FH University of Applied Sciences TECHNIKUM WIEN

MASTER THESIS

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Engineering at the University of Applied Sciences Technikum Wien - Degree Program Medical Engineering & eHealth

Development of an *ex-vivo* lung perfusion system focusing on the preservation of fresh animal lungs for experiments and storage

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Vienna, May 30, 2022

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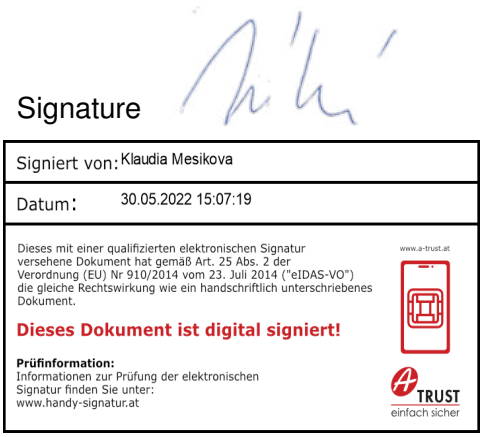

Kurzfassung

Ein mechanisches kombiniertes Lungenmodell ist eine Art von Modell, das in der Simulation der menschlichen Atmung verwendet wird. Die größte Stärke dieses Modells ist die große Ähnlichkeit mit der menschlichen Lunge. Um mit der tierischen Lunge über einen längeren Zeitraum zu arbeiten und so den Prinzipien der 3R zu folgen, wird ein Perfusionssystem in das Verfahren einbezogen. Das Perfusionssystem, das mit einer ausgewählten Perfusatlösung gefüllt ist, ist dafür verantwortlich, den Zeitraum zu verlängern, in dem die Tierlunge für Experimente und die Lagerung in der *ex-vivo*-Umgebung lebensfähig ist.

Die Entwicklung eines gut funktionierenden Perfusionssystems basiert auf mehreren Komponenten, die in den Prozess eingebunden sind. Die Wahl der richtigen Lösung für die Perfusion der inneren Umgebung der Lunge ist einer der wichtigsten Punkte, die es zu berücksichtigen gilt. Die Rollenpumpe ist der Antriebsmotor des Systems. Druck- und Durchflusssensoren sind für die Überwachung der Prozessparameter verantwortlich, die die Funktionalität und die Fähigkeit zur Erhaltung der Tierlunge in der *ex-vivo*-Umgebung beschreiben könnten. Die Validierung des entwickelten Systems durch die Verwendung frischer Tierlungen ist ebenso Teil der Arbeit wie die Überprüfung des Einflusses der Lösung auf die Lagerdauer.

Das Perfusionssystem wurde erfolgreich aufgebaut und getestet. Die bei der Messung gewonnenen Druck- und Flussparameter wurden bei Verwendung der Kochsalzlösung, der Ringerlösung und von Histofix im System verglichen. Die Compliance-Parameter der Lunge wurden sowohl während der Perfusion als auch während der Lagerung überwacht, um das Verhalten der konservierten Lunge im Laufe der Zeit und die Auswirkungen der gewählten Lösung darauf zu bestimmen. Die Compliance nahm zunächst ab und stabilisierte sich dann über den gesamten Lagerungszeitraum auf einem bestimmten Wert. Bei der Perfusion mit Kochsalzlösung und Ringerlösung sank sie um ein Drittel. Bei der Konservierung mit Histofix betrug der Rückgang die Hälfte der ursprünglichen Compliance. Die Konservierungszeit ohne Auftreten von Gewebenekrose betrug 120 Stunden bei der Kochsalzlösung, 240 Stunden bei der Ringerlösung und mindestens 268 Stunden bei der Histofix-Lösung.

Das Perfusionssystem könnte weiterhin in der medizinischen Forschung eingesetzt werden und einen positiven Aspekt im Hinblick auf den geringeren Verbrauch von Tierorganen für Versuchszwecke in verschiedenen Forschungsbereichen darstellen. Für die künftige Forschung wird die Verbesserung des Perfusionssystems und der Zusammensetzung der Lösung zur Gewährleistung einer noch längeren Konservierung begrüßt.

Schlagworte: Perfusionslösung, Perfusionssystem, Tierische Lungen, Bewahrung, Mikrocontroller

Abstract

A mechanical combined lung model is a type of model used in human breathing simulation. The biggest currency of the model is a high similarity with the human lungs. In order to work with the animal lungs for a longer time and so follow the principles of the 3Rs, a perfusion system is involved in the procedure. The perfusion system filled with a chosen perfusate solution is responsible to prolong the period in which the animal lungs are viable for experiments and storage in the *ex-vivo* environment.

The development of the properly functioning perfusion system is based on the several components included in the process. Choosing the right solution for the perfusion of the inner environment of the lungs is one of the most important things that need to be taken into account. The roller pump is considered the drive motor of the system. Pressure and flow sensors are responsible for monitoring the process parameters that could describe the functionality and the ability to preserve the animal lungs in the *ex-vivo* environment. The validation of the developed system by using the fresh animal lungs is a part of the thesis as well as the checking procedure of the solution's influence with the time of the storage.

The perfusion system was successfully created and tested. The pressure and flow parameters gained during the measurement were compared while using the saline solution, the Ringer's solution, and Histofix in the system. The compliance parameter of the lungs were been monitored during the perfusion as well as during the storage with the aim to determine the behaviour of the preserved lungs with the time and the impact of the chosen solution on it. Compliance initially decreased and then stabilized at a certain value throughout the storage period. For the perfusion with the saline and Ringer's solution, it dropped by one-third. For Histofix preservation, the drop was by half of the initial compliance. The preservation time without the presence of the tissue necrosis was 120 hours using the Saline solution, 240 hours using the Ringer's solution, and at least 268 hours using Histofix.

The perfusion system could further be used in medical research and make a positive aspect in terms of less consumption of the animal organs for experimental purposes in various fields of the research. For future research, the improvement of the perfusion system and solution composition to ensure even longer preservation is welcomed.

Keywords: Perfusate solution, Perfusion system, Animal lungs, Preservation, Microcontroller

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Contents

1 Introduction

Respiration is characterized as the automatic process necessary for living. Three systems of the human body are involved in the respiration process, the human respiratory tract, the cardiovascular system, and the CNS. An important role in the transfer of respiratory gases through the human body is played by the motile component of blood.

The human respiratory system is mainly responsible for supplying the human body with the necessary oxygen and for removing the waste product together with the carbon dioxide from the human body. The system also participates in many other processes in the human body such as maintenance of the acid-base balance, thermoregulation, creation of speech and sound expression, and many more.

The respiratory system is often affected by various diseases of different types. This is the reason why the research aims for the adequate simulation of the breathing pattern in various conditions. Many types of the lungs model are used for the simulation process in this topic, but the one which is with his similarly irreplaceable in this area is a combined mechanical model.

A combined mechanical model of the lungs is the model that is represented by the animal lungs. The animal lungs provide almost all properties that can be found in the real human lungs and so the simulation process is very similar to the real human breathing that takes place in the human body. The animal models are usually used immediately after they are obtained and are measured once. The aim of the perfusion system involved in the simulation process is to prepare the lungs before simulation and preserve the tissue of the lungs. After the lungs' tissue is preserved, the lungs have the possibility to be stored for some time after the measurement process and can be remeasured. This process could have a positive impact on the less consumption of animal lungs and also reduction the preparation requirements of the lungs for repetitious measurements.

For these purposes, the perfusion system should be developed and validated by using the fresh animal lungs in this master's thesis.

1.1 Anatomy of Respiratory Tract

The human respiratory tract, figure 1, comprises several parts which are involved in respiration. Each part is responsible for performing a specific function that is necessary for the gas moving process in both directions, into and out of the human body.

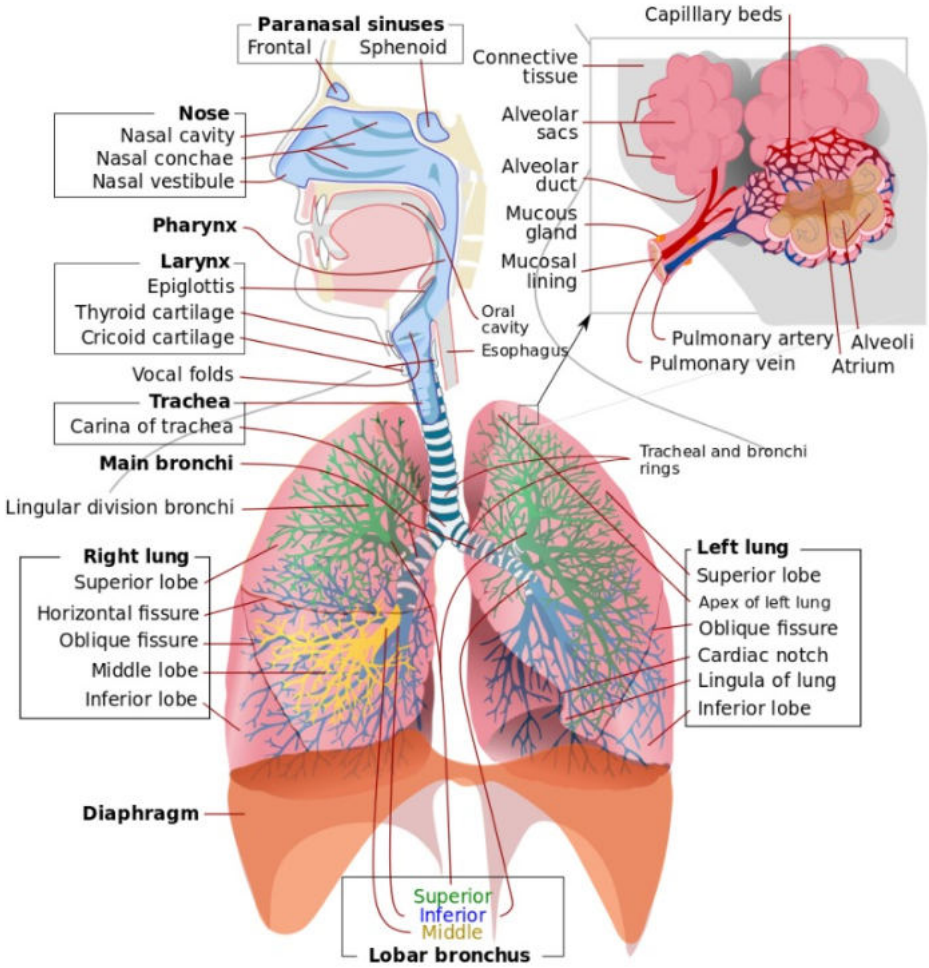


Figure 1: Human Respiratory Tract [1]

Anatomically, the human respiratory tract can be separated into two big parts.

