustic fields passively manipulate organoids, achieving contact-free, label-free, and minimally impactful rotation, transport, and fusion. In modeling the human midbrain-to-forebrain mesocortical pathway, human forebrain organoids (hFOs) and human midbrain organoids (hMOs) underwent acoustic fusion with controllable neuroepithelial bud alignment. Post-assembly, successful tyrosine hydroxylase neuron projection from hMO to hFO was observed, accompanied by increased firing rates and synchrony of excitatory neurons. The passive controllable fusion method regulates characteristics such as neuron projection, projection maturation, and neural progenitor cell division in resulting assembloids through initial spatial control (Ao, *et.al.*, 2021).

Analysis of the effects of acoustic levitation to simulate the microgravity environment on the development of early zebrafish embryos. In this review, the author discusses how an acoustic standing wave field(ASWF) is used to simulate

the space environment, which shows characteristics such as microgravity and the absence of containment and contact. Zebrafish embryos, used as the species under study in this work, were raised within the acoustic field by the authors, allowing the biological effects on such early zebrafish embryos at each developmental stage and within the ASWF creating the acoustic levitation (AL) technology used to be studied. In this way, the biological safety of the specimens, simulating the space environment, could be carefully evaluated. Some important indexes of the process of zebrafish development, such as mortality, malformation rate, hatching rate, voluntary movement, and heart rate, were detected and analyzed (Li, *et.al.*, 2020).

High-throughput fabrication of cell spheroids with 3D acoustic assembly devices. The review discusses, a novel 3D acoustic cell assembly device, integrated with gelatin methacrylamide (GelMA) hydrogels, was developed for the high-throughput production of cell spheroids. Utilizing three orthogonal piezoelectric transducers, three standing bulk acoustic waves were generated by the acoustic device, forming a 3D dot-array of levitated acoustic nodes. The potential efficacy of these acoustically assembled spheroids in drug response was demonstrated through drug testing. In conclusion, promise lies in the scalability of the production of cell spheroids or organoids using this 3D acoustic cell assembly device, presenting versatile applications in various biomedical fields, including high-throughput screening, disease modeling, tissue engineering, and regenerative medicine (Miao, *et.al.*, 2023).

On evaporation dynamics of an acoustically levitated multicomponent droplet: Evaporation-triggered phase transition and freezing. This review discusses Multi-component droplet evaporation has received significant attention in recent years due to the broad range of applications such as material science, environmental monitoring, and pharmaceuticals. The selective evaporation induced by the different physicochemical properties of components is expected to influence the concentration distributions and the separation of mixtures, thereby leading to rich interfacial phenomena and phase interactions. A ternary mixture system containing

hexadecane, ethanol, and diethyl ether is investigated in this study. The diethyl ether exhibits both surfactant-like and co-solvent properties. Systematic experiments were performed using an acoustic levitation technique to achieve a contact-less evaporation condition. The evaporation dynamics and temperature information are acquired in the experiments, using high-speed photography and infrared thermography technologies (Zeng, *et.al.*, 2023).

Emerging biofabrication approaches for gastrointestinal organoids towards patient specific cancer models. This review explores advancements in the creation of three-dimensional (3 D) artificial cell constructs for the purpose of modeling gastrointestinal dynamics for cancer diagnosis. The focus is on tissue-engineered organoids, simple biomodels capable of replicating the structural and functional complexity of specific organs. The discussion includes bottom-up approaches involving the use of biochemical and physical cues to guide organoid self-assembly, along with engineering techniques like 3D printing/additive manufacturing and external field-driven protocols. The paper concludes by outlining the primary challenges and potential risks associated with translating gastrointestinal organoids from laboratory settings to patient-specific models in clinical applications (Soto, *et.al.*, 2021).

Multi-frequency acoustic levitation and trapping of particles in all degrees of freedom. The author's review discusses the stabilization of the position and orientation of non-spherical, sub-wavelength particles in mid-air required for using acoustic levitation forces in applications such as automation of micromanufacturing processes, 3-D scanning, and inspection. Using this method, objects made from high-density materials across a wide range of geometries can be locked acoustically with increased stability compared with state-of-the-art methods (Röthlisberger, *et.al.*, 2022).

Use and application of 3 D-organoid technology. The review discusses the capacity of the 3D-organoid cultures to resemble a near-physiological tissue organization

and to mimic – to a certain degree – organ functionality, make organoids an excellent model for applications spanning from basic developmental/stem cell research to personalized medicine. It also concerns key findings achieved through organoid technology, and we discuss applications such as disease – and tumor modeling, correction of genetic mutations, and understanding gene – and cell functions. Finally, discusses future developments in the field (Artegiani and Clevers, 2018).

Patient-specific organoid and organ-on-a-chip: 3D cell-culture meets 3D printing and numerical simulation. The review describes two novel 3D cell culture models: Organoid, promoted mainly by the developments of stem cell biology, and Organ-on-a-Chip, enhanced primarily due to microfluidic technology, have emerged as two promising approaches to advance the understanding of basic biological principles and clinical treatments. The comparable distinct differences between these two models provide more insights into their complementarity and integration to recognize their merits and limitations for applicable fields. The convergence of the two approaches to produce multi-organoid-on-a-chip or human organoid-on-a-chip is emerging as a new approach for building 3D models with higher physiological relevance. Furthermore, rapid advancements in 3D printing and numerical simulations, which facilitate the design, manufacture, and results translation of 3D cell culture models, can also serve as novel tools to promote the development and propagation of organoid and organ-on-a-chip systems (Zheng, *et.al.*, 2021).

