Experimental device provided with a computerized analysis system of biomedical images for obtaining cardiac biomatrices

PhD Thesis – Summary

for obtaining the scientific title of doctor at
Universitatea Politehnica Timișoara
in doctoral domain of Electronics, Telecommunications and Information Technology engineering

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month 11 year 2023

The Ph.D. thesis addresses a novel and important topic in the field of tissue engineering. The aim of this study is to develop a modified Langendorff device for rat heart decellularization, along with a computerized biomedical image analysis system for obtaining cardiac biomatrices.

The thesis includes a presentation of the automated decellularization process, the optimized software algorithm, and the improvements made to the device by integrating a vibrating fluid column. Additionally, the last chapter of the thesis presents an innovative aspect and focuses on optimizing the decellularization process through neural networks. This thesis makes a significant contribution to the field of tissue engineering by developing a modified Langendorff device and a computerized biomedical image analysis system that can be used in the rat heart decellularization process.

1. INTRODUCTION

In recent decades, medical research had a significant impact on increasing life expectancy, but contemporary medicine faces challenges, such as chronic degenerative pathologies and organ shortages for transplantation. The waiting list for suitable donors is long, prompting the search for new options in transplantology.

Tissue engineering has emerged as a new field of study, offering the possibility of creating functional organs and tissues in vitro through decellularization and recellularization. These methods rely on the use of biomatrices obtained by decellularizing various organs and tissues, thereby eliminating the need for compatible donors and reducing the risk of immune rejection.

Decellularization methods have been successfully applied depending on the thickness and type of tissue or organ, and experimental protocols have yielded promising results in the case of cardiac tissue. The decellularization process involves treatments such as static incubation, which exposes the tissue to certain forms of turbulence.

Tissue engineering holds promising potential in medicine, but technical and economic obstacles need to be overcome to reach a large number of patients. The development of biomaterials capable of controlled oxygen release and angiogenesis stimulation can provide the necessary oxygen and nutrients for modified tissues. The use of decellularized organs with vascular channels and improved extracellular matrix components represents another interesting strategy. Furthermore, the use of autologous cells eliminates the risk of immune rejection but may be limited in certain applications, leading to the exploration of other cell sources, such as progenitor cells and stem cells. Bioreactors play an essential role in replicating the native

environment and obtaining viable and functional tissues in vitro.

Studies in developmental biology and cellular biology have provided the foundation for reproducing the native environment in tissue engineering. Understanding gene expression and the regulatory pathways of cellular function and tissue development has led to significant progress in this field. The tissue engineering approach covers microscopic and cellular levels, as well as macroscopic levels of tissues and organs, mimicking their native environment.

Bioreactors are used to simulate the natural environment of tissue development and regeneration, allowing the formation of viable and functional tissues in vitro. They involve controlling multiple factors that influence cellular activity and provide a means to enhance tissue formation that is not achievable in vivo or with traditional tissue culture techniques. However, achieving suitable bioreactors requires innovative approaches and considering physiological systems from an engineering perspective.

Designing bioreactors is essential to ensure the proper transport of nutrients and the elimination of metabolic waste products. Mechanical and electrical factors also play an important role in tissue development and function. Advanced bioreactors enable the generation of functional tissue grafts and the study of complex biological responses in vitro, opening new possibilities in tissue engineering and regeneration for clinical applications [1].

Decellularization experimental protocols vary depending on the objectives and types of tissues or organs. The first stage of the protocol involves cleaning and preparing the tissue or organ. Then, a suitable decellularization medium is selected and maintained for a specific period. The decellularization process can be monitored using techniques such as microscopy and spectroscopy.

Efficient decellularization protocols involve physical, chemical, and enzymatic approaches. A typical protocol starts with the cell membrane lysis, followed by the separation of cellular components and the removal of cellular debris. To avoid negative host tissue reactions, residual chemicals need to be removed. There is no universal decellularization protocol, and each protocol has specific advantages and disadvantages.

There are several categories of decellularization methods, including physical, chemical, and combined mechanisms, which depend on the tissue type and research objectives. A rigorous and controlled evaluation of the decellularization process is essential to achieve precise and reliable results. As each organ and tissue has different characteristics, finding a universal and efficient decellularization agent is challenging. Physical methods such as freezing, direct pressure, ultrasound, electric field, and agitation can be used in decellularization.

Mechanical force can be utilized to separate tissue layers with natural dissection planes. Ultrasound and mechanical agitation have been combined with chemical treatments to lyse cells and remove cellular debris. Additionally, electroporation can be used to increase the permeability of the cell membrane and introduce decellularizing chemicals into the organ or tissue. These methods have been employed in various studies and show promise in tissue and organ decellularization.

Another important aspect of tissue engineering is the use of biomaterials. Biomaterials can be tailored for controlled oxygen release and angiogenesis stimulation, thereby providing the necessary oxygen and nutrients for modified tissues. The development of improved biomaterials and the utilization of decellularized organs with vascular channels and enhanced extracellular matrix components represent other interesting strategies.

The use of autologous cells eliminates the risk of immune rejection but may be limited in certain applications, leading to the exploration of other cell sources, such as progenitor cells and stem cells. Bioreactors play an essential role in replicating the native environment and obtaining viable and functional tissues in vitro.

Studies in the field of developmental biology and cellular biology have provided the foundation for reproducing the native environment in tissue engineering. Understanding gene

expression and the regulatory pathways of cellular function and tissue development has led to significant progress in this field. Biomedical engineers utilize this knowledge to investigate, study, and enhance biological responses and generate functional tissues. The tissue engineering approach encompasses microscopic and cellular levels, as well as macroscopic levels of tissues and organs, mimicking the native environment of the tissues.

In conclusion, tissue engineering represents a promising approach to addressing issues related to chronic degenerative pathologies and the need for transplants. By utilizing decellularization and recellularization methods, advanced biomaterials, and bioreactors, functional organs, and tissues can be created in vitro, reducing the dependence on donors and the risk of immune rejection. However, technical and economic obstacles need to be overcome to achieve widespread clinical implementation of these technologies, but ongoing research and development in this field show promising potential for the future of regenerative medicine.

2. VARIOUS LABORATORY DEVICES AND DECELLULARIZATION SYSTEMS

Biomatrices need to respect the specific architecture of the respective organ and be minimally populated with native cells to allow for subsequent repopulation with cells obtained from the patient in the laboratory, who is scheduled to receive the transplant.

The Langendorff method, developed in 1895, is used for perfusing an isolated heart in vitro, providing it with nutrients and oxygen to maintain its functionality [2].