- **The upper respiratory tract** includes the nose, pharynx, epiglottis and larynx. This passage filter, warms and humidifies incoming air.
- **The lower respiratory tract** consists of trachea, bronchi (primary, secondary, tertiary), bronchioles, alveolar duct and alveoli [30]. It is the place where the gas exchange actually takes place.

Functionally, the respiratory tract is separated into two parts, conducting zone and the respiratory zone.

- **Conducting zone** provides a path for the incoming and outgoing air. It consists of structures from the nose to bronchioles. Several structures within conducting zone perform other functions as well. The epithelium of the nasal cavity is essential to sensing odors. The bronchial epithelium that lines the lungs can metabolize some carcinogens that are transmitted by air.
- **The respiratory zone** is a part that is involved in the gas exchange. It begins at the place where the terminal bronchioles join a respiratory bronchiole. The zone then leads to an alveolar duct and a cluster of alveoli at the end [30].

1.1.1 Upper Respiratory Tract

The nose

The nose is the first contact environment of the respiration system and transports inhaled air into the nasal cavity that is subdivided by the nasal septum into the right and left segments. Air is inhaled through the nostrils and is warmed on the way to the nasal cavity. Epithelial cilia, which is located inside of the nasal cavity, trap unwanted particles with help of mucus produced by seromucous. The path of the air then continues through the oral cavity to the pharynx.

The pharynx

The pharynx is the passage connecting the nasal and oral cavity to the larynx and esophagus. It is separated into three floors, the upper, the middle, and the lower. The upper floor is called the nasopharynx and it is the place of secretions from the nose to the oral cavity. It is also connected to the middle ear to equalize the pressure difference on both sides. The middle one is called the oropharynx and it is directly connected to the oral cavity. The last part of the pharynx is the bottom part and it is called the hypopharynx. The hypopharynx is the pathway of the pharynx through which the air, as well as food, passes. That means the pharynx is a part of the respiratory tract as well as of the digestive tract. The selection of the tract which is in use at the time is the responsibility of epiglottis.

The epiglottis

The epiglottis is a cartilaginous flap that is located in the larynx and is attached to the thyroid cartilage. The function of the epiglottis is to close the laryngeal inlet during swallowing. This serves to prevent the penetration of water and food into the lungs.

The larynx

The larynx is considered as the transitional tube that helps the air to get into the lungs. It can be understood as the protection of the trachea. It is also known as the voice box.

1.1.2 Lower Respiratory Tract

The trachea

The trachea is the airway that leads from the larynx to the bronchi. The trachea is also known as the windpipe. It is a tube that is around 12 cm long with a diameter of about 1.5 cm. The inner environment of the trachea is composed of tough cartilages in the C-shape. The interior of trachea is lined by ciliated columnar epithelium. The trachea leads from the larynx to the breastbone. It is then divided into two bronchi. One bronchus for the right lung and one for the left lung.

The bronchi

The bronchi are a continuation of the trachea in the air pathway to the lungs. The trachea is divided into two stem bronchi. The structure of the bronchi is very similar to that of the trachea has. They serve to direct air into the alveoli, which are located inside the lungs [36], [30], [35].

The lungs

The lungs, figure 2, are the foundational organs of the lower respiratory tract and is located in the thorax where they are protected by the thoracic cage that is formed by the 12 thoracic vertebrae, 12 pairs of ribs and associated costal cartilages and the sternum. The lungs are a place where the exchange of gas between the atmospheric air and blood is made.

The lungs is a paired cone shaped organ. They are formed in two parts, the right and the left half. Each lung is divided by deep fissures into lobes. The right one is composed of three lobes, the lower, the middle and the upper. The left one consist of two lobes, the lower and the upper. The bronchi that enter the lungs are simplified to the smallest branches of the respiratory tree that are called bronchioles. Bronchioles pass through the alveolar duct and form clusters of sacs that are labeled as alveoli.

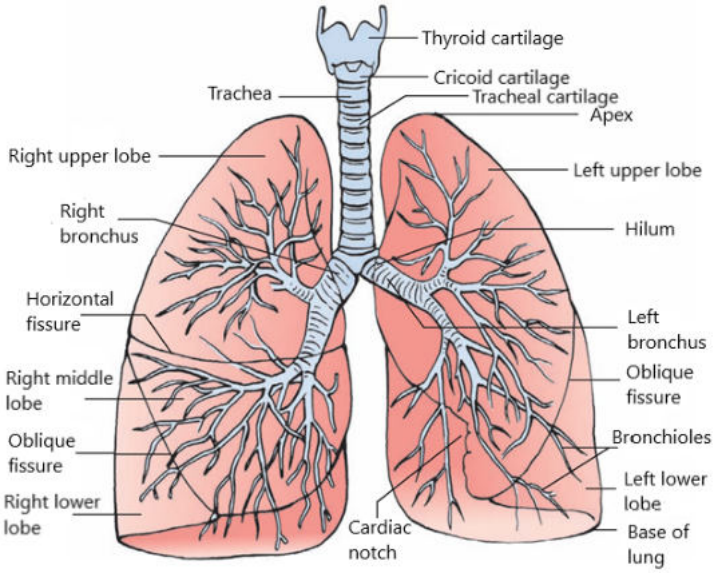


Figure 2: Human Lungs [41]

Alveoli, figure 3, are pulmonary sacs in which the molecules of O_2 diffuse from inhaled atmospheric air through the alveolar wall into the bloodstream. Alveoli are surrounded by tiny blood vessels called capillaries. The blood vessels are then responsible for the distribution of oxygen to the body. The molecules of CO_2 are moved in the opposite direction from the blood to the alveoli and then they can be exhaled. An adult human being has on average 600 million of alveoli [29].

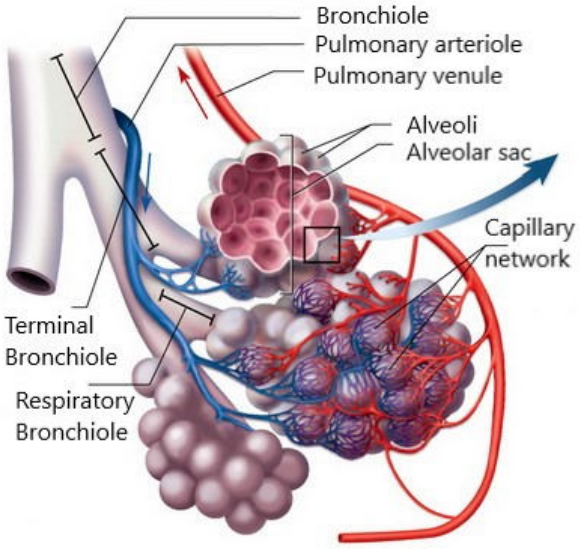


Figure 3: Alveoli [44]

Alveoli are made up of two types of cells. Each type is responsible for different function.

- **Type I pneumocytes** - these cells are involved in the process of gas exchange at the level of alveoli and capillaries. They are extremely thin, around 15 µm, which has a positive impact on minimizing the distance for gas diffusion. The connection between these cells is made by occluding junction. This kind of junction prevents the leakage of tissue fluid into the alveolar space.

- **Type II pneumocytes** - these cells are responsible for the secretion of surfactants into the alveolar space. Secreted surfactant decreases surface tension in alveoli. Their shape is cuboidal and many granules are recognizable inside of them. If it is required, type II pneumocytes can be differentiated into type I pneumocytes [3].

The layer that protects the lungs is called pleura. Pleura is a double serous membrane that envelops the lungs. Between two layers of pleural membrane, there is fluid. The area in which the lungs are embedded in the thoracic cavity is known as the pleural cavity.

An integral part of the respiratory system is a diaphragm, that separates two cavities, thoracic and abdominal. The diaphragm, as well as intercostal muscles, is a part of the respiratory muscular system. They both help the thoracic cavity to expand during inhalation and shrink in the case of exhalation [21].

1.2 Physiology of Respiratory System

The respiration is the basic process for the functioning of a living organism. The respiratory system provides us with the fundamental ability to breathe. The body cells need a continuous supply of oxygen. Oxygen is responsible for the metabolic processes that are responsible for maintaining the function of cells inside the human body. Metabolic processes play a principal role in the production of adenosine triphosphate (ATP), which is necessary to maintain life.

In physiology, respiration is perceived as the movement of the oxygen (O_2) from the outside environment to the cells within tissues and the movement of the carbon dioxide (CO_2) in the opposite direction in order to ensure their removal from the human body.

During respiration four main processes occur:

- **Pulmonary ventilation** or breathing is an automatic process that can be influenced by the will. Ventilation is divided into two main parts that follow each other in the direct way. Inspiration is the process when the air flows in the direction of the human body. Expiration is the process when the air moves out of the human body.

- **External respiration** is an exchange of gases between an inhaled air and the helping vehicle, the blood. The movement of gases is in both directions, into and out of the human body.

- **Internal respiration** is a process of distribution of inhaled gases between the blood and internal tissue cells. The gained O_2 is used in this process.

- **Cell respiration** is the energy-releasing process by which molecules of ATP are produced. It occurs within cells and consumes O_2 [43]. The respiratory gas flow rate through wall of blood capillaries depends on the partial pressure of O_2 and CO_2.

From a physiological point of view, pulmonary ventilation is also known as breathing and comprises an expiration and inspiration. The respiratory rate of the average human is from 14 to 16 breaths per minute. The total lungs capacity is from 4 to 7 liters depending on the physiology of the human being. The breathing process is mainly influenced by pressure differences. The direction of the gas flow gradient is determined by the pressure difference between the atmospheric environment pressure and pressure in lungs [12].

During inspiration, the air is drawn into the lungs based on the active contraction of the diaphragm and external intercostal muscles of the ribcage. These muscles are in cooperation to achieve the expansion of the lungs. The inspiration procedure is performed while the pressure remaining inside of the lungs is lower than the pressure of atmospheric air. The gradient of the gas flow is set in the direction of the lungs. Based on the pressure differences air is moved to the lungs and the expansion of the chest wall is visible. The interior of the abdominal cavity is pushed downward and outward. The expansion of the chest walls and abdominal cavity should be coordinated.

The expiration is a normally passive process. It is known as the process while the higher pressure inside of the lungs is produced. In this case, the gradient of the gas flow in the direction of the outside environment is created. The intercostal muscles, as well as the diaphragm, are relaxed. The chest wall and abdominal cavity are returned to the original state. The gas is moved out of the lungs [5].