Acoustic levitation in mid-air: recent advances, challenges, and future perspectives. The author's review discusses mid-air acoustic levitation is becoming a powerful tool to suspend and manipulate millimetric objects. Because of its unique characteristics, acoustic levitation is suitable to trap a wide variety of materials, such as liquids, solids, soap bubbles, and even living creatures. Acoustic levitation can also be combined with noncontact measurement systems, allowing contactless analysis and characterization of levitating samples. The author also reviewed some of the advances that have been made over the last decade. We also present the

technical challenges that must be overcome in order to extend the capability of current acoustic levitation devices, and, finally, we point out future directions for acoustic levitation (Brizzotti Andrade, *et.al.*, 2020).

Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. The review discusses the considerable attention garnered by three-dimensional (3D) stem cell culture systems, which aim to faithfully replicate the intricate interactions between individual cells and the extracellular matrix (ECM) observed *in vivo*. The unique properties of these systems play a crucial role in guiding specific functions, growth, and processes of stem cells, such as embryogenesis, morphogenesis, and organogenesis. This facilitates fundamental research on various tissues and organs. The focus of this review is on the advanced therapeutic applications of stem cell-based 3D culture systems, providing a summary of their historical progression and discussing engineering techniques for tissue repair and regeneration, particularly through the use of stem cell-based spheroids and organoids (Kim, *et.al.*, 2023).

Levitodynamics: Levitation and control of microscopic objects in vacuum. The author's review discusses the control of levitated nano- and micro-objects in vacuum is of considerable interest, capitalizing on the scientific achievements in the fields of atomic physics, control theory, and optomechanics. The ability to couple the motion of levitated systems to internal degrees of freedom, as well as to external forces and systems, provides opportunities for science and technology. Attractive research directions, ranging from fundamental quantum physics to commercial sensors, have been unlocked by the many recent experimental achievements, including motional ground-state cooling of an optically levitated nanoparticle. We review the status, challenges, and prospects of levitodynamics, the multidisciplinary research devoted to understanding, controlling, and using levitated nano- and micro-objects in vacuum (Gonzalez-Ballester, *et.al.*, 2021).

The acoustic droplet printing of functional tumor microenvironments. This study introduces an innovative acoustic droplet printing method to address challenges in current 3D bioprinting. The nozzle-free approach ensures stable printing of high-concentration cells, including spheroids, with exceptional cell viability exceeding 94%. The method's compact design allows flexible integration for different bioinks, overcoming limitations in printing low-concentration gelatin methacryloyl materials. As a proof of concept, the study demonstrates the construction of a tumor microenvironment, highlighting the method's potential to advance bioprinting for creating functional native tissues, organoids, or disease models (Chen, *et.al.*, 2021).

Acoustic manipulation of dense nanorods in microgravity. The review discusses the behavior of dense gold nanorods under ARF during a parabolic flight campaign. Because the absence of sedimentation in zero-gravity makes the culture and the manipulation of cells or particles challenging, an attractive alternative we studied the behavior of dense gold nanorods under ARF during a parabolic flight campaign. Using dense objects enhances the effect of gravity on the axial position of the so-called "levitation plane," which is the equilibrium position at which ARF balances gravity in the laboratory. The experiments clearly show a different collective organization and individual behavior of the rods in micro-gravity conditions (Dumy, *et.al.*, 2020).

Ultrasonic Based Tissue Modelling and Engineering. This review emphasizes the potential of ultrasonic standing wave (USW) particle manipulation in tissue modeling and engineering for in vitro applications. Recognizing these tools as valuable assets that leverage controlled laboratory environments and mimic tissue organization in vivo, the exploration of device-based tissue engineering for future explant culture in regenerative medicine is acknowledged. The critical requirements for platforms in tissue modeling and engineering, particularly in shaping and maintaining cell aggregates over long-term culture, are highlighted. Ultrasonic standing wave particle manipulation is praised for its label-free and gentle

positioning and aggregation of cells, with stable pressure nodes over time, accommodating both static and dynamic configurations based on actuation schemes (Olofsson, *et.al.*, 2018).

The waves that make the pattern: A review on acoustic manipulation in biomedical research. In this review, the novel approaches integrating technology, biomaterial design, and advanced cell culture techniques for tissue engineering and regenerative medicine. Acoustic manipulation, a promising method, enables remote control of spatial cellular organization within a carrier matrix by leveraging hydrodynamic forces in standing waves. This technique has evolved from arranging micro- or nanoparticles in 2D to assembling multiple cell types or organoids into complex in vitro tissues. The review highlights past achievements in acoustic manipulation, emphasizing biomedical applications and surveying devices for arranging non-living and living units in buffers or hydrogels. Challenges and prospects for understanding in vitro tissue formation in biomedical engineering are also discussed (Guex, *et.al.*, 2021).