Decellularization is a crucial process in obtaining cardiac biomatrices but must be carefully performed to preserve the structural integrity of the extracellular matrix and vascular network. The devices used in decellularization are based on the Langendorff principle and involve establishing an appropriate perfusion pressure to allow decellularization without damaging the biomatrix architecture. Since decellularization is still poorly understood, the associated technologies and devices are mostly non-standardized and limited to small, laboratory-specific sizes. There is a growing interest in obtaining biomatrices from organs and tissues, and decellularization protocols have been optimized for various situations.

Decellularization systems can be divided into two main categories: perfusion-based decellularization and immersion or agitation-based decellularization. Important control parameters in decellularization include the type and concentration of the decellularization agent, as well as the exposure time, which needs to be optimized to achieve efficient removal of DNA and RNA without affecting the structure of the extracellular matrix.

Perfusion-based decellularization systems represent automated methods used to remove cellular material from organs while preserving the extracellular matrix (ECM) structure. Several scientific studies have presented different approaches in this field. One of the early automated perfusion systems focused on decellularizing whole porcine kidneys [3]. The method involved alternating cycles of SDS (sodium dodecyl sulfate), sodium chloride, and deionized water, with solenoid valves controlling the perfusion rate. This system reduced the exposure time to SDS from 36 hours to just 5 hours and achieved complete decellularization of porcine kidneys.

Another fully automated system was created for decellularizing porcine lungs [4]. This system automated the entire decellularization process of organs with intact vascular systems. Using software programming, valves were controlled to direct the perfusion of the decellularization fluid through the vascular system and airways. This system demonstrated increased efficiency and significantly reduced decellularization time compared to manual methods.

Another system for decellularizing large-sized organs, such as porcine kidneys, was developed using individual chambers with intact vascular systems [5]. This system allowed

simultaneous decellularization of multiple organs and consistently and reproducibly operated.

Overall, the development of diverse laboratory devices and decellularization systems has contributed to advancing the field of tissue engineering by enabling the production of biomatrices with preserved extracellular matrix architecture. These systems have the potential to improve the availability of functional organs and tissues for transplantation, reducing the reliance on donor organs and minimizing the risk of immune rejection. However, further research and standardization are necessary to optimize these systems and ensure their broad applicability in regenerative medicine.

To shorten the time required for porcine aorta decellularization, a hybrid method was used, involving the use of SDS solutions and treatment with supercritical carbon dioxide (scCO2) [6]. This treatment accelerated the washing process and allowed for the efficient removal of cellular material from the aortic tissue.

In the case of mouse and porcine liver, a patented device was used for decellularization under oscillating pressure conditions [7]. By pumping the reagents into the liver and creating a condition of oscillating pressure, cellular material was efficiently removed.

These perfusion-based decellularization systems offer advantages such as complete process automation, reduced decellularization time, and preservation of the extracellular matrix structure. However, each system also has specific disadvantages, such as higher costs for reagents and waste management. Nevertheless, these approaches represent significant progress in the development of decellularization techniques and open new perspectives in the field of tissue regeneration and engineering.

Immersion and agitation-based decellularization systems involve the use of physical agents, such as cutting or chopping the organ of interest and immersing the tissue in reagents, followed by mixing. This brute-force method accelerates the decellularization process by allowing rapid penetration of detergents into the tissue and removal of cellular content. An example of using this approach is the study by Skardal et al. ([8]) who developed a bio-ink for constructing bio-printed tissue. In this study, the tissue was cut into small pieces and placed in deionized water, then agitated for 3 days. Triton X-100 and NH4OH solutions were applied for decellularization, and then the tissue was rinsed with deionized water. The resulting decellularized extracellular matrix was used to create a bio-ink.

Similar studies have been conducted by Pati and Choi, who investigated the use of this approach for porcine cardiac tissue, skeletal muscles, cartilage, and adipose tissue [9, 10]. This approach allows for complete and efficient decellularization, with the potential to be adapted to different types and forms of tissue. However, further research is needed to standardize and expand the use of these decellularization techniques.

To date, there is no literature reviewing commercial decellularization systems and tools. Commercial companies producing extracellular matrix (ECM)-based biomaterials focus on the approaches and processes used in decellularization. Some examples of such companies include Miromatrix, ADInstruments, Langendorff, and Xylyx Bio. Miromatrix, ADInstruments, and Langendorff have adapted perfusion devices for their use in various studies. For instance, Miromatrix produces decellularized ECM from whole organs, which is then used in the recellularization process [11]. Xylyx Bio focuses on producing ECM for pigs and humans, but the methods and tools used in the decellularization of organs and tissues have not been disclosed yet.

There are a few devices developed by companies such as Harvard Apparatus (HA) and Ebers that focus on commercial decellularization systems. One example is the Ebers tubular chamber, initially designed for cell culture but now used for the decellularization of tubular-shaped tissues and organs like the trachea and esophagus [12]. The Harvard HPC-3 apparatus is a hydrostatic perfusion chamber used for the decellularization of organs and tissues [13]. Harvard Apparatus also developed ORCA (Organ Control and Acquisition Bioreactor) in 2013,

which can manage the decellularization and recellularization of organs of different sizes [14].

These commercial devices offer solutions for the decellularization and recellularization of organs and tissues on a small scale, but information and resources about this equipment can be limited and difficult to find.

The lack of universal standards for characterizing decellularized materials is one of the challenges in the decellularization process. The variability of organs and their different DNA and tissue content makes it difficult to establish a common standard for evaluating decellularization. However, the amount of remaining DNA in the decellularized extracellular matrix serves as the main evaluation criterion, and this can be measured through methods such as staining and residual length measurement.

Most systems used in research for decellularization are specialized and involve the use of a small number of dedicated bioreactors. Currently, there are only a few options available on the market. Different research teams use varied methods for evaluating decellularized extracellular matrices. For example, some approaches include histological analyses, macroscopic evaluations, biochemical assessments, and CT scanning, while others utilize staining, immunofluorescence, DNA tests, and imaging.

In addition to DNA content evaluation, there are other recommended methods for characterizing decellularized materials. These include histological analysis of extracellular matrix components, ultrastructure analysis, quantitative analysis of collagen and glycosaminoglycan content, as well as biomechanical property analysis. To develop relevant standards and recommendations for the safe use of decellularized extracellular matrix, it is important to consider these additional evaluation methods.

3. DESIGN AND DEVELOPMENT OF AN EXPERIMENTAL DEVICE USED FOR THE DECELLULARIZATION OF RAT HEARTS

The decellularization process has the potential to provide an acellular platform for organ and tissue regeneration. However, there is a trade-off between maintaining biomechanical integrity and eliminating immunologic cells. Currently, there is no standardized and well-optimized protocol for decellularization, which poses a challenge for both medical professionals and biomedical engineers. The protocols used allow for an advanced degree of decellularization but can also cause damage that affects the quality of the organ intended for transplantation.