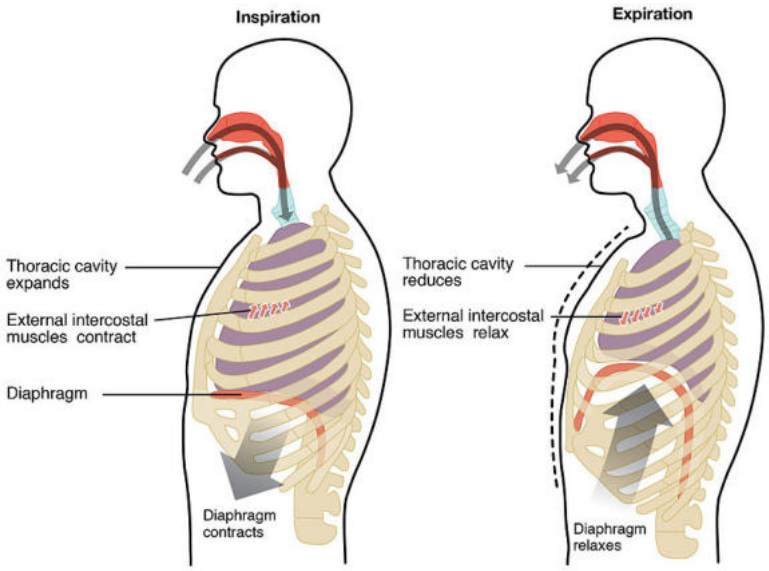


Figure 4: Diagram showing the process of inspiration and expiration [28]

1.2.1 Physics of Gas Diffusion

The movement of the gases in the airways is not organized in one specific direction. The type of movement which is applied in the respiratory tract is diffusion. Diffusion is defined as the passive movement of molecules that is random but at the end resulting in one direction. The resulted direction is from the environment with a higher concentration to the one with a lower concentration of ions.

The rate that is reliable to the diffusion process is affected by several parameters:

- Length of diffusion pathway - the greater the pathway is, the faster the rate is.
- Concentration gradient - the greater the gradient is, the faster the rate is.
- Surface area during diffusion - the greater the surface is, the faster rate is.

Diffusion of Hydrogen

The partial pressure of the O_2 in alveoli is lower in comparison with the partial pressure of the atmospheric environment. It is achieved by the continuous transit of the O_2 ions through the alveolar membrane. This movement is controlled as well by the ions of CO_2 to leave the human body. Since the partial pressure is still higher in alveoli than in capillaries, the diffusion in the direction of capillaries is performed. The ions of O_2 are transported to the capillaire's blood. They are mixed with hemoglobin. The oxygen is then transported by the bloodstream to the target tissue and cells.

Diffusion of Carbon Dioxide

The partial pressure of CO_2 in the capillaries is higher than in the alveolar environment. Based on the gradient ratio, the diffusion is taken in the direction from capillaries into the alveoli. After the transfer to the alveoli, the CO_2 can be exhaled. The gradient is still in the direction out of the human body because the partial pressure in alveoli is higher than the partial pressure in the external environment.

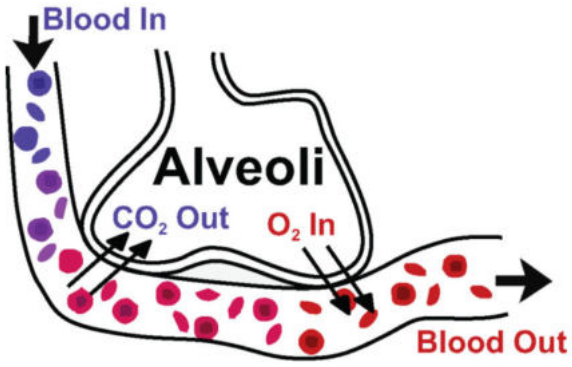


Figure 5: Diffusion process between alveoli and blood [26]

1.2.2 Pulmonary Pressures

The inspiration and expiration processes are dependent on differences between the pressure remaining in individual parts of the respiratory tract. The occurrence of different pressures in the respiratory tract is the force for the gas movement throughout the airways. The pressures that are involved in the ventilation process are the atmospheric pressure, intra-alveolar pressure, transpulmonary pressure and intrapleural pressure.

Atmospheric Pressure

The atmospheric pressure is the pressure of the air surrounding the body. It is the pressure defined as 101.325 Pa, which is equivalent to 760 mmHg or 14.696 psi. In the breathing process, it is taken as a reference for all other pressures within airways. For this reason, the negative pressures are pressures lower than the atmospheric pressure and the positive pressures are pressures that are greater than the atmospheric pressure is. The pressure ratings are therefore pressure differences in the relation to this reference.

Intra-Alveolar Pressure

The intrapulmonary pressure is the pressure of the air within alveoli. The changes in this pressure are recorder in the different phases of the breathing cycle. This pressure is responsible to keep the lung from collapsing that could be caused by the natural elasticity of the lungs tissue.

Transpulmonary Pressure

The transpulmonar pressure is the pressure difference between the intra-alevolar pressure and intrapleural pressure. The role is to separate the pressure delivered to the lungs from the one from abdominal cavity and wall chest. The higher transpulmonary pressure is typical for the bigger lung [9].

Intrapleural Pressure

The intrapleural pressure is the pressure that is situated in the pleural cavity. This pressure is also influenced by the phase of the breathing process. Due to the characteristics of the lungs, the pressure is lower than the intra-alveolar pressure is. The value of the pressure is around -4 mmHg and is almost stable.

The reason why the intrapleural pressure is negative is competing forces in the thorax. One from forces is in relation to the elasticity of the lung tissue and one in the relation to the fluid situated in the alveoli that creates surface tension. The first force pulls the lungs inward and the second force pulls the lungs outward.

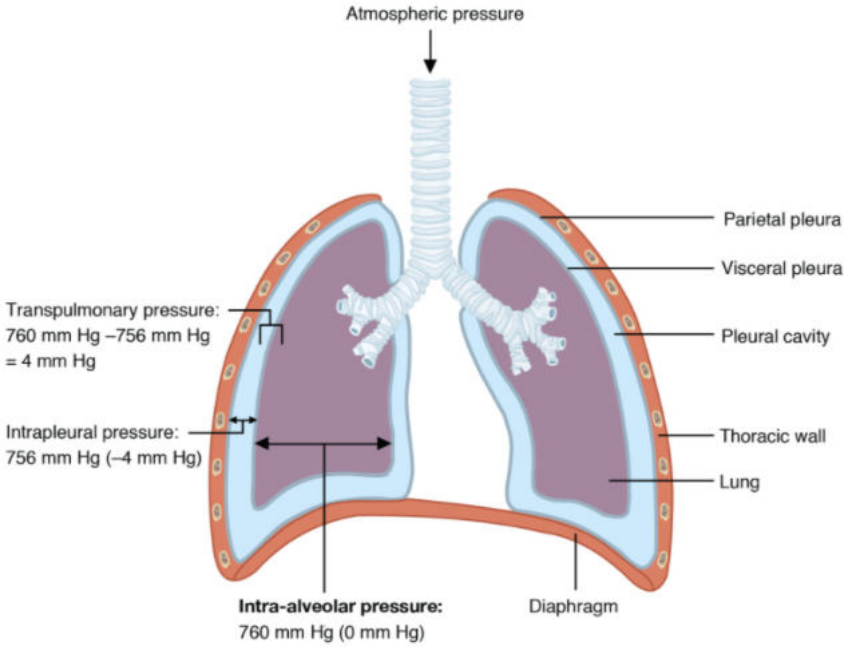


Figure 6: Different types of pressures in the airways [40]

1.2.3 Respiratory Physics

Physic is a part of the respiratory tract and events happening during the respiration process.

Pulmonary Resistances

Pulmonary resistances are characterized as the degree of resistance to the flow of air on its way through the respiratory tract. Basically, it is the force that is associated with slowing the flow of air in airways. These events are present during the expiration and inspiration processes. Resistance in the respiratory tract is divided into two main groups. It is the resistance of the airways and the resistance of the lungs. The degree of resistance is dependent on many things. The determinants that are related to the degree of resistance are the diameter of the airways, whether the flow of gases is laminar or turbulent, and the surfactant in the airways.

Certain equations and relations could be used to describe the determinants that express the degree of resistance:

- Ohm's law - the law that is determined as the relationship between airflow, pressure and resistance.

$$airflow = \frac{pressure}{resistance} \tag{1}$$

- Poiseuille's law - the law that is determined as a relationship between the resistance in airways (R) and its diameter (r).

$$R = \frac{8\eta l}{\pi r^4} \tag{2}$$

In the context of the respiratory system, a couple of conclusions could be determined from these laws. In order to follow Ohm's law, equation 1, with the increase of resistance, the pressure gradient must also increase to maintain the same flow rate into the alveoli. According to Poiseuille's law, equation 2, the airway resistance is inversely proportional to the radius with a power of 4. Based on this fact, the small changes in diameter have a huge impact on the resulting resistance.

Lungs are eqquiped by the elastic tissue. The humidity of the lung tissue is one from the most important need of the tissue to avoid compliance character. The tissue of the airway is then lined by fluid and the compliance of lungs is limited by the surface tension. In water, the surface tension is generated by hydrogen bonds that is responsible for pulling the molecules together. The higher the surface tension is, the harder stretching of the lungs is present. This effect is overcome by the surfactant that is present in the respiratory tract. This surfactant is produced by alveolar cells and has the hydrophilic and hydrophobic parts to perfectly rise to the surface of the fluid in airways, the gas-fluid interface. The basic role of the surfactant is to disrupt the hydrogen bonds on the surface and to make an allowance for the lungs to expand.

Pulmonary Compliance

Compliance is know as the most important property of the lungs. The compliance of the respiratory system is described as the expandability of the lungs and chest wall. Lungs compliance is defined as the change in volume (ΔV) in relation to the change of the transpulmonary pressure (ΔP). There are two types of compliance: dynamic and static. Dynamic compliance is defined as the compliance measured during the breathing process. It is a combination in which lung compliance and airway resistance are involved. This type of compliance can be also described as the change in lung volume per unit change in pressure while the flow is present. Static compliance is described as pulmonary compliance when there is no presence of airflow. This period is called inspiratory pause. Defined as the change in lung volume per unit change in pressure with the absence of flow. The physiological value of compliance for an adult is in range 0.1-0.4 l/cmH_2O. The relation between changes in volume and pressure can be seen below, equation 3:

$$C = \frac{\Delta V}{\Delta P} \tag{3}$$

The lung compliance changes are usually related to pulmonary illness. A decreased lung compliance might be associated with restrictive lungs disease. Restrictive lung disease can be related to mechanical issues with peripheral hypoventilation, insufficient muscular effort, or structural dysfunction. Common causes of decreased lung compliance are pneumonia, pulmonary fibrosis, and pulmonary edema. An increased lung compliance can be present while the degeneration of the tissue in the lungs is appeared. The degenerative lung disease is responsible for the harder expansion of the lungs because of less elastic recoil. The examples of this kind of dissease are emphysema, asthma, and bronchitis [8], [23].