A Non-Contact Manipulation for Robotic Applications: A Review on Acoustic Levitation. The author's review discusses contact and non-contact manipulation with their most common robotic applications and focusing on the non-contact manipulation since it can do specific tasks which contact manipulation cannot do. The main core of this article is acoustic levitation which is considered one of the most important levitation and manipulation method due to the recent advancements that made it proper to deal with any kind of materials (Al-Nuaimi, *et.al.*, 2022).

Application of an acoustofluidic perfusion bioreactor for cartilage tissue engineering. The author's review discusses how cartilage grafts generated using conventional static tissue engineering strategies are characterized by low cell viability, suboptimal hyaline cartilage formation and, critically, inferior mechanical competency, which limit their application for resurfacing articular cartilage defects.

To address the limitations of conventional static cartilage bioengineering strategies and generate robust, scaffold-free neocartilage grafts of human articular chondrocytes, the present study utilized custom-built microfluidic perfusion bioreactors with integrated ultrasound standing wave traps. The study has demonstrated the first successful application of the acoustofluidic perfusion bioreactors to bioengineer scaffold-free neocartilage grafts of human articular chondrocytes that have the potential for subsequent use in second generation autologous chondrocyte implantation procedures for the repair of partial thickness cartilage defects (Li, *et.al.*, 2014).

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The main goal of this project is to create a new system using sound waves to grow tiny organ-like structures called organoids. This system will create a special environment where organoids can grow in 3D and organize themselves properly. We want to make sure the system can work with different types of cells, provide them with the right nutrients, and be easy for scientists to use. By improving how organoids are grown, we hope to learn more about how bodies develop, model diseases, and test new medicines. This project aims to push forward the way we grow cells and organoids, leading to important discoveries in biology and medicine.

3.2 SCOPE

This project's objective is to create advanced equipment customized for organoid culture using acoustic levitation technology. It will involve optimizing acoustic parameters to effectively suspend organoids in a chamber that meets biocompatibility standards, accommodating various cell types, and improving nutrient delivery while maintaining aseptic conditions. A user-friendly interface will enable real-time control, and potential applications in developmental biology, disease modeling, and drug screening will be explored. Safety measures will be integrated to adhere to ethical and biosafety standards, and comprehensive documentation will be provided for user guidance. Collaboration with experts in relevant fields will be pursued, with the ultimate goal of contributing an innovative and adaptable solution to advance organoid culture techniques, thereby driving progress in biological research and therapeutic applications.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS

4.1.1 ARDUINO NANO

The Arduino Nano, a compact and adaptable microcontroller board based on the ATmega328P processor, assumes a pivotal role in the functionality of an acoustic levitator. Serving as the central processing unit, the Nano executes the control algorithm and orchestrates the seamless operation of the system. Through its interfaces, the Nano engages with sensors, such as ultrasonic sensors, to collect crucial data regarding the position of the levitated object. This data is instrumental in a closed-loop control system that continuously adjusts parameters to maintain the object in a stable position. Moreover, the Nano takes charge of modulating the acoustic transducers or actuators, directing signals to achieve the desired standing wave pattern for levitation. With communication interfaces and programming capabilities, the Arduino Nano enables not only real-time feedback control but also customization of the control algorithm to suit specific requirements. In essence, the Arduino Nano functions as the intelligence hub, seamlessly coordinating sensor input, actuator response, and closed-loop control to achieve the precise and stable levitation of objects in an acoustic levitator setup.

4.1.2 DRIVER (L297N)

The L297N, functioning as a specialized stepper motor controller integrated circuit, plays a pivotal role in applications requiring meticulous control over stepper motor operations. In the specific context of an acoustic levitator, this versatile IC can be instrumental in orchestrating the precise positioning and movement of objects through the manipulation of acoustic waves. Boasting features such as microstepping, the L297N ensures finer positioning and smoother motion, crucial

attributes in scenarios where exact control over object placement is paramount. With its ability to interface with various control systems via input signals, including those dictating direction and step pulses, the L297N seamlessly integrates into digital control circuits or microcontroller-based setups. Applied to an acoustic levitator, it can extend its influence to regulate transducers generating acoustic waves, modulate wave parameters based on feedback from sensors, and leverage microstepping for enhanced precision in maintaining the stability of levitated objects. In essence, the L297N, designed for stepper motor control, emerges as a valuable component contributing to the stability, precision, and control of object levitation in the dynamic environment of an acoustic levitator setup.

4.1.3 ULTRASONIC TRANSMITTER

The piezo ultrasonic transmitter functions as a pivotal component in the operation of an acoustic levitator, leveraging the piezoelectric effect within piezoelectric crystals. The transmitter, utilizing a piezoelectric crystal, generates ultrasonic waves when subjected to an electrical voltage, forming a standing wave pattern in the medium. This standing wave pattern is critical for the levitation of objects within the acoustic field. Simultaneously, the piezo ultrasonic receiver, employing a similar piezoelectric crystal but operating in reverse, detects ultrasonic waves by converting mechanical vibrations into electrical voltage. This feedback mechanism enables precise control and adjustment of the transmitted waves in real-time, ensuring the stability and controlled levitation of objects. The receiver's ability to analyze the characteristics of received ultrasonic waves, such as amplitude and phase, provides valuable information for maintaining the desired position of the levitated object. In essence, the synergy between the piezo ultrasonic transmitter and receiver facilitates the creation of a stable acoustic field, offering a means to precisely manipulate objects in an acoustic levitator setup.