The development of an automated system for decellularization is a primary objective, thereby eliminating manual operations and constant supervision. The overall research goals include the development, calibration, and testing of a Langendorff-type experimental device, pressure-controlled, for the decellularization of rat hearts. The decellularization method will be validated using a spectrophotometric method to assess the progression of the decellularization process.

The research also proposes the improvement and optimization of the experimental device by introducing a vibrating fluid column, which will reduce the decellularization time by applying a mechanical effect on the cell membranes. Ultraviolet spectroscopy using the NanoDrop ND-1000 instrument will be employed for real-time determinations of the decellularization process. This instrument allows direct measurements of nucleic acids and proteins without the need for additional reagents.

The proposed objectives will be validated through experiments using rat hearts, and the obtained results will be confirmed and validated using the equipment and methods developed within the research. The research is conducted in collaboration between the Faculty of Electronics, Telecommunications, and Information Technology, the Department of Measurements and Optical Electronics at Politehnica University of Timișoara, and the

OncoGen Research Institute in Timișoara.

4. DESIGN AND DEVELOPMENT OF A MODIFIED LANGENDORFF DEVICE FOR THE DECELLULARIZATION OF RAT HEARTS

Chapter 4 focuses on the design and development of a modified Langendorff device for the decellularization of rat hearts. The aim of the study is to utilize the decellularization process for organ and tissue regeneration, providing an acellular tissue and organ platform for regenerative medicine. It is mentioned that the decellularization process involves the removal of immunologic cells while preserving biomechanical integrity.

For this purpose, a low-cost and user-friendly experimental Langendorff device has been developed [15, 16]. The device consists of two main components: the hydraulic assembly and the electronic control part. The decellularization process utilizes an ionic detergent solution (sodium dodecyl sulfate) that is recirculated during heart perfusion. By monitoring the concentration of DNA and proteins in the solution, the kinetics of decellularization can be determined.

The description of the experimental device highlights its components and functionality. The decellularization chamber contains the heart prepared for decellularization and the decellularization solution, which is perfused into the heart through a peristaltic pump. A pressure transducer monitors the perfusion pressure, and an automation module controls the operation of the peristaltic pump.

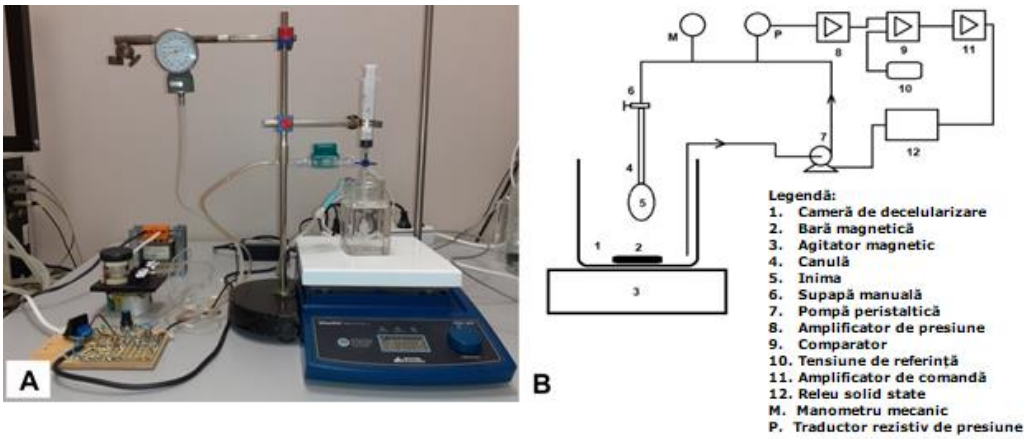

Figure 1. A. Experimental device. B. Block diagram (adapted from D. D. Bonciog et al.) [15].

An experimental protocol was conducted to validate and test the device. The hearts of five rats were decellularized by excising them and infusing them with a 1.5% SDS solution for 600 minutes. The hearts of these rats were removed in accordance with international legislation (the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, publication number 85-23) and the recommendations of the Ethics Committee of the "V Babeș" University of Medicine and Pharmacy Timișoara.

Samples of the solution were collected at regular intervals to determine the concentration of nucleic acids and proteins. These determinations were performed using ultraviolet spectroscopy. The endpoint of decellularization was established when the concentration of these analytes became constant.

The results and discussions regarding the decellularization of the rat heart are presented succinctly and are not included in this summary.

In conclusion, this chapter focuses on the design and implementation of a modified Langendorff device for the decellularization of the rat heart. The developed experimental device

proves to be effective in the decellularization process, providing the basis for obtaining organs with good functional capacity for regenerative medicine.

5. DESIGNING AN AUTOMATED SYSTEM FOR THE DECELLULARIZATION PROCESS OF THE RAT HEART

Chapter 5 focuses on the design of an automated system for the decellularization process of the rat heart.

Within this chapter, the creation of the software application for the acquisition, processing, and analysis of biomedical images is presented, along with the optimization of the software algorithm for automated decellularization.

The software application's main purpose is to enable the acquisition, processing, and analysis of biomedical images obtained during the decellularization process. The ideal decellularization system should produce a completely decellularized three-dimensional biomatrix with maximum efficiency and precision.

The intermediate stage for automating the decellularization process involves the development of a complex experimental Langendorff-type device for obtaining cardiac biomatrices. This pilot study uses a latex balloon to simulate a rat heart, initially filled with a dye. Then, through controlled pumping of distilled water, the dye is gradually replaced with distilled water. The goal is to validate the software application by automatically detecting the moment when the simulated heart becomes completely decolorized and stopping the system.

The overall objectives of this software application include simulating the decellularization process in an accessible and cost-effective manner to avoid sacrificing real hearts. By using a simulated heart model, the software algorithm can be tested and modified in real-time to meet the requirements and be ready for use in a real-world setting. The software application should allow real-time visualization of the performed actions and provide options for selecting an area for image processing and analysis. The acquired and processed images will be stored internally and graphically represented to visualize the dye's evolution in the simulated heart.

The experimental device involves hardware implementation and consists of an imaging acquisition, processing, and analysis system. It utilizes a Raspberry Pi board and a Raspberry camera module for automating the decellularization process. The latex balloon simulating the heart is placed in a decellularization chamber and supplied with distilled water to simulate decellularization. For process control, a clinical-grade injector and a peristaltic pump are used, controlled by an electronic control module.

The block diagram of the automation system is illustrated in the following figure.