1.2.4 Lung Volumes and Capacities

The mechanical properties of the respiratory apparatus and their conditions are evaluated by several tests. Some volumes and capacities of the lungs are the first indicators of whether the lung function is sufficient or if there is some disorder presence.

The volumes, figure 7, which are evaluated are inspiratory reserve volume (IRV), tidal volume (TV), expiratory reserve volume (ERV), and residual volume (RV). Capacities that need to be monitored are inspiratory capacity (IC), functional residual capacity (FRC), total lung capacity (TLC), and vital capacity (VC). TV is known as the volume of a normal breath. IRV is the volume that could be inhaled after a normal breath. ERV is described as the volume that could be exhaled after a normal breath. RV is known as the volume that remains in the lungs. VC is the measurable lung capacity. IC is the capacity of the lung to inhale the air. FRC is the capacity of the lungs in which expiratory reserve volume and residual volume are included. TLC is represented as the total lung capacity that includes vital capacity as well as residual capacity.

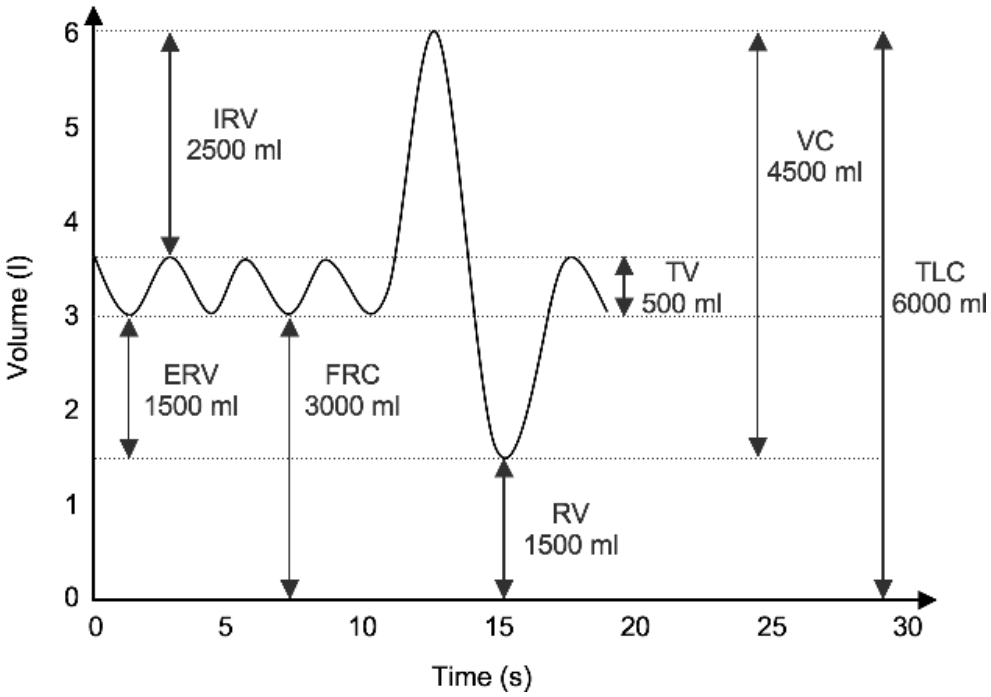


Figure 7: Lung Capacities and Volumes [24]

Lung capacities and volumes can be measured by several techniques. Spirometry is one of the most used techniques. It is a measurement of the rate of airflow and estimation of lung size. The patient is breathing with regular and maximal effort through the tube and the result is monitored by computer. The body plethysmography is a method for the measurement of FRC using Boyle's law. The patient is seated in the airtight box where the measurement of changes in pressure and volume can be measured accurately. Nitrogen washout is the technique that is based on washing out the nitrogen from the lungs of the patient. The patient is breathing O_2

without any admixture using dilution properties. The last technique is helium dilution which is based on the equilibration of gas while the volume of helium in gas is known.

1.2.5 Breathing Control

Breathing process is controlled to maintain the constant value of the necessary gases that are involved in this procedure. Several types of respiratory controls are known.

Central Control

The respiratory system and its working process are controlled by the central nervous system (CNS). The respiratory centers, involving afferent and efferent nerves, are responsible to collect information about the level of O_2 and CO_2 in the blood and to determine signals that are sent to the respiratory muscles. The centers are located in the medulla oblongata and pons. The stimulation of the respiratory muscles is necessary to create respiratory movement and so to provide alveolar ventilation [37].

The medulla oblongata is the primary respiratory control center. Its principal role is to send signals to the breathing muscles about the breathing process. The medulla also controls non-respiratory reflexes such as swallowing, coughing, sneezing, and vomiting. Two regions in the medulla oblongata that are responsible for controlling respiratory reflexes are the ventral respiratory group and the dorsal respiratory group. The ventral group stimulates expiratory movement and the dorsal group stimulates inspiratory movement.

The pons is the other control center and is responsible to control the speed and rate of involuntary respiration. There are two regions in the pons that perform the task, the apneustic center, and pneumotaxic center. The apneustic center is responsible for controlling the long and deep breaths. It increases the TV. The pneumotaxic center is responsible for the inhibition of inspiration to control the respiratory rate. It decreases the TV.

Chemical Control

The chemical control of the breathing process is operated through the chemoreceptors. These receptors are described as the receptors that give the response when chemical constituents inside of the blood are changed. Chemoreceptors are basically divided into two groups, to central chemoreceptors and peripheral chemoreceptors [37].

Central chemoreceptors are very sensitive to increased concentrations of hydrogen ions in the blood. Whenever the concentration of O_2 is increased, chemoreceptors are responsible for stimulation of the respiratory center, and an increase of the respiratory rate and forced breathing is the result of this process.

On the other side, peripheral chemoreceptors are more sensitive to the reduction of hydrogen ions. Whenever, the concentration of O_2 is decreased, peripheral receptors are activated and they are used for the stimulation of responsible neurons in the CNS.

1.3 Pulmonary Circulatory Pathway

Every cell, tissue, organ, and system is impacted by the circulatory system, figure 8. It is the system that transports nutrients, respiratory gases and metabolic products in the human body. The cardiovascular system is defined as the closed system. The system is composed of the blood vessels and the heart. Functionally, the circulatory system is divided into two circuits, pulmonary circuit and systematic circuit.

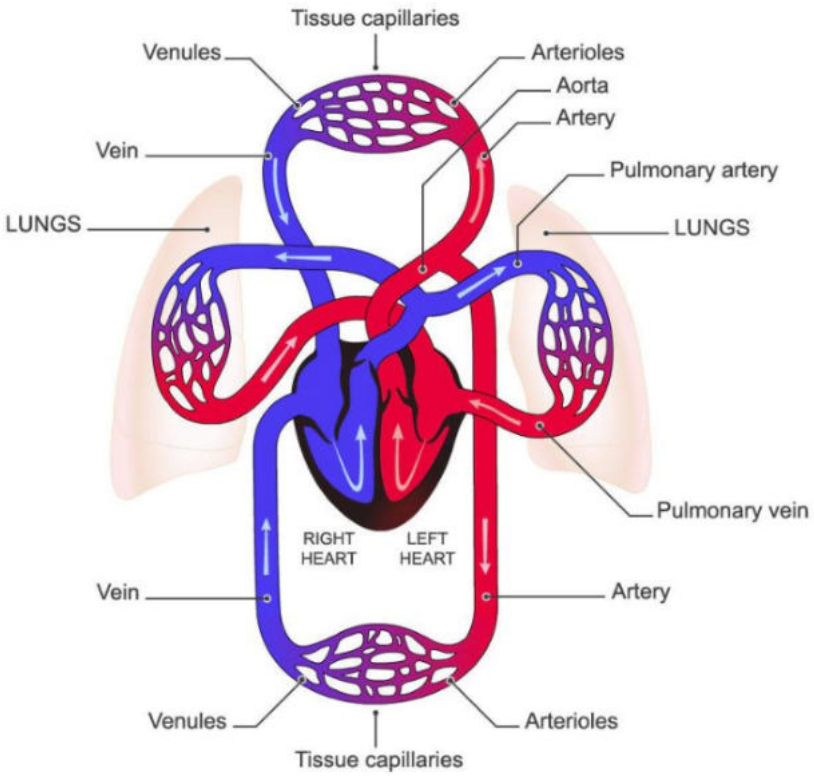


Figure 8: Cardiovascular System [18]

The pulmonary circuit is involved in the process of gas oxygenation. The oxygen-poor blood is pumped by the right ventricle into the pulmonary artery. The pulmonary artery branches off into arteries, smaller arteries, and capillaries at the end. Capillaries form a network. The network is wrapped around the pulmonary vesicles. This junction is the place where CO_2 is released from the blood to the gas inside of vesicles to leave the human body and also the place where O_2 molecules enter into the bloodstream. The oxygen-rich blood is then through the pulmonary veins, the left atrium to the left ventricle.

The systemic circuit is defined as the power supply pathway for all tissues and cells over the whole human body. The O_2 and other nutrients are carried to the target cells and CO_2 and waste products are picked up from the cells. The molecules of O_2 and nutrients are transported to the body cells and tissues. The way of oxygen-rich blood starts in the left ventricle which is

the pump for the circuit. The blood is pumped into the aorta. The blood passes from the aorta to the smaller arteries and capillaries that form a network. The oxygen passes from the blood to cells and the CO_2 together with the waste product are transported in opposite direction. At that moment, the blood is oxygen-poor and travels through veins to the right atrium and ends in the right ventricle [13].

The heart is the driving force of the system. It is located in the middle of the chest and slightly towards the sternum. The heart is divided into two main parts separated by septum, right and left heart. Each part of the heart has two chambers. The right heart is separated by valves to the right atria and right ventricle. The left heart is separated by valves to the left atria and left ventricle. The pump for the pulmonary circuit is the right ventricle. The systemic circuit is pumped by the left ventricle [11], [14].

1.3.1 Vascular Pressures

In the vascular pathway, pressures are varying according to the part of the pathway, in arteries, arterioles, capillaries, venules and veins. It is the pressure of the blood to the walls of the vessels in the pathway. The pressure is also varying based on the cardiac cycle. The pressure is measured as in the case of pulmonary pressures in relative to the reference value. The reference value is the atmospheric pressure that surrounded the human body.