4.2 METHODOLOGY

1) 3D Print the Base:

- a) Use a 0.4mm or 0.6mm nozzle to print the base for the levitator.
- b) Use a brim but no support, and ensure it can be printed in one piece.

2) Polarity Detection:

- a) Connect one wire to A0 and another to GND on the Arduino.
- b) Run the Arduino with the code provided and open the Serial Plotter.
- c) When a transducer is connected between A0 and GND:
- d) If the signal remains at 0 or goes down, mark the leg connected to GND.
- e) If the signal remains at 1023 or goes up, mark the leg connected to A0.
- f) Avoid touching the transducer legs or wires during this process.

3) Transducer Installation:

- a) Apply hot glue on the side of the socket.
- b) Push the transducer into the socket and apply pressure to make it lay flat.
- c) Ensure all marked legs are pointing towards the center of the device.

4) Wire Wrapping:

- a) Wrap the exposed wire around the legs of the transducers in six concentric rings.

5) Soldering:

- a) Solder the pins to the wires.
- b) Prepare two red wires and two black wires, each around 1 meter long.
- c) Expose the tip on one side and three segments on the other side of the wires.

6) Connecting Wires to Transducers:

- a) Solder the long wires to the transducers, with one segment for each ring.
- b) Each side of the levitator should have a black and a red wire.

- 7) Code Upload:
 - a) Upload the provided code into the Arduino Nano.
- 8) DC Connector and Switch:
 - a) Solder the DC female connector to the switch.
 - b) Prepare two wires to supply power to the driver board.
- 9) Connecting Power:
 - a) Connect the red wire from the supply to the 12V input of the driver.
 - b) Connect the ground from the supply to the middle connector of the driver.
 - c) Insert a male-female jumper into the 5V input of the driver.
 - d) Connect the jumpers from the driver to ground and 5V of the Arduino.
- 10) Driver Board Connections:
 - a) Connect female jumpers from Arduino (A0, A1, A2, A3) to the inputs of the driver (IN1, IN2, IN3, IN4).
 - b) Connect a female-male jumper from ground of the Arduino to D2, D3, or D4.
 - c) Connect D10 to D11 with a jumper for synchronized signal emission.
- 11) Driver Board Testing:
 - a) Ensure the driver outputs a 40kHz square wave of twice the voltage provided to the circuit.
- 12) Check for Short Circuits:
 - a) Test for any shortcuts between the red and black wires of the levitator.
- 13) Levitator Connection:
 - a) Connect the levitator to the driver board and switch it on (between 6V and 12V).
- 14) Particle Placement:
 - a) Provide up to 10V for testing.

b) Use tweezers or a metallic grid to place particles into the levitator.

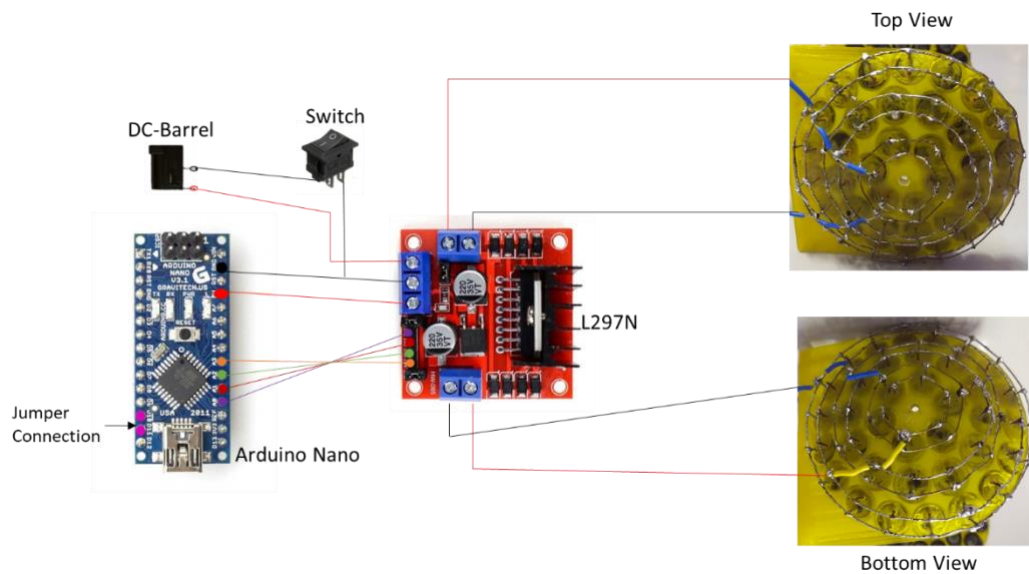


Fig: 4.1. Circuit Diagram for acoustic levitator

4.3. ALGORITHM

Step 1: Initialization

- 1) Initialize the AVR microcontroller.
- 2) Configure pins:
 - a) Set pins A0 to A3 (PC0 to PC3) as output for LED signals.
 - b) Set pin 10 (PB2) as output for generating a 40kHz sync signal.
 - c) Set pin 11 (PB3) as input with a pull-up resistor for sync input.
 - d) Set pins D2 to D7 (PD2 to PD7) as input with pull-up resistors for button inputs.
- 3) Set up Timer 1:
 - a) Configure Timer 1 for Fast PWM mode.
 - b) Set Timer 1 to generate a 40kHz PWM signal for synchronization.
- 4) Disable unused peripherals:
 - a) Disable ADC, SPI, TWI, and Timer0 to save power.
- 5) Initialize serial communication at 115200 baud rate.