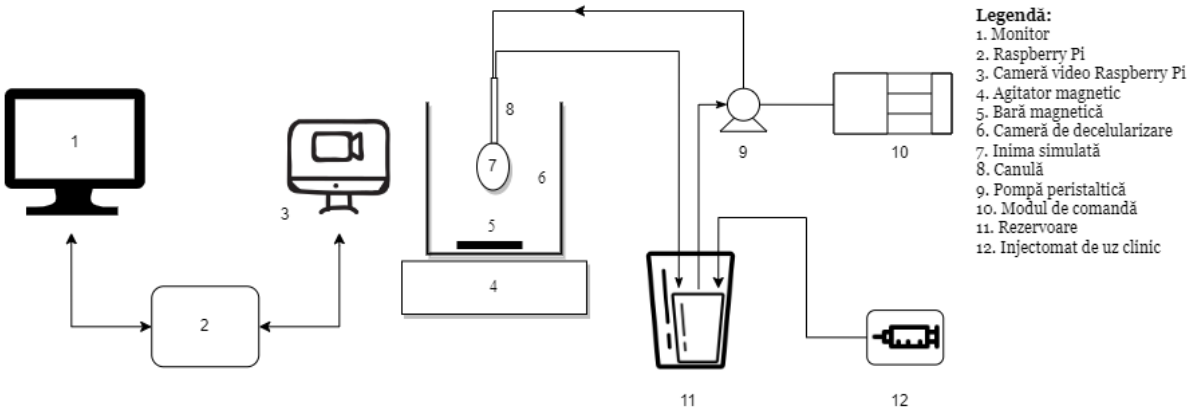

Figure 2. Block diagram of the system (adapted from D. D. Bonciog et al.) [17].

The graphical user interface allows real-time visualization of the heart containing the dye, positioning the heart within the decellularization chamber, and setting the time interval for image acquisition. The user can also select a region of interest from the heart image to be subsequently processed and analyzed.

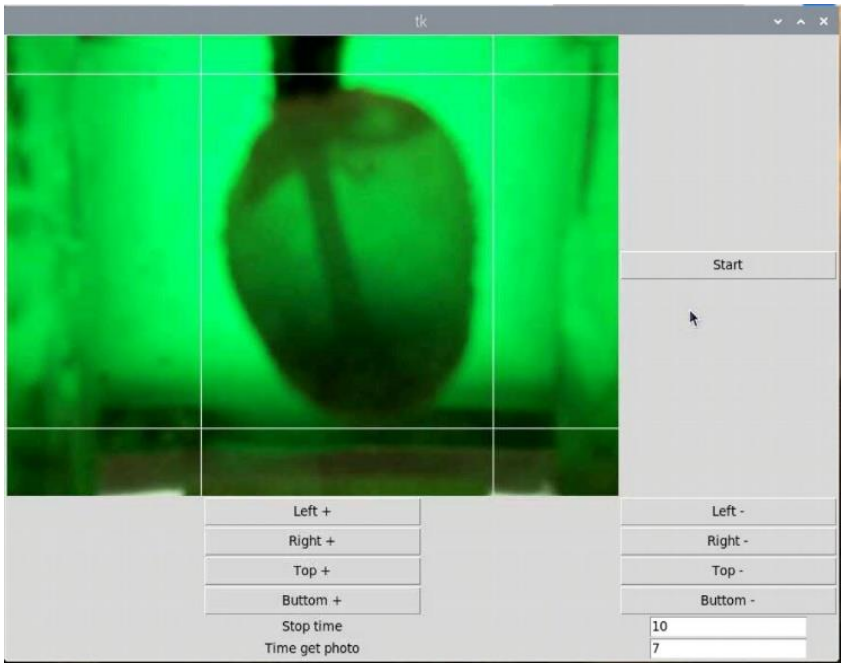

Figure 3. Graphical User Interface (adapted from D. D. Bonciog et al.) [18].

The second part of the software application deals with the acquisition and processing of biomedical images. This involves real-time image capture by the Raspberry Pi camera and storage of the images on the micro SD card. The acquired images are then processed to extract relevant information about the progression of the dye in the heart. This information can be graphically represented to visualize and analyze the decellularization process.

For the software implementation, several Python libraries and modules such as "OpenCV" and "VideoCapture (0)" were used for image capture and processing, "Tkinter" for creating the graphical interface, and "matplotlib" for data visualization.

In conclusion, this section of the project focuses on developing a software application that allows real-time acquisition, processing, and analysis of biomedical images during the decellularization process of the rat heart. Through this application, the user can control and monitor the progression of decellularization and obtain relevant information about the stage of the process. This software application represents an important step in automating and optimizing the decellularization process, opening the way for complex and innovative applications in regenerative medicine.

After the development of the software application is completed, it can be used in the decellularization process of the rat heart. The user can configure application parameters such as the time interval for image acquisition and the region of interest in the heart for analysis. Then, the application will start acquiring real-time images using the Raspberry Pi camera and process them to extract the necessary information.

As the colored heart image evolves over time (decolorization), the application will update the real-time visualization of the heart and display the graph of the dye's progression. This provides the user with the ability to monitor decellularization progress and identify any anomalies or issues that may arise during the process.

Additionally, the application can save the acquired images and associated data on a micro SD card, allowing the user to access and analyze this information later on. This feature is useful for conducting analyses and comparisons during multiple decellularization sessions or for sharing the data with other researchers and specialists in the field of regenerative medicine.

In conclusion, the developed experimental device, together with the software application developed in this project, provides a powerful and versatile tool for monitoring and analyzing the decellularization of the rat heart. The overall experimental device can be observed in the figure below.

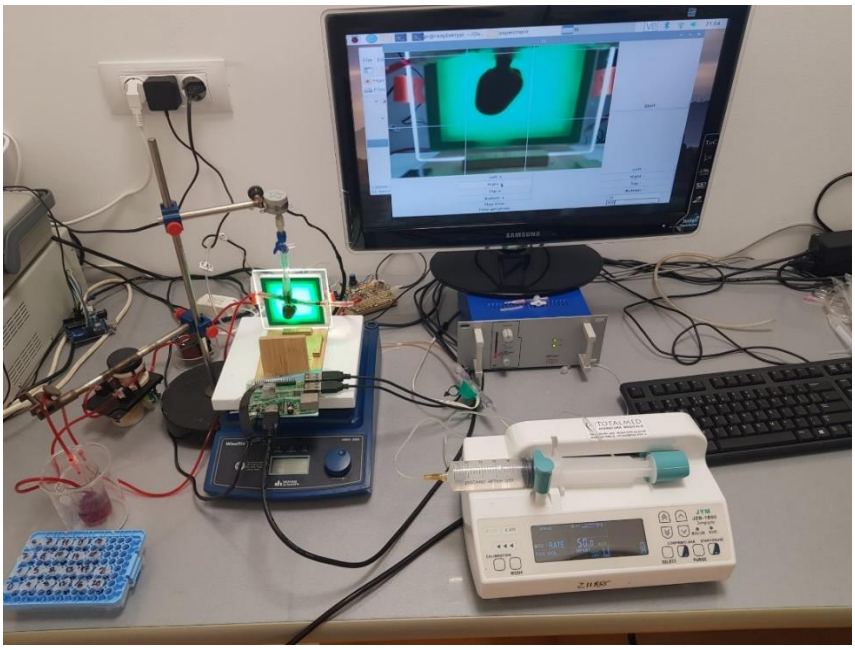

Figure 4. The experimental device used to automate the decellularization process (after D. D. Bonciog et al.) [17].