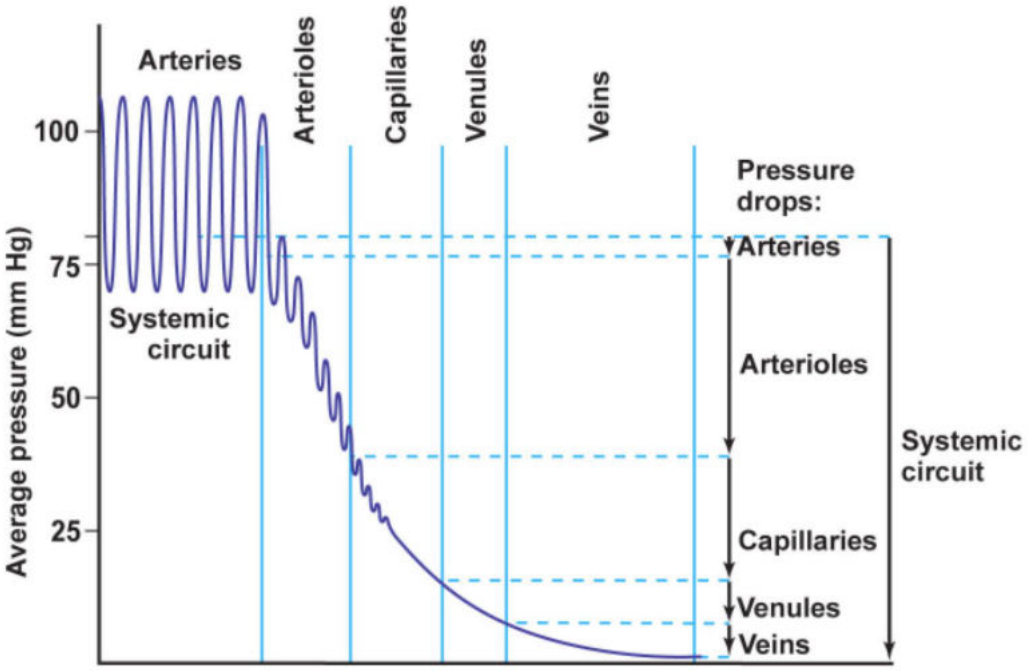


Figure 9: Pressures in the circulatory pathways [2]

The arterial pressure is the pressure that is measured by all blood pressure measurement device. The maximum value is called systolic pressure (SP), 120 mmHg, because it is occuring

during systole and minimal value is called diastolic pressure (DP), 80 mmHg, because it is occuring during diastole. Usually, an average pressure is used to calculate some important physiological values. This value is known as the mean arterial pressure (MAP), 93 mmHg [25].

$$MAP = \frac{2DP + SP}{3} \tag{4}$$

The pressure value decreases with increasing distance from the aorta. The smallest blood pressure on the walls is in the veins.

1.4 Lung Preservation

The preservation in general is a technique that is used to maintain any organ in physiological conditions in an *ex-vivo* environment. In order to preserve organs, the system which perfuse the inner environment of organs is used. The *ex-vivo* lung perfusion (EVLP) is the method of the lung preservation in *ex-vivo* environment. The basic principle of EVLP is to maintain the viability of the lungs without additional injury and the presence of edema or bacteria. The preservation can be either with the focus on the total physiological form of lungs with all physiological conditions and internal events or only with the focus on avoiding necrosis tissue growth to be able to use the lungs for repetitious measurement. The lungs that are preserved are usually used in transplantation process or are preserved to be used in research for measurements.

The lungs which are the subject of transplantation must be taken care of more because there are a lot more parameters to monitor to make sure the lungs are appropriate to be transplanted to the recipient body. In this case, the lungs need to be evaluated. If there is some dysfunctionality present, the functions of the lungs have to be reconditioned. The perfusion system that prepares lungs is usually composed of several parts. The system includes the pump, the gas exchange module, heater-cooler unit, leukocyte filter, the solution reservoir, and oxygenator [19], [34].

The lungs which will be involved in medical research do not need to be preserved by perfusion system as complex as it is in the case of transplantation and then the question of components selection is on the place. In this case, we need to clarify which component is really needed in the system to achieve the required result.

1.4.1 Perfusion System

The perfusion system is widely used in pulmonary research. This technique is useful and is often part of the characterization of pulmonary physiology as well as of pathology by measuring metabolic activities and respiratory functions. The technique includes interactions between circulatory substances and the effects of inhaled or perfused substances. The system which is used to preserve lungs in an *ex-vivo* environment is usually composed of several components, as was mentioned above. Except for the tubing system and lungs which will be perfused there

are components involved in the system that are important to perform some particular functions. The pump is considered as the driving force of the system and its role is to pump the solution into the system. The perfusate solution circulates through the gas-exchanger membrane. The gas-exchange module is responsible for the supply of breathing gases. The heater-cooler unit is understood as the unit for maintaining the appropriate temperature in the system, in the case of Toronto and Lund protocol it is between 32 °C to 37 °C. This unit is connected to the membrane of the gas exchanger to cool/heat the perfusate solution directly. The leukocyte filter is used to reduce cytokine-induced lung injury. The solution reservoir is needed for the storage of the required amount of the perfusate solution. The ventilator is an important compartment of the system due to its ability to ventilate the lungs [31].

1.4.2 *Ex-vivo* Lung Perfusion Protocols

Among the most well-known protocols dedicated to the EVLP procedure are Toronto, Lund, and Organ Care System (OCS) protocols. These protocols are different from each other in the composition of the perfusate solution, in the perfusion and ventilation settings, and in the equipment used in the system.

Toronto Protocol

Toronto protocol is characterized by using the STEEN Solution™ at the place of the perfusate solution. STEEN Solution™ is an extracellular solution supplemented by human albumin. Due to this composition, the perfusate solution is responsible for maintaining the optimal pressure and protecting the epithelium from cell injury. According to the settings of the system, Toronto protocol is working with the target flow 40 % of cardiac output. Another parameter that is controlled is pulmonary artery pressure (PAP) and for Toronto protocol, the value is remaining about 12 mmHg. The last difference in protocols is either the left atrium (LA) is open or it is closed by suturing the atrial cuff. In Toronto protocol, the closed LA is used to maintain a positive LA pressure in the range of 3 to 5 mmHg.

Lund Protocol

Lund protocol is another standard from protocols used for perfusion of lungs in *ex-vivo* environment. This protocol is characterized by using the same STEEN Solution™ as in is in Toronto with one change. The red blood cells are added to the perfusate solution to better mimic physiological conditions. The target flow is set to 100 % of the cardiac output. The PAP is moving below 20 mmHg. The LA is opened to discharge effluent from the lungs.

OCS Protocol

The perfusate used in OCS protocol is OCS™ Solution® or Perfadex®. Both perfusate solutions are low potassium dextran without human albumin but with the addition of red blood cells for the

same reason as in the Lund protocol. The target flow is in this case set to 2-2.5 l/min. The PAP needs to be between 15 to 20 mmHg. A part of this protocol is also LA which remains opened [34], [6].

All these protocols play an important role in the total preservation and perfusion of the lungs. The lungs are preserved and all functions are physiologically maintained. These protocols could be used in the case of transplantation. If the perfused and so preserved lungs are not used for transplantation the procedure of perfusion can vary.

1.5 Artificial Lung Models

The current state-of-the-art in the modeling of the breathing simulation provides several approaches to understanding the human breathing process and the possible impact of related diseases. The approaches to simulate human breathing patterns are based on the lung models. The basic types of lung simulators can be divided into three principal groups. Electrical, mathematical, and mechanical approaches are the main focused groups for simulation of breathing as well as the behavior of the lungs in order to understand the impact of some pulmonary diseases [38].

1.5.1 Electrical Equivalent

The electrical equivalent of the lungs for the simulation of respiration can be considered as a simplified model of the respiratory tract. It contains only basically linear components in a simple circuit. The compliance of the lung is described by the capacitor (C). The resistance present in the airways is described by resistor (R). The values of these electrical components need to be set based on real values of the compliance and resistance in the human respiratory system. These values can be determined using pneumotachography. The advantage of the model is the simplicity of the system. The disadvantage of the model is that only passive behaviour of the lungs is described. There is no presence of spontaneous activity.

1.5.2 Numerical Equivalent

The mathematical models discover other issues in respiration modeling. Basic problems for modeling are the highly complex structure of the human lungs, asymmetric structure, and varying shape per individual. The only way to the model lung is to make an assumption, simplifying the model or modeling only a specific part of the lung. The most popular mathematical models are Weibel and Lattice-Boltzmann models. The Weibel model is the most popular mathematical model for simulation of the lung but has several limitations and uses a lot of assumptions. This model considers only the symmetric structure of the lung and make often only planar representation. The Lattice-Boltzmann model added some innovations to the Weibel model to make a better approximation of the lung function. The model considers also the asymmetric structure

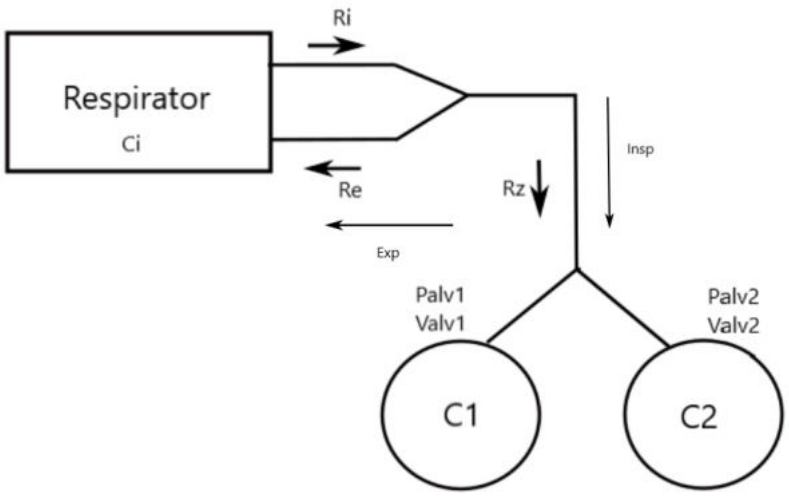


Figure 10: Electrical model of the lungs

of the lungs and uses finite-difference and finite-volume methods. The flow field is simulated via the Lattice-Boltzmann model. The steady flow was simulated using the constant Reynold's number. The biggest advantage is low computational costs. One of the disadvantages is that the method is not usable in high Reynold's number regime. The stability of the system depends non-linearly on the viscosity, local velocity, and grid resolution of the model [4].

1.5.3 Mechanical Equivalent

The last group of basic models is a mechanical model. This group of models are specific because of its ability to simulate breathing process by volume displacement. Mechanical models can be divided into three subgroups.