Step 2: Main Loop

- 1) Wait for a sync signal on pin 11 (PB3).
- 2) Once a sync signal is detected, proceed to the animation loop.
- 3) Animation Loop:
 - a) Iterate through each frame of the animation:
 - b) Set the output pins (A0 to A3) according to the current frame of the animation.
 - c) Read the state of the button inputs (D2 to D7).
 - d) Check if any button is pressed and track the duration of the button press.
 - e) If a button is pressed for a certain duration:
 - f) Decrease the animation frame index by STEP_SIZE for Button 0, looping back to the last frame if necessary.
 - g) Increase the animation frame index by STEP_SIZE for Button 1, looping back to the first frame if necessary.
 - h) Reset the animation frame index to 0 for Button 2.
 - i) Repeat the animation loop continuously.

Step 3: End of Program

- 1) The program continues running indefinitely, playing the LED animation synchronized with external signals and responding to button inputs.

4.3.1. Program

```
#include <avr/sleep.h>
#include <avr/power.h>

#define N_PORTS 1
#define N_DIVS 24

#define WAIT_LOT(a) __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop");
__asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__
__volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop")
```

```

#define WAIT_MID(a) __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop");
__asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__
__volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop");
__asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop")
#define WAIT_LIT(a) __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop");
__asm__ __volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__
__volatile__ ("nop"); __asm__ __volatile__ ("nop"); __asm__ __volatile__
("nop")

#define OUTPUT_WAVE(pointer, d) PORTC = pointer[d*N_PORTS + 0]

#define N_BUTTONS 6
//half a second
#define STEP_SIZE 1
#define BUTTON_SENS 2500
#define N_FRAMES 24

static byte frame = 0;
static byte animation[N_FRAMES][N_DIVS] =
{{0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0xa,0xa,0xa,0xa,0xa,0xa,0
xa,0xa,0xa,0xa,0xa,0xa},
{0x9,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x6,0xa,0xa,0xa,0xa,0xa,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x6,0x6,0xa,0xa,0xa,0xa,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x6,0x6,0x6,0xa,0xa,0xa,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x6,0x6,0x6,0x6,0xa,0xa,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x5,0x5,0x5,0x5,0x5,0x5,0x6,0x6,0x6,0x6,0x6,0xa,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x9,0x5,0x5,0x5,0x5,0x5,0x6,0x6,0x6,0x6,0x6,0x6,0x
a,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x5,0x5,0x5,0x5,0x6,0x6,0x6,0x6,0x6,0x6,0x
6,0xa,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x5,0x5,0x5,0x6,0x6,0x6,0x6,0x6,0x6,0x
6,0x6,0xa,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x5,0x5,0x6,0x6,0x6,0x6,0x6,0x6,0x
6,0x6,0x6,0xa,0xa,0xa},
{0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x5,0x6,0x6,0x6,0x6,0x6,0x6,0x
6,0x6,0x6,0x6,0x6,0xa},

```

```

{0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x9,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6,0x6},
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{0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x9,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0x6,0x6,0x6,0x6},
{0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x5,0x9,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0xa,0x6,0x6,0x6,0x6}};

```

```

void setup()
{

    DDRC = 0b00001111; //A0 to A3 are the signal outputs
    PORTC = 0b00000000;

    pinMode(10, OUTPUT);
    pinMode(11, INPUT_PULLUP);

    for (int i = 2; i < 8; ++i){
        pinMode(i, INPUT_PULLUP);
    }

    noInterrupts();
    TCCR1A = bit (WGM10) | bit (WGM11) | bit (COM1B1); TCCR1B = bit (WGM12)
| bit (WGM13) | bit (CS10);
    OCR1A = (F_CPU / 40000L) - 1;
    OCR1B = (F_CPU / 40000L) / 2;
    interrupts();
    ADCSRA = 0;
    power_adc_disable ();
}

```

```

power_spi_disable();
power_twi_disable();
power_timer0_disable();

Serial.begin(115200);

byte* emittingPointer = &animation[frame][0];
byte buttonsPort = 0;

bool anyButtonPressed;
bool buttonPressed[N_BUTTONS];
short buttonCounter = 0;

LOOP:
    while(PINB & 0b00001000);

    OUTPUT_WAVE(emittingPointer, 0); buttonsPort = PIND; WAIT_LIT();
    OUTPUT_WAVE(emittingPointer, 1); anyButtonPressed = (buttonsPort &
0b11111100) != 0b11111100; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 2); buttonPressed[0] = buttonsPort &
0b00000100; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 3); buttonPressed[1] = buttonsPort &
0b00001000; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 4); buttonPressed[2] = buttonsPort &
0b00010000; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 5); buttonPressed[3] = buttonsPort &
0b00100000; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 6); buttonPressed[4] = buttonsPort &
0b01000000; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 7); buttonPressed[5] = buttonsPort &
0b10000000; WAIT_MID();
    OUTPUT_WAVE(emittingPointer, 8); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 9); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 10); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 11); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 12); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 13); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 14); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 15); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 16); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 17); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 18); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 19); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 20); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 21); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 22); WAIT_LOT();
    OUTPUT_WAVE(emittingPointer, 23);