By automating the image acquisition and processing process, users can obtain real-time information and make better-informed decisions regarding the stage and progress of decellularization. This opens up new perspectives in the field of regenerative medicine and can contribute to the development of innovative treatments for human heart conditions.

Subchapter 5.2 focuses on optimizing the software algorithm for automatic decellularization in the process of decolorizing simulated rat hearts. The aim of the study is to improve the software algorithm to determine the endpoint of decolorization in simulated hearts with greater accuracy. Preliminary studies were conducted where the region of interest was converted to grayscale, and processing was performed to determine the endpoint of decolorization. This approach was chosen to closely approximate the classical spectroscopic method, which involves determining the concentration of the analyzed chemical species at a specific optimal wavelength. The validation of this software application and the calculation of the precision level for these experimental processes will be carried out through comparative studies using ultraviolet spectroscopy for the dye samples taken at the same time intervals.

For the experimental device, the same hardware architecture was maintained, and the software implementation was improved for the automated validation of the decellularization process. The user can outline the region of interest, and the acquired images are processed using the created software algorithm. The images are saved in separate directories and processed to obtain a complete and accurate image of the simulated heart.

The monitoring system utilizes the values obtained from the acquired images and graphically represents them. With each acquired image, the graph is updated, and the progression of the color in the simulated heart is represented. The process is automatically

terminated when the heart becomes transparent, and the final graph is saved.

For the validation of the software application, spectroscopic tests of the dye concentration used in the experimental process were conducted for each experiment. The measured values were compared with the progression of the color in the simulated heart, and no significant differences were observed, confirming that the software application meets the experimental requirements.

The software architecture for optimizing the software algorithm is presented in the figure below.

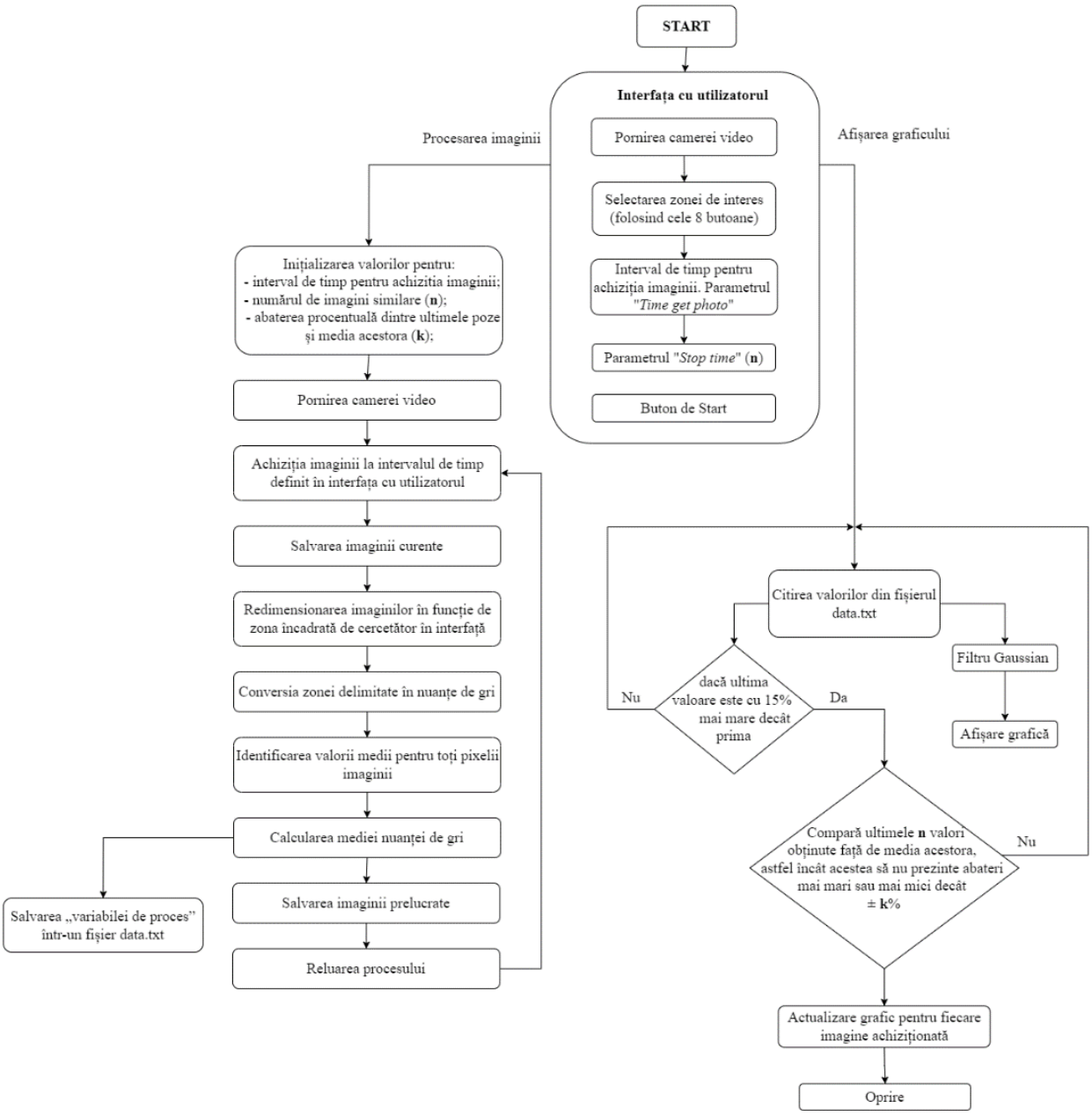

Figure 5. Program operation diagram of the software algorithm (adapted from D. D. Bonciog et al.) [19].

The obtained results demonstrate that the proposed software algorithm can be successfully used in monitoring the decellularization process of real rat hearts. It has been proven that the utilization of the enhanced software algorithm in monitoring the decellularization process is accurate and reliable, as its results match those obtained through spectroscopic methods, which are commonly used to evaluate the success of the

decellularization process. This indicates that the improved software algorithm meets the necessary experimental criteria in a physiological environment and can be considered a valid tool for the decellularization process.

This optimization of the software algorithm for automated decellularization brings benefits in terms of accuracy and process efficiency, providing a promising method for monitoring the decellularization of real hearts in further studies.