Rigid Model

The first type of mechanical model is a rigid model. The basic setup of the rigid model is defined by the rigid volume of the compartment. Usually, the syringe or cylinder-piston system is used. The breathing process is simulated by volume displacement within defined ranges. The biggest advantage is that the volume of the compartment is known, which could help during calibration process. The disadvantage of the model is the structure that does not represent anatomical lung situation. The thing that could influence the accuracy of measurement is the fact that no compliance factor is present in the model.

Flexible Mechanical Model

The second group of the mechanical models is a flexible mechanical model. This model is in contrast to the rigid model defined by the maximal possible volume. A part of the model are

balloons, latex or polymer bags that are involved in the simulation process. The breathing is simulated by volume displacement within defined ranges including flexible retraction. Advantages are flexibility of the lung material, which allows the inclusion of the compliance factor which is necessary to consider to obtain relevant results of measurement. The flexible model can be used for calibration measurements and simulations as well. The disadvantage of the model is that no internal structure is present in the model. This means that no anatomical factor is included.

Combined Mechanical Model

The last type of mechanical model is the combined mechanical model. This model is represented by animal lungs. The respiratory system of the animal is somehow similar to that of humans. This is the reason why animal lungs found application in respiratory research. Due to the similarity between the properties of animal lungs and human ones, they are usually used on the place of the combined lung model. Based on the research type it can be porcine lungs, rabbit lungs, rat lungs, sheep lungs, or many more. The most similar in structure and physiological parameters in comparison to humans are the porcine lungs and porcine respiratory system in general.

The breathing process is simulated by volume displacement caused by inflation and deflation of the lung. Lots of advantages are related to this kind of model. The bigger one is that the model is capable to be used for actual measurements and simulations. Another thing that makes the combined model the best option to simulate breathing patterns and simulation of pulmonary injuries is the presence of the natural compliance factor. In comparison to the other models, this model is the only one that is typical for its high similarity to the human lung anatomy and also similarity in the structure of the tissue. Among disadvantages are included a high variance of the volume and compliance of the organ based on the specific animal model. The repeated measurement results could be affected by a single previous measurement.

For this type of lung model, it is necessary to mention that the use of animals, as well as animal organs for research, is following the three Rs principle: replacement, reduction, and refinement. Replacement means that wherever it is possible, it is necessary to replace the use of animals and on its place use alternative models. Reduction stands for minimize the number of animals per experiment. Refinement express the need for minimalizing the pain and distress for animals. Refinement is applied to all aspects during experiment to animals, to housing, to husbandry and also to the scientific procedures performed on them [39].

1.6 Animal Lungs Models

1.6.1 Porcine Lungs

The porcine lungs, figure 11, are used as the mechanical lung model for many decades. These lungs have contributed significantly to biomedical research in several sectors. The size and anatomy that are really similar to human lungs are responsible for making this model particularly beneficial for research in the field of medical devices, therapeutics, and transplantation. In recent years, the publication of the porcine genome overcome the limitation of the model. The result is that the role of the model is likely to become even more important.

The function of the porcine lungs is the same as human lungs and so delivering the oxygen to the organs and removing the carbon dioxide from the blood. The air is moved through the larynx and trachea to the lungs.

The lobular division of the porcine lungs is different from human ones. The right lung is composed of four lobes, cranial, middle, accessory, and caudal. The left lung is composed of two lobes, cranial and caudal. The lungs have a more spongious texture than the human ones. The intention of these surfaces is to take the greatest benefit from the air that is passed through pig airways. The porcine trachea is much longer than the human one is. It is divided into the bronchus. The porcine airway tree is considered as the monopodial branching pattern where each larger bronchus is split into smaller side branches called bronchi that branch off at obtuse angles. The morphological structure of the lungs is dependent on the age of the pig. The airways structure of the porcine respiratory tract are more cartilaginous [27], [20].

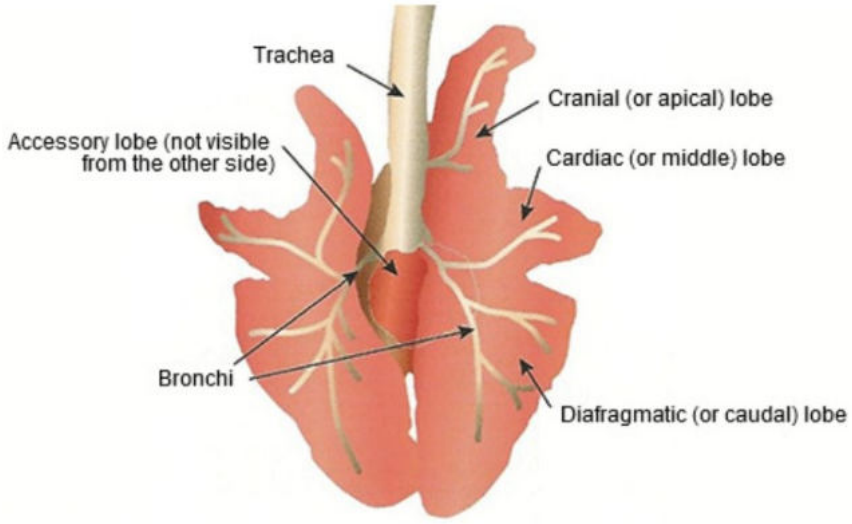


Figure 11: Porcine Lungs [10]

The histological structure of the porcine airway is also very similar to humans. The type of tissue lining the airways is varying by the part of the respiratory tract. Types of epithelia that

are present in the porcine respiratory tract are squamous epithelium, ciliated pseudostratified columnar epithelium, nonciliated columnar epithelium, and olfactory epithelium. The contrast to other animals is the large number of glands that are situated in bronchi [16].

1.6.2 Rabbit Lungs

The rabbit lungs, figure 12, are used as one from the mechanical combined lung model in some types of research to simulate the breathing process and disease related to the respiratory tract of humans. The principal difference is that rabbit nostrils are eqquiped by sensory pads. These pads are very sensitive for touch. The nostrils are exceptional in the ability to twitch at up to 150 twitches per minute. Rabbits have a well developed sense of smell. They are obligated to breathe by the nose because of their epiglottis situated rostrally to the soft palate. The inhlaed air is moved through the nostrils to the nasal cavity. The nasal cavity of rabbits is divided into left and right cavity by the cartilaginous septum. The oral cavity, that is the following part, is separated from the nasal cavity by the hard palate cranially and the soft palate caudally. Epiglottis is situated right after the soft palate. This fact is responsible for possibility to move air directly from nostrils to the rima glottis that is situated directly after the epiglottis. The mucosa that is covering the rabbit airways is sensitive to the trauma related to the intubation process.

Each lung is divided into several lobes. The right one has four lobes, cranial, middle, caudal and one accessory lobe. The left one has tree of them, cranial, middle and caudal [15].

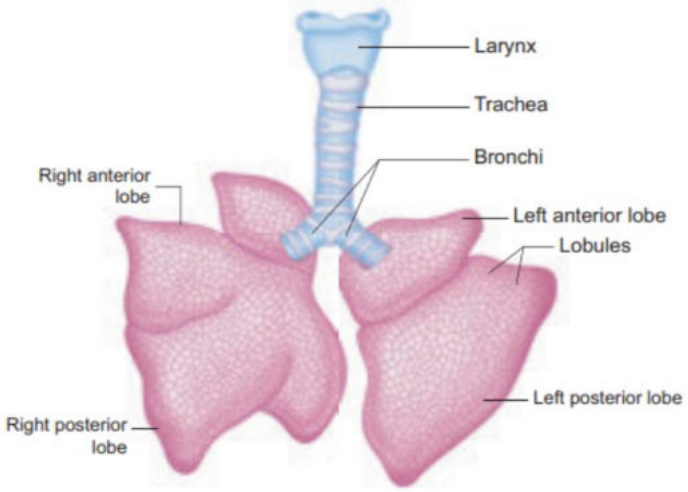


Figure 12: Rabbit Lungs [33]

1.7 Analogy of Animal Lungs with Human Lungs

Animal models have been used to explore physiology, pathophysiology of human disorders, and to test the safety and efficacy of new therapies in preclinical studies. The animal organs are

usually used in research in many fields of study. In the field of respiratory research, the animal lungs are considered as combined models for research in the simulation of human breathing patterns and impact of related diseases where also impact of the anatomy and physiology of the living organ is included.

Mammals' organs are very similar to humans in terms of anatomy, genetics, and physiology. The similarity makes the model suitable for advancing translational medicine. An increasing number of conditions in human lungs are studying and these conditions are modeled with the help of the animal lungs. Small differences of animal lungs concerning human ones, that are physiologically present, need to be taken into account to make the research relevant [17].

The biggest perspective has porcine lungs. They are more than similar to human ones which makes them suitable to be relevant as the alternative model of the human lungs in the research. In recent years, the porcine organs have found potential also in the field of transplantation to the human being. This could be a breakthrough in tackling the lack of donor organs. However, the practical application is still in the research stage.

2 Materials and Methods

The perfusion system is designed so that the animal lungs are inserted into the system that includes all necessary components. The components are responsible for monitoring parameters during the perfusion of the lungs for relevant evaluation of the preservation process and its condition.

2.1 Measurement Setup

The proposed system, figure 13, is composed of several components which are needed to be incorporated into the system. The principal component of the system is animal lungs. The lungs need to be firstly cannulated. The cannulated lungs are placed in the system and are connected to the circuit in both directions. One direction is at the input point to the lungs where the pulmonary artery is situated and the second is at the output point of the lungs to the left atrium.

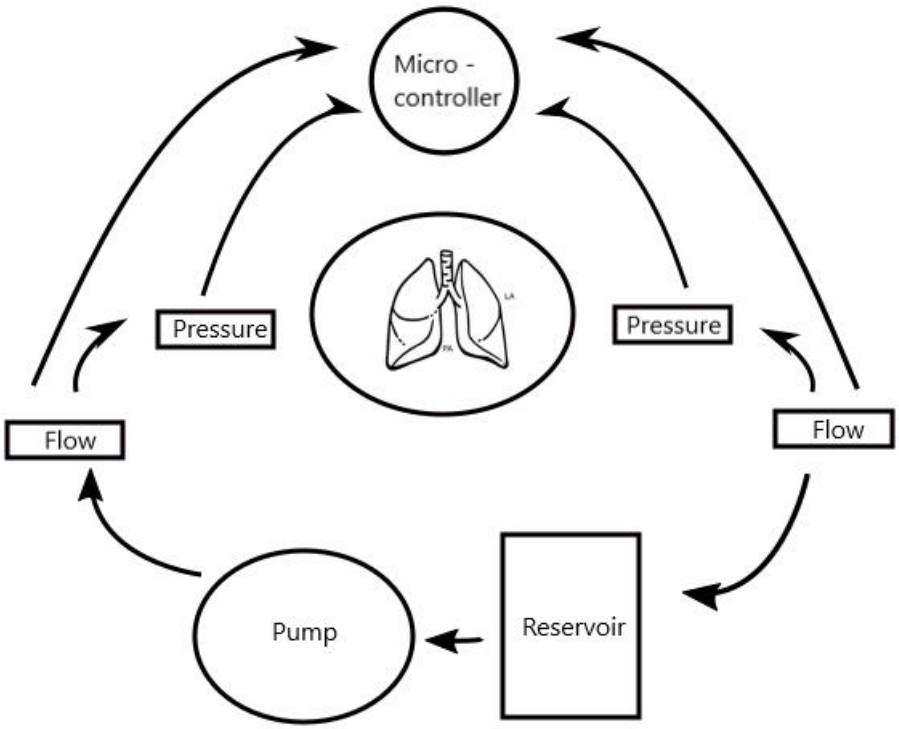


Figure 13: Diagram representing the created perfusion system

The flow and pressure sensors are also placed on both sides of the lungs for monitoring the flow rate and pressure in the system. Two types of pressure sensors are connected in the system, one is an on-chip pressure sensor and the second one is an invasive pressure transducer. An integral part of the system is the roller pump and the reservoir filled with the solution. The reading of the sensors and control of the pump is secured by the microcontroller connected to the system.