```

```

if( anyButtonPressed ){
    ++buttonCounter;
    if (buttonCounter > BUTTON_SENS){
        buttonCounter = 0;

        if (! buttonPressed[0] ) {
            if( frame < STEP_SIZE ) {
                frame = N_FRAMES-1;
            }else{
                frame-=STEP_SIZE;
            }
        }
        else if (! buttonPressed[1] ) {
            if ( frame >= N_FRAMES-STEP_SIZE ) {
                frame = 0;
            }else {
                frame+=STEP_SIZE;
            }
        }
        else if (! buttonPressed[2] ) {
            frame = 0;
        }
        emittingPointer = & animation[frame][0];
    }
    }else {
        buttonCounter = 0;
    }

    goto LOOP;

}

void loop(){}

```

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 RESULTS

After completing the setup, the heat sink was removed from the driver because the instrument might need to operate for extended periods or provide more than 10 volts of power. However, upon changing the heat sink, the instrument failed to make the foam float, and the Arduino Nano board caught fire upon powering up. This occurred with three Arduino Nano boards in total. It was later discovered that the new heat sink wasn't properly connected, causing it to direct heat towards the Arduino Nano board, resulting in the fire. Consequently, the original heat sink was reinstated for the driver. After several attempts, the device successfully achieved foam levitation, as depicted in Figure 5.1, but the foam later fell due to loose connections. The instrument was soldered, ensuring all connections were secure, and upon retrying, the foam floated as required, as shown in Figure 5.2. Several more foam pieces were tested, with a total of six managing to float, indicating the formation of six pressure nodes.

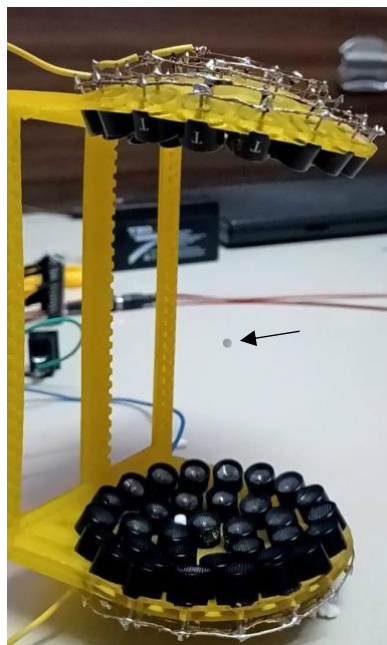


Fig: 5.1. Acoustic levitator levitates the foam.

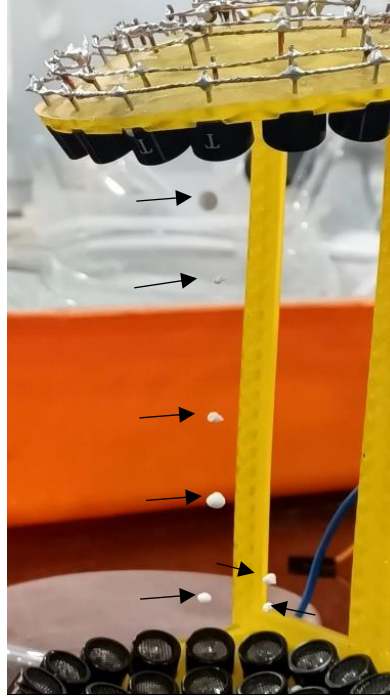


Fig: 5.2. Foams levitated at the pressure nodes

5.2 DISCUSSION

The project outlined presents a pioneering endeavor in utilizing acoustic levitation for the precise manipulation and growth of organoids, offering a novel approach to address the limitations of traditional organoid culture methods. This initiative aligns with recent advancements in acoustic levitation technology, as evidenced by studies exploring its application in various biomedical research domains. For instance, Ao et al. (2021) demonstrated the controllable fusion of human brain organoids using acoustofluidics, highlighting the potential of acoustic manipulation techniques in tissue engineering applications. Furthermore, Chen et al. (2021) showcased the acoustic droplet printing of functional tumor microenvironments, indicating the versatility of acoustic-based methods in generating complex biological constructs.

While existing research provides valuable insights into the feasibility and benefits of acoustic manipulation in biomedical contexts, there remains a notable gap in specialized equipment tailored specifically for growing organoids with acoustic

levitation. This gap underscores the significance of the proposed project, which aims to design and build a dedicated system to facilitate organoid growth and organization in a 3D environment. Drawing inspiration from advancements in acoustic levitation technology (Al-Nuaimi et al., 2022; Andrade et al., 2018), the project seeks to address technical challenges and optimize nutrient delivery to enhance organoid functionality.

Moreover, the proposed system's integration of user-friendly features and compatibility with various cell types echoes recent efforts to develop advanced bioreactor platforms for organoid culture (Cai et al., 2020; Guex et al., 2021). These endeavors underscore the importance of creating accessible and adaptable tools to support innovative research approaches in biomedicine.