6. IMPROVEMENT OF THE EXPERIMENTAL DEVICE CONTROLLED BY PRECISION PRESSURE EQUIPPED WITH A VIBRATING FLUID COLUMN.

In this chapter, a summary of the improvement of the pressure-controlled experimental device equipped with a vibratory fluid column is presented.

Previous studies have highlighted that a significant proportion of transplanted organs and tissues are lost within a relatively short period of time, while others suffer from immune system disorders. To address this problem, techniques for decellularization of organs and tissues have been developed, leading to the emergence of tissue engineering.

The aim of this study was to design and implement a pressure-controlled experimental Langendorff-type device equipped with a vibratory fluid column. By applying oscillating perfusion pressures to the cellular membranes, the decellularization process can be accelerated by generating mechanical pressure waves that can disrupt the cellular membranes and extracellular matrix components. The use of ultrasonic frequencies was investigated for this purpose.

The main objective of this study was to evaluate the kinetics of the decellularization process of the rat heart under the influence of low-frequency oscillating perfusion pressure. The designed experimental device was based on the Langendorff device principle and consisted of a decellularization chamber containing the cell lysis solution, a magnetic stirrer and bar for solution homogenization, an electromagnetic assembly that induced vibrations in the heart by generating a vibratory liquid column, a peristaltic pump for the circulation of the decellularization solution, and a perfusion pressure control system.

The device was designed to allow the superimposition of an oscillating pressure with a frequency of 18Hz onto the perfusion pressure. The use of low-frequency ultrasound for decellularization presented advantages in terms of speed and avoiding the use of harsh chemicals or high pressures that could damage the ECM structure of the organs.

The block diagram of this experimental device is illustrated in the figure below.

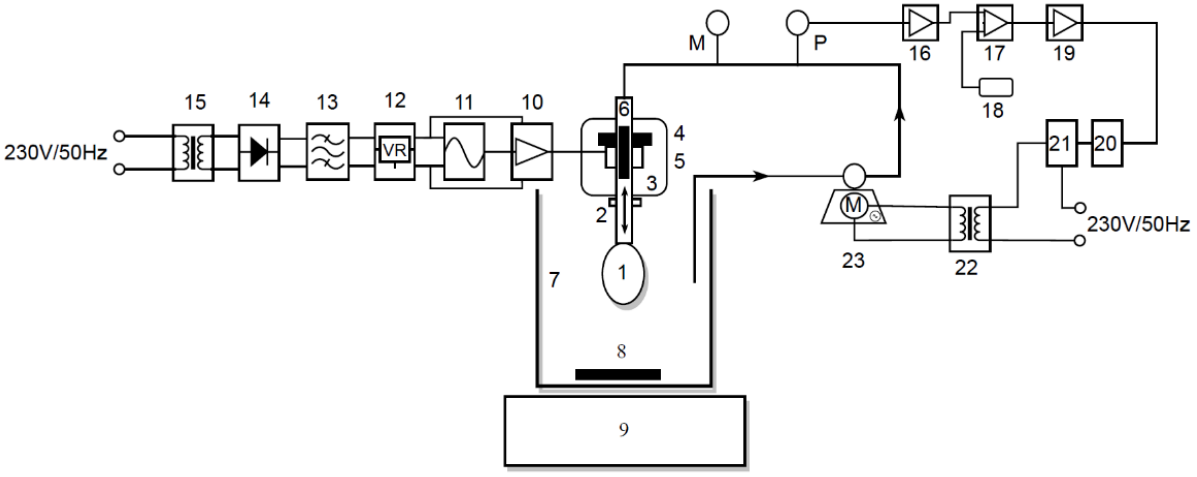

Figure 6. Scheme of the proposed system (adapted from D. D. Bonciog et al.) [17].

For the interpretation of the block diagram shown in Figure 6, a table containing its legend is presented below.

Table 1. Block diagram legend (adapted from D. D. Bonciog et al.) [17].

Nr.	Name	Nr.	Name
1	Heart	14	Bridge rectifier
2	Cannula	15	Center-tapped transformer
3	Electromagnetic assembly	16	Pressure transducer amplifier
4	Permanent magnet	17	Comparator module
5	Coil	18	Reference voltage
6	Ferromagnetic bar	19	Power amplifier 2
7	Decellularization chamber	20	Solid-state relay
8	Magnetic bar	21	Snubber circuit
9	Magnetic stirrer	22	Step-down transformer
10	Power amplifier 1	23	Peristaltic pump
11	Wien oscillator	M~	Peristaltic pump AC motor
12	Voltage regulator	M	Mechanical pressure gauge
13	Capacitor filter	P	Pressure transducer

The components of the system included a decellularization chamber, a magnetic stirrer and bar for solution homogenization, an electromagnetic assembly for generating vibrations, a peristaltic pump for solution circulation, a pressure transducer for monitoring perfusion pressure, and an automation system for controlling the operation of the peristaltic pump and other system components. All these components were integrated into a central control unit that was responsible for coordinating and controlling the system's operations.

The decellularization chamber was designed to house the heart that was to be decellularized. Here, the process of removing cells and cellular components from the tissue was carried out, leaving only the extracellular matrix.

The magnetic stirrer and bar were used to homogenize the decellularization solution and ensure uniform distribution of the active substance in the tissue. The magnetic stirrer generated vibrating movements and oscillations in the solution, while the magnetic bar contributed to the dispersion and uniform mixing of the solution in the tissue.

The electromagnetic assembly was used to generate vibrations that facilitated the penetration of the decellularization solution into the tissue and stimulated the decellularization process. The generated vibrations helped to break down the cellular structure and facilitate the removal of cells from the tissue. This assembly consists of a permanent magnet in the form of a perforated disk, to which a coil is attached. The coil is traversed by a 1 cm diameter glass tube, inside of which there is a cylindrical ferromagnetic bar wrapped in Teflon. As long as the coil is not powered, the bar is held in a fixed position by the magnetic field produced by the permanent magnet. When the coil is powered with an alternating voltage, the bar will start oscillating around the equilibrium position. The maximum amplitude of these oscillations, for a certain current intensity, depends on the constructive characteristic of the apparatus and was experimentally determined to be approximately 9 mm at a frequency of 18 Hz. The measurements were made after the device was filled with the decellularization solution.

The decellularization solution is aspirated from the decellularization chamber by the peristaltic pump. This pump was responsible for circulating the decellularization solution inside the system and through the cardiac tissue placed in the chamber. This ensured a uniform distribution of the solution in the tissue and an efficient decellularization process.

The pressure transducer was used to monitor the perfusion pressure of the decellularization solution. It provided information about the pressure exerted on the tissue and helped in adjusting and controlling the flow of the solution.