2.1.1 Perfusate Solution

One of the principal components of the system is a perfusate solution. The perfusate solution is responsible for bringing all necessary ions to the lungs tissue in an *ex-vivo* environment and so maintain the lungs viable enough to be used for many measurements. Perfusate solution which can be used for perfusion is usually divided into cellular and acellular. In the system, an acellular solution is used. The measurement of the lung properties is performed several times based on the changes in the previous cycles. Gradually, the three different perfusate solutions are used, table 1.

First lungs are perfused using Saline. As saline, a mixture of sodium chloride and water, is the solution that has a number of uses in the medicine, it is tested also in the perfusion system to see what is the impact of the saline solution on inner tissue in the *ex-vivo* environment.

The second lungs are perfused with Ringer's solution in the place of perfusate solution. The Ringer's solution is an isotonic solution, several salts dissolved in water, and for this reason is used in the system to perfuse the lungs' tissue to balance the pH of the inner environment, treat dehydration, and replace the extracellular losses.

The last perfusate solution used to perfuse the third lungs in the system is called Histofix. Histofix is a fixative solution usually used in pathological anatomy. It contains three principal components, 4 % formaldehyde, methanol, and sodium phosphate (Na_3PO_4). The formaldehyde meets the need to fix the tissue of the preserved organ and so avoid the growth of bacteria. The solution is buffered with sodium phosphate and stabilized using methanol in it. The respiratory laboratory does have not ventilation sufficient for the work with the solution that contains alcohol. For this reason, the utilization of Histofix in the place of the perfusate solution is performed in the cell and tissue engineering laboratory which is ready for work with such solutions.

Perfusate Solution	**Name**	**Composition**
1. solution	Saline solution	NaCl, water
2. solution	Ringer's solution	NaCl, KCl, $CaCl_2$, $NaHCO_3$, distilled water
3. solution	Histofix	4 % formaldehyde, methanol, Na_3PO_4

Table 1: Perfusate solutions used in the system

2.1.2 Solution Reservoir

During the perfusion procedure, the perfusate solution needs to be stored in a container. The container has a volume of 5 l. This volume is sufficient to store enough solution for the perfusion cycle.

The reservoir has two openings. One is served as the outflow of the solution to the system and the second one is served as the inflow of the solution. The openings are secured using silicon on both sides. At the end of the openings, stopcocks are used on both sides to ensure no leakage at the time when the reservoir is not connected to the system. Stopcocks ensure also an easy connection between the reservoir box and the system.

2.1.3 Lungs Container

The lungs are connected to the system using cannulas. They are placed in the closed box to avoid the impact of the surrounding environment on the conditions in which lungs are measured. The reservoir has two openings that need to be secured against leakage. The openings for cannulas are secured using silicon and insulation.

2.1.4 Roller Pump

The roller pump is considered the driving force for the whole perfusion system. The pump is provided by the respiratory laboratory at UAS Technikum Wien. The pump consists of two main parts, the peristaltic pump, and the geared motor. The motor which is used to run the pump is identified by m44 x 40 s+ pv42. Inside there is a hose through which the solution passes with the help of wheels.

The pump is controlled using an Arduino microcontroller. The PWM function is used in the Arduino code to control the rotation of the pump. The power supply switch is realized using a transistor IRF520N. The transistor is also controlled by the Arduino. The guided control is secured by the potentiometer connected to the board.

As the pump with others electrical component is supplied using socket, the integrated power supply HRPG-300-12 produced by MeanWell is connected to the system. The role of this device is to reduce the output supply voltage to the limit to 12V to make the work with the input current more safe.

2.1.5 Sensors

Two types of sensing are used in the perfusion system, the flow and pressure sensor. For sensing the system pressure, two types of sensors are used, one is a monolithic silicon sensor and one invasive transducer.

Flow Sensor

The two flow sensors that are used in the system are sensors FCH-M-POM-LC NR.150392 from BIO-TECH. These sensors are able to measure the flow rate of the air, water, oil, and other liquid media. The sensor has 3 pins connector. The output pin of the sensor is connected to the digital input on Arduino microcontroller. It is powered by the 5V supply on the Arduino microcontroller.

The measurement principle is based on the turbine placed inside the plastic body of the sensor and is combined with the Hall effect. During the measurement, impulses from the turbine are detected. According to the data sheet, 2500 impulses correspond to 1 l. Impulses are then transformed into the flow rate value. The reading range is between 0.05 and 3 l/min [22].

Characteristic	**Min**	**Typ**	**Max**	**Unit**
Reading Range	0.05	-	3	l/min
Supply Voltage	5	-	24	Vdc

Table 2: Operating characteristics for BIO-TECH flowsensor

Pressure Sensor

In the system, two pressure sensors are used. One is invasive pressure transducer and one is on-chip piezoresistive transducer. The first idea was to use only invasive pressure sensors. Because of the difficulty of correctly transforming measured impulses by these sensors to relevant pressure values without the patient monitor that is usually a part of this sensor in a medical facility, another sensor is used to make a comparable value of the gained values.

The two invasive pressure sensors that are used in the perfusion system are TruWave transducers from the company Edwards [7]. These sensors are disposable sensors usually used for the measurement of the blood pressure of critically ill patients. Information about blood pressure is transferred from the catheter to the patient monitoring system. Transducers are usually used in the intensive care units and operative rooms [42]. Transducers are supplied by 5 VDC.

The transducer is supplied with a connector compatible with the patient monitor. For this reason, the original connector is cut off and replaced by jumper wires to simplify working with it and created the possibility to connect it to the Arduino microcontroller.

Characteristic	Min	Typ	Max	Unit
Pressure Range	-50	-	300	mmHg
Sensitivity	-	5	-	µV/V/mmHg
Supply Voltage	4.75	5	5.25	Vdc

Table 3: Operating characteristics for TruWave sensor

The pressure sensor MPX5050DP from NXP is piezorestitive monolithic silicon sensor. It is designed for a wide range of applications. The sensor is connected directly to the Arduino microcontroller, to analog input. This single element sensor is a combination of several techniques applied in it. Thin-film metallization and bipolar processing are advantages of the sensor that provide a high-level analog output. The analog output signal is proportional to the pressure which is applied to the sensor.

Characteristic	Min	Typ	Max	Unit
Pressure Range	0	-	50	kPa
Supply Voltage	4.75	5	5.25	Vdc
Sensitivity	-	90	-	mV/kPa
Response Time	-	1.0	-	ms

Table 4: Operating characteristics for MPX5050DP sensor

2.1.6 Microcontroller Arduino UNO

Microcontroller Arduino UNO is one from low-cost and open-source microcontroller board. It is integrated circuit that is based on ATmega328P. It is equipped by 16 digital input pins, 6 analog pins, USB connection, 16 MHz quartz crystal, a power jack and a reset button. UNO is the first from Arduino boards that is in a series of Arduino boards with USB connectors. This microcontroller can be programmed by C++ in Arduino IDE software.

Diagram of the Flow Sensor Reading

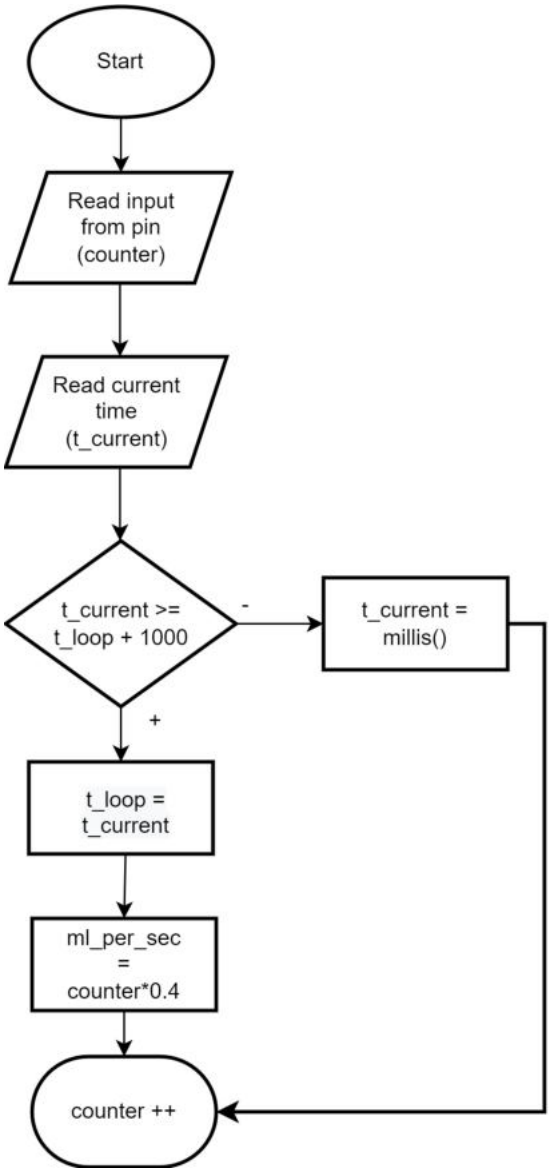


Figure 14: Diagram of the flow sensor reading

Diagram of the Pressure Sensor Reading

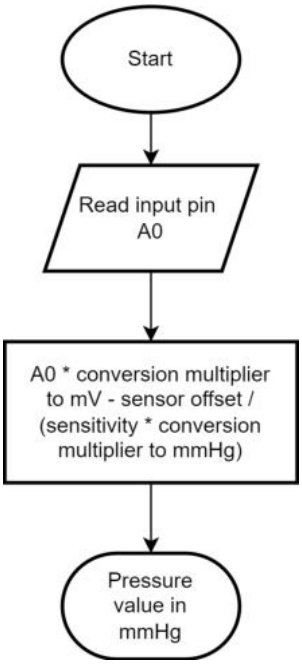


Figure 15: Diagram of the pressure sensor reading

2.1.7 Logging of the Data

CoolTerm is a serial-port terminal application that is used for data exchange with hardware connected to serial ports. In this case, the CoolTerm terminal is working as the exchange platform to log the data from the Arduino microcontroller gained during the measurement process. The data from Arduino are directly transferred to the CoolTerm terminal at the same time as they are measured and then they are saved to a separate file.