While existing studies have explored the applications of acoustic levitation in biomedical research, the proposed project represents a significant step towards addressing the specific challenges associated with growing organoids using this technology. By leveraging insights from previous research and integrating cutting-edge acoustic manipulation techniques, the project has the potential to advance our understanding of organ development and disease pathology, ultimately contributing to the broader field of medical research.

5.3 PERFORMANCE ANALYSIS

TRAIL 1:

In the first attempt, the HC-SR04 transducer was utilized with transmitters on both sides. A straightforward program was developed and uploaded to the Arduino Nano, adhering to the connection diagram. Upon activating the setup, it was observed that the foam shifted slightly, but it did not achieve the desired floating state.

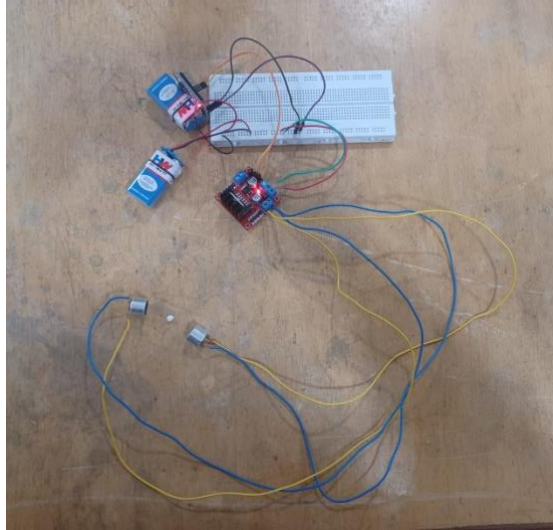


Fig: 5.3. Circuit connection of trial 1.

TRAIL 2:

In the subsequent attempt, the same program and setup were maintained from the previous trial. However, the HC-SR04 transducer was replaced with a piezo ultrasonic transducer, while retaining transmitters on both sides. This alteration resulted in successful foam levitation, as depicted in the picture.

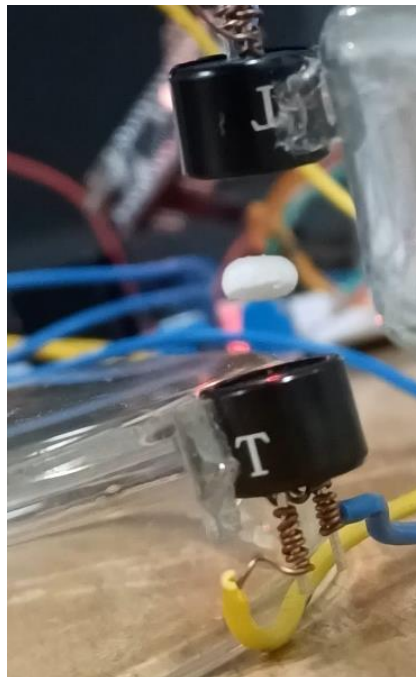


Fig: 5.4. Levitation of the foam in the trial 2 circuit connection.

TRAIL 3:

For the third attempt, a 3D model base was constructed. The same piezo ultrasonic transducer used previously was utilized, but this time, 36 transmitters and 36 receivers were placed on each side of the base. The program in the Arduino was modified accordingly, and all connections were made following the provided instructions. To verify proper functioning, tests were conducted to ensure the LD driver received power and each transducer was correctly connected. However, upon activating the system, instead of achieving foam levitation, it unexpectedly pushed the foam away. Unfortunately, this attempt did not yield the desired outcome.

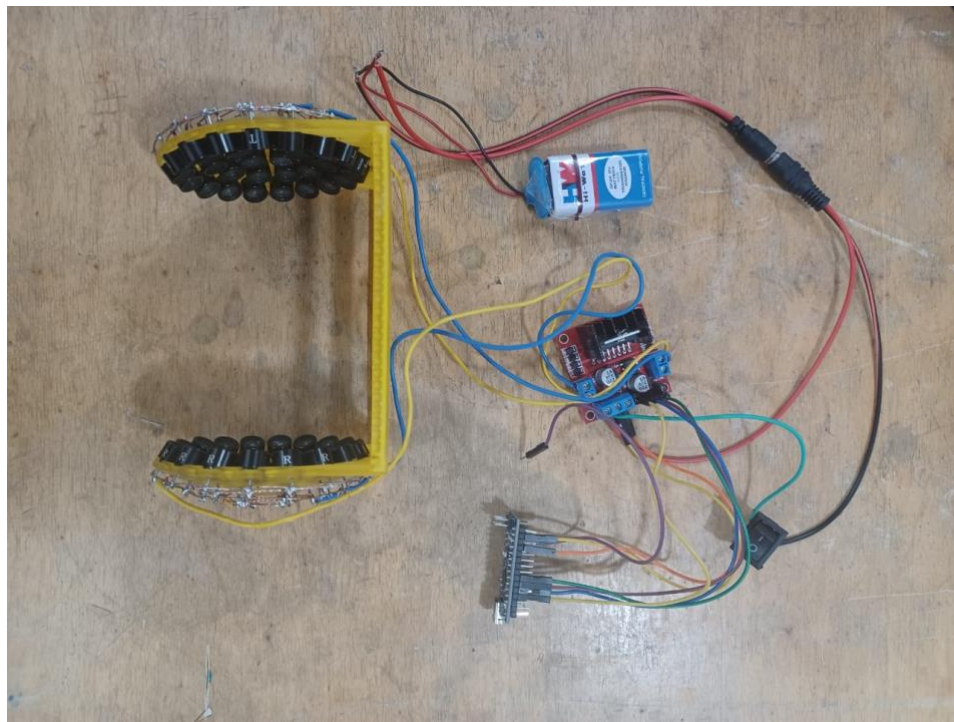


Fig: 5.5. Circuit connection of the trial 3.