The automation system consists of a series of components listed in Table 1. The sizing of the components for the designed circuits is not included in this summary. Their role is to enable the control and coordination of the system's operation automatically. This system is responsible for controlling the operation of the peristaltic pumps, the electromagnetic assembly, and the other components of the system. Additionally, the system allowed for programming and managing the decellularization process in a precise and controlled manner. Overall, these components worked together to efficiently and controlled achieve tissue decellularization, ensuring cell removal and preservation of the extracellular matrix.

The entire experimental setup can be observed in the figure below.

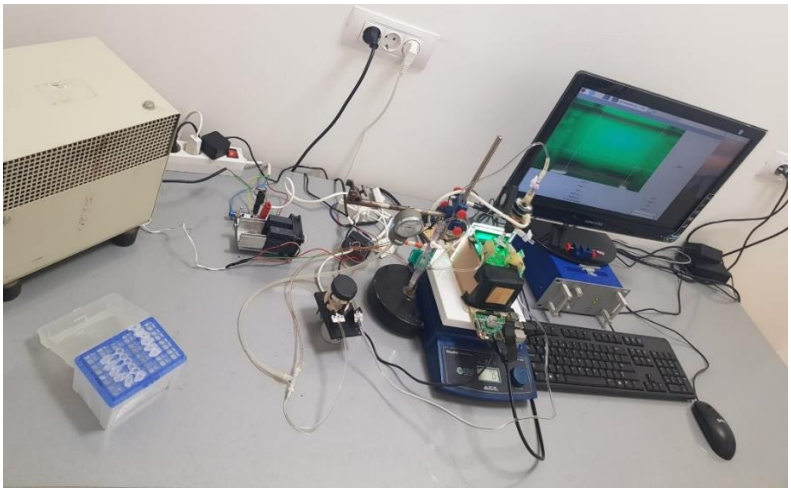

Figure 7. The improved experimental device for the automation of the decellularization process (after D. D. Bonciog et al.) [17].

The monitoring system developed in this study aims to evaluate and compare the effectiveness of the decellularization process of rat hearts using an improved experimental device. Testing and validation of the experimental device were performed on six Sprague Dawley rats, divided into two equal groups. The experimental device used in this study consists of a vibrating column of fluid that applies oscillatory hydrostatic pressure at a frequency of 18Hz above the perfusion pressure. The device was used in Group A, while Group B served as the control group, where the vibrating fluid column was not activated. Images during the decellularization process were acquired at 10-second intervals, and the monitoring system allowed real-time tracking of decellularization progress for both groups.

The obtained results were presented as mean values to enable comparison and analysis. It was found that the use of the vibrating fluid column led to a reduction in the decellularization time by approximately 30% compared to the control group. This percentage was calculated based on the automated results provided by the developed software algorithm. Additionally, these results were validated using a second option. Spectrophotometric studies in ultraviolet were conducted, measuring the concentration of nucleic acids and proteins in the decellularization solution. The results obtained using this method reduced the decellularization time by approximately 29% (a percentage determined and validated using the ultraviolet spectrophotometric method employed).

The study concludes that the improved experimental device, together with the software algorithm, represents an efficient and reliable method for rat heart decellularization. This approach can have significant applications in tissue engineering and regenerative medicine, where decellularized tissues and organs are used as scaffolds for producing functional replacement tissues and organs.

In conclusion, the monitoring system developed in this study has demonstrated its efficiency and utility in the decellularization process of rat hearts. By integrating the

experimental device with the software algorithm, an automated and precise method has been achieved, which can contribute to the advancement of research in the field of tissue engineering and regenerative medicine.

7. OPTIMIZING THE DECELLULARIZATION PROCESS THROUGH A NEURAL NETWORK

Research in the field of medical imaging is advancing rapidly, and deep learning and machine learning algorithms play a crucial role in enhancing medical imaging applications. These approaches utilize artificial intelligence algorithms to evaluate medical images and extract useful data for diagnosis and therapy. Convolutional neural networks (CNNs) are commonly used in medical imaging for image segmentation and classification, while generative adversarial networks (GANs) are employed for generating synthetic images and enhancing the dataset. These techniques can be applied in the field of tissue engineering to improve the quality, segmentation, and reconstruction of the extracellular matrix.

The presented study aims to utilize deep learning for optimizing the decellularization process of rat hearts. By analyzing the kinetics of the decellularization process and utilizing a neural network built based on experimental data, the objective is to achieve an automated and accurate method for evaluating the degree of decellularization.

The architecture of the convolutional neural network is based on the adapted AlexNet model for regression. The images used in the study have a resolution of 1024 x 768 pixels, and the DNA and protein concentration data have been normalized and remapped to ensure data standardization and comparability. Data augmentation techniques have been employed to improve the performance of the model. The model was trained on 46,938 images using data augmentation techniques such as rotation, flipping, and scaling.

The neural network model was tested on a separate set of 2000 images, and the obtained results show a mean squared error of 0.14%, indicating high precision in predicting continuous values for DNA and proteins. The predicted values fall within the range of [0, 100], suggesting that the model is well-calibrated. The use of a neural network in the decellularization process can contribute to improving the cardiac transplant process and estimating the time required for complete decellularization of the heart. Furthermore, this approach can be expanded and applied in other areas of medical imaging for analyzing and monitoring various types of tissues and organs.

The figure below illustrates the structure of the implemented neural network architecture.

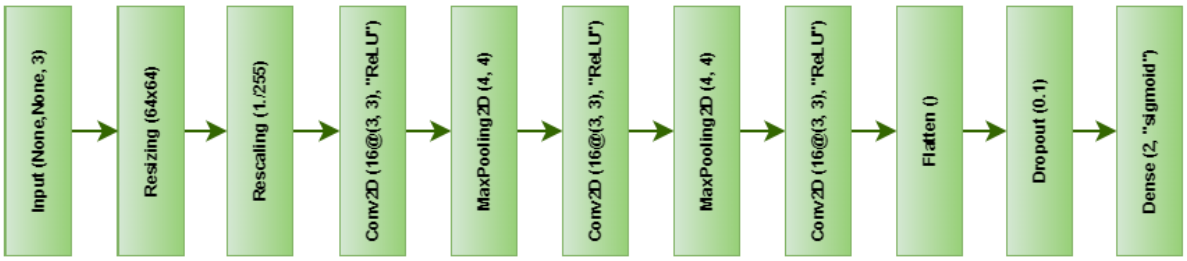

Figure 8. Convolutional neural network architecture (after D. D. Bonciog et al.) [20].

The study presents great potential for optimizing the decellularization process and mass production of bioartificial tissues and organs. Additionally, it can contribute to the development of new medical imaging techniques and real-time image analysis automation for guiding and controlling the decellularization process.