2.2 Measurement Procedure

The measurement process is done according to the procedure specified in the measurement protocol, appendix A. The measurement is repeated four times, every time using new lungs and another solution.

2.2.1 Cannulation Procedure of the Lungs

The animal lungs must be subjected to the cannulating process before they will be placed in the perfusion system. The very first thing is to prepare the block for the cannulation. The trachea, the pulmonary artery and the left atrium need to be cut off. The cannulating process continues with the cannulation of the left atrium. The cannula is inserted into the left atrium. The

pulmonary artery is then cannulated and secured by the suture. The lungs are then placed in the perfusion system. The left atrium is connected first to the system to de-airing the pulmonary artery cannula using the retrograde flow of the perfusate solution. After de-airing the PA cannula is connected to the circuit and the flow of the solution is increased.

2.2.2 Measurement Steps

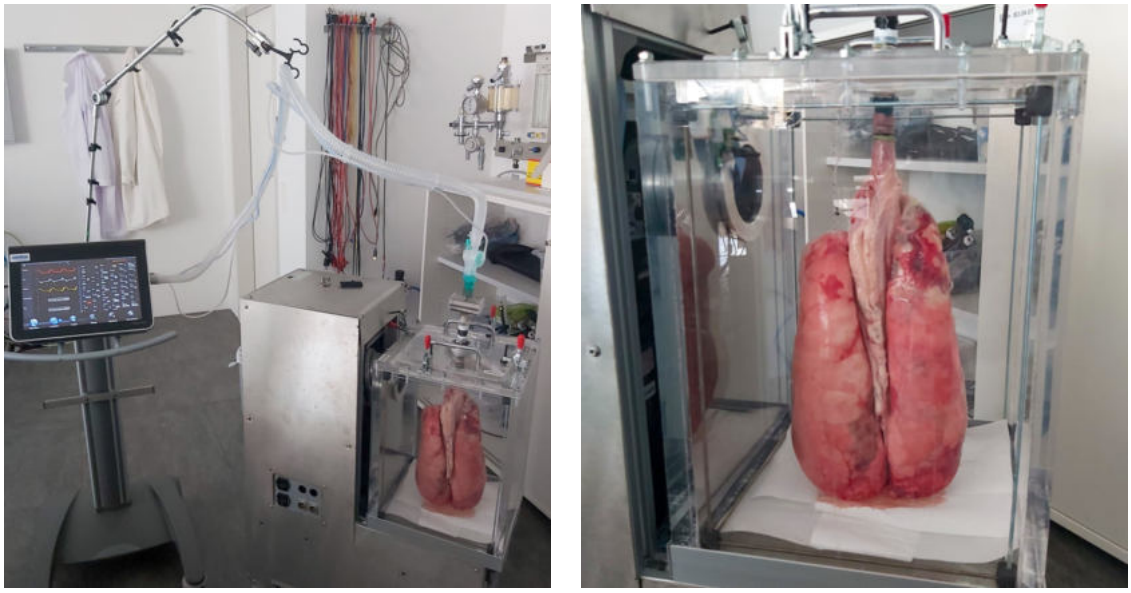
After the cannulation procedure, the lungs are ready to be measured. Firstly, the compliance using BellaVista ventilator is performed before placing the lungs into the perfusion system. When the compliance measurement is done, the lungs are ready to be connected into the system. The lungs are connected to the perfusor lines and are placed in the lung container in the lying position to ensure that the solution reaches all parts of the inner environment of the lungs. The solution reservoir is filled with specific volume of the perfusate solution. In the case the rabbit lungs are used it is 1 liter of the perfusate solution, in the case the porcine lungs are used, it is 2 liters. Gradually, the saline solution, the Ringer's solution and Histofix are used in the place of the perfusate solution. The temperature of the solution needs to be measured before the measurement procedure starts. The temperature of all solutions should be similar to make similar measurement conditions for all solutions.

The roller pump is set to the constant speed that lasts during all measurement cycle. The perfusion procedure lasts for 20 minutes for the rabbit lungs, for 30 minutes for the porcine lungs. The flow and pressure parameters are monitored in the system and are saved with the help of the microcontroller and CoolTerm serial port terminal software for future evaluation. Sensors are placed in both directions, in the direction to the pulmonary artery and in the direction to the left atrium.

After the determined time unit, the pump is turned off and used solution is disposed. The lungs are connected to the BellaVista ventilator, figure 16, to measure mechanical properties of the perfused lungs, especially the compliance. The measurement on ventilator lasts for 10-15 minutes. The measurement cycle on the same lungs with the same type of the solution is repeated at least once and then the compliance, flow and pressure parameters are evaluated and compared. The compliance is a crucial aspect. The cycle is repeating while the changes in compliance are present. When there is no change in the compliance, the measurement is done and the lungs are stored.

So, compliance is measured before perfusion, after the first perfusion, and after the second perfusion. The compliance parameter is also monitored after every 24 hours of storage until visible tissue necrosis appears.

Parameters obtained during the perfusion process related to the perfusate solution placed in the reservoir are the temperature, the resistance, and the conductivity. The temperature of the perfusate solution in the solution reservoir was been measured using the multimeter Multi 3620 IDS with the temperature probe on. The second multimeter that was used for monitoring the solution resistance was the multimeter Amprobe 510-AM. The conductivity (σ) of the solution



(a) **(b)**

Figure 16: Compliance measurement using the Bellavista ventilator: **(a)** measurement setup **(b)** placement of the lungs in the setup

was then calculated from resistance (ρ) based on the following relation:

$$\sigma = \frac{1}{\rho} \tag{5}$$

2.3 Storage of the Lungs

After all measurement process, the lungs need to be stored for the future uses. The lungs are placed in the resealable bag filled with a small quantity of the Ringer's solution to ensure humid environment for stored lungs and also the presence of necessary ions during the storage. The bag is then placed in the closed box and it is stored in the fridge to create a necessary condition for good preservation of the lungs.

It is important to wash the resealable bag and fill it with fresh Ringer's solution after each removal of the lungs from the resealable bag.

2.4 Evaluation of Preservation: Solution Influence

The measurement of the compliance of the lungs perfused using the saline solution, the Ringer's solution and Histofix on the BellaVista ventilator are repeated also after 24, 48 and 72 hours and so on until the necrosis of the tissue is present to determine if there are any changes in compliance with the time and to compare how the chosen perfusate solution influ-

ences the preservation of the lungs. Between all of these measurements, the lungs are still stored in the fridge.

Before every compliance measurement procedure, the lungs are taken out of the box and are also visually evaluated. The visual evaluation is dedicated to the change in color and the appearance of the lung tissue.

3 Results

The practical part of the thesis is divided into two main parts. The first is the creation of the proposed perfusion system and the second one is the validation of the system by placing the porcine lungs into the system. The rabbit lungs were not available.

The parameters that were monitored during the perfusion process were the temperature and the resistance of the perfusate solution, the flow and pressure parameters. The conductivity of the solution was calculated from resistance values.

3.1 Developed Perfusion System

The designed perfusion system, figure 17, is composed of several components that are connected to each other to fulfill some specific task in the system as a complex for perfusing the animal lungs. Among these components are the reservoir for a solution, the lung container, the roller pump, flow sensors, pressure sensors, and the microcontroller include. The electrical connection between all components used in the developed perfusion system is shown in the figure 18.

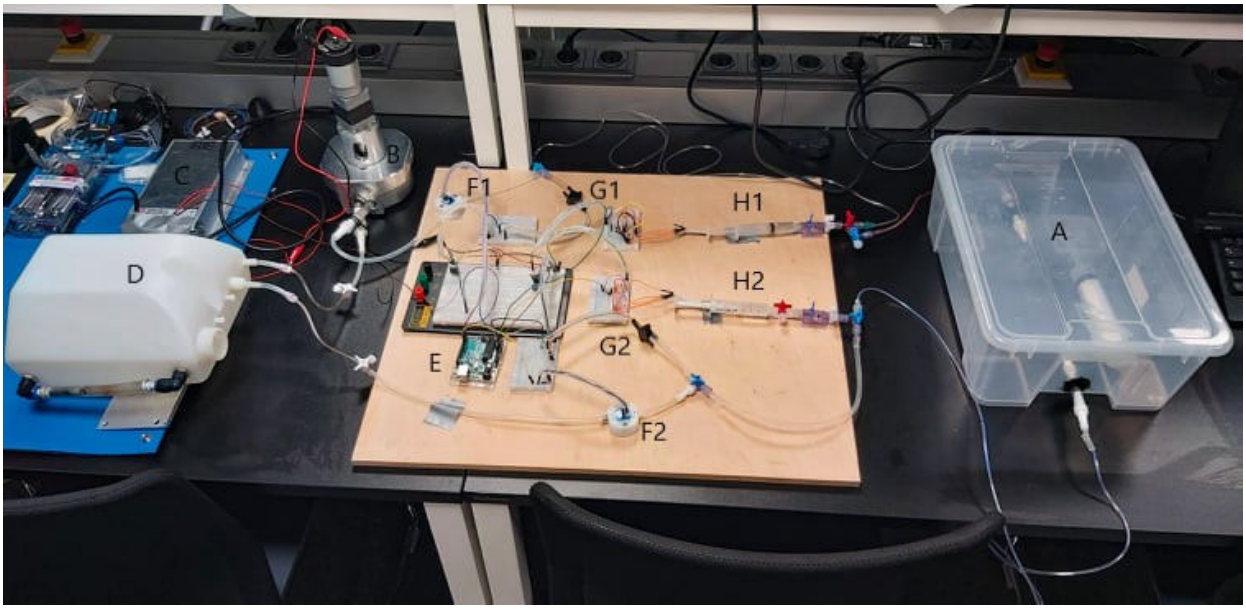


Figure 17: Built perfusion system: **(A)** lungs container **(B)** roller pump **(C)** integrated power supply **(D)** solution reservoir **(E)** microcontroller Arduino **(F1**/**F2)** flow sensors **(G1**/**G2)** pressure sensors MPX **(H1**/**H2)** pressure sensors TruWave