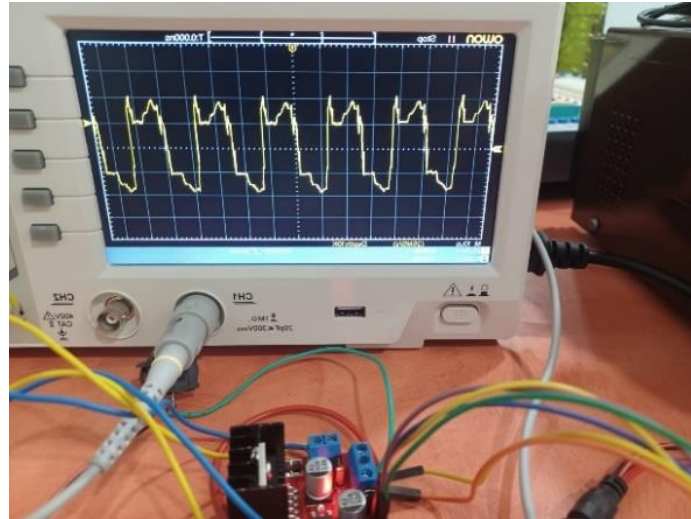


Fig: 5.6. Testing the current flow in the driver using oscilloscope.

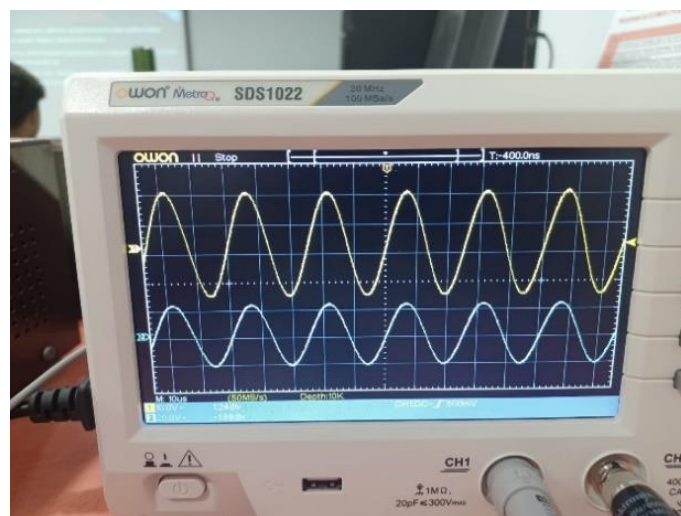


Fig: 5.7. Checking the polarity of the transducers using the oscilloscope.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

In today's medical research, there has been a notable shift towards utilizing 3D cell cultures, such as organoids, to study diseases. However, conventional methods of creating organoids often fail to accurately replicate the complexities of real organs, thereby limiting their utility. To address this issue, innovative techniques like acoustic levitation are explored. This cutting-edge approach employs sound waves to manipulate objects in mid-air without physical contact, offering immense potential for growing organoids without conventional support structures. Acoustic levitation enables precise control over cell positioning and movement within a 3D space, minimizing contamination risks and facilitating uniform nutrient distribution, thus more closely mimicking physiological conditions. Despite its promise, the absence of dedicated equipment tailored for growing organoids using acoustic levitation poses a significant challenge. This gap motivates ongoing efforts to design and construct specialized systems aimed at optimizing organoid growth and functionality. By integrating principles of acoustic levitation, these systems aim to enhance nutrient delivery, accommodate diverse cell types, and ensure user-friendly operation. While initial demonstrations have shown the capability of acoustic levitation to levitate objects like foam, further refinement is required to optimize its application for organoid cultivation. Overcoming technical hurdles and innovating new methodologies for leveraging this technology hold the promise of advancing our understanding of organ development and drug testing in medical research. Ultimately, these attempts seek to deepen insights into human physiology and elevate the impact of organoid studies on medical progress.

6.2 CONCLUSION

The integration of acoustic levitation into organ culture represents a promising frontier within biomedical research. Leveraging sound waves to manipulate organs within a non-contact, three-dimensional environment introduces an innovative approach offering a unique advantage over conventional methodologies. This innovative approach not only reduces contamination risks but also encourages the creation of environments more closely resembling physiological conditions. The potential impact spans across various domains, from enhancing our comprehension of organ development to refining disease modeling and drug testing procedures. Despite existing challenges, such as protocol optimization and scalability concerns, the transformative benefits of acoustic levitation are unmistakable. As efforts persist in exploring and perfecting these techniques, unlocking the complete potential of organ research. Ultimately, this progress paves the way for advancement in personalized medicine and therapeutic interventions.

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