In conclusion, the utilization of a neural network in the decellularization process of rat

hearts has demonstrated its efficiency and usefulness in evaluating the degree of decellularization. This approach holds significant potential for enhancing the decellularization process and the production of bioartificial tissues and organs. Furthermore, it can be extended and applied in other areas of medical imaging, contributing to the development of novel techniques and methods for the analysis and monitoring of decellularized tissues and organs.

8. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The Ph.D. research presented in this paper focuses on the field of tissue engineering, with an emphasis on the development and implementation of an experimental device used in the decellularization process of rat hearts.

In the first chapter, various aspects related to this topic were described, including current challenges, protocols, methods, and agents used so far.

Chapter 2 presented the current state of research in the field, classifying and describing various decellularization devices and systems. Devices and systems used worldwide are largely based on the concept created by Langendorff, adapted to meet the perfusion needs of the decellularized organ.

Although interest and research activity in tissue engineering have increased recently, the lack of universal standards for evaluating the completion of decellularization or the adequacy of this process is a significant problem.

Chapter 3 aimed to outline the general objectives of designing and implementing the experimental device for the decellularization of rat hearts, presenting the method of ultraviolet spectroscopy using the NanoDrop ND-1000 device for sample determinations at different stages.

The design and implementation of the experimental device, a modified Langendorff type, represented one of the key steps in applying the knowledge acquired in the field of biomedical electronics.

The results obtained in this study are of particular importance as they provided me the opportunity to collaborate with physicians from the OncoGen research center in Timișoara and allowed me to conceive and implement an experimental device in the field of biomedical electronics. To obtain biomaterials that can be subsequently repopulated with autologous cardiac muscle cells produced through in vitro differentiation of mesenchymal stem cells, rat hearts can be decellularized using the designed experimental device and the described procedure. The resulting three-dimensional matrix from this process will be a useful tool for future research in tissue engineering.

Furthermore, I improved the device by creating a software application that facilitates the acquisition, processing, and analysis of biomedical images used in automating the rat heart decellularization process through the application of various experimental decellularization protocols.

Since decellularization is a laborious procedure that involves numerous manual operations and monitoring, Chapter 5 was dedicated to designing an automation system. This system aims to acquire, process, and analyze biomedical images to automate the decellularization process of rat hearts using different experimental decellularization protocols.

In subsection 5.1, the overall objectives of validating the proposed scope were addressed. The necessary hardware components were selected, and the existing Langendorff device was modified to enable the implementation of the software application used in simulating rat hearts. The following two subsections describe the experimental device that combines the two essential parts of the system: the physical part, represented by the hardware implementation, and the logical part, represented by the software implementation.

Chapter 5.1.6 presents the results obtained for this software prototype, which allows for the detection of the final moment of decolorization in simulated hearts. To validate this software

system, the ultraviolet spectroscopy method was used to determine the dye concentration in the simulated heart. In Chapter 5.2, the software algorithm was optimized to achieve a higher precision in determining the final moment of decolorization in the simulated hearts and to closely match the classical spectroscopic method.

The monitoring system described in Chapter 5.2.5, along with the spectroscopic test in Chapter 5.2.6 used for determining dye concentrations, provides validation with a very high level of precision for detecting the optimal moment of decolorization in the simulated hearts.

Considering that decellularization is a time-consuming process, chapter 6 presents an improvement made to the created device. By overlaying oscillating pressure at a frequency of 18Hz on the perfusion pressure, the time required for decellularization was reduced by approximately 29-30%. The results generated by the software algorithm and those obtained using the spectroscopic method were identical, demonstrating that the software algorithm meets the experimental requirements in a physiological context.

This innovative approach to automating the process of decellularizing rat hearts represents a significant step towards improving the efficiency and precision of this technique. Through the use of the software application and the optimized algorithm, the decellularization process can be monitored and controlled in a more precise and efficient manner.

Throughout this research, it has been highlighted that the experimental device, improved by the addition of the software application and the automation system, is suitable for decellularizing rat hearts. It accelerates the decellularization process and automatically determines the final moment of decellularization, generating a stable and biomechanically suitable three-dimensional structure.

Furthermore, the developed software application can be expanded for real-time image analysis, which could be beneficial for the mass production of bioartificial tissues and organs, as well as the development of new medical imaging techniques.

The last chapter of the thesis presents the design and validation of a neural network for optimizing and automating the process of decellularizing rat hearts. The obtained results show that the created convolutional neural network model is well-calibrated and exhibits high accuracy in detecting and predicting continuous values for DNA and proteins.

The use of this neural network can improve the heart transplantation process by providing important information about the time required for complete decellularization of the heart. Additionally, future studies can utilize this neural network for real-time automated image analysis, with the potential to revolutionize the production of bioartificial tissues and organs.

In conclusion, the Ph.D. thesis presents extensive research in the field of tissue engineering, focusing on the development and improvement of an experimental device for decellularizing rat hearts. By adding a software application and an automation system, the decellularization process has become more efficient and precise.

The results obtained demonstrate the efficiency and accuracy of the developed software algorithm in this application, enabling the determination of the optimal moment of decellularization in rat hearts and generating a stable and biomechanically suitable three-dimensional structure. This innovative and integrated approach, which combines the improved experimental device, the software application, and the automation system, opens new perspectives in scientific and medical research in the field of tissue engineering.

The results obtained in this Ph.D. thesis make significant contributions to the field of tissue engineering and the decellularization of rat hearts. By designing and implementing the improved experimental device, its efficiency, and utility in accelerating the decellularization process have been demonstrated, opening new possibilities in regenerative medicine.

Furthermore, the development of the software application and automation system has brought significant advantages in monitoring and controlling the decellularization process, ensuring greater precision and increased efficiency in determining the final moment of

decellularization. The use of convolutional neural networks for optimizing and automating the decellularization process represents a remarkable contribution, offering an advanced and promising approach to estimating the time required for complete decellularization of the rat heart. This can improve the heart transplantation process and can be applied in the production of bioartificial tissues and organs, holding significant potential in the field of regenerative medicine.

In conclusion, the Ph.D. thesis presents a comprehensive and rigorous study in the field of tissue engineering, focused on the development and improvement of an experimental device for decellularizing rat hearts. By integrating a software application and automation system, the decellularization process becomes more efficient, precise, and easily monitored.

The contributions made by this research open new directions and perspectives in the field of tissue engineering and regenerative medicine. These can be applied not only in scientific research but also in medical practice, contributing to the improvement of treatments and therapies in the cardiac and tissue regeneration domains.

In the future, furthering these research efforts can lead to the development of advanced techniques and technologies that allow efficient and precise decellularization of human/porcine organs, thus opening new perspectives in regenerative medicine and the treatment of severe cardiovascular conditions.

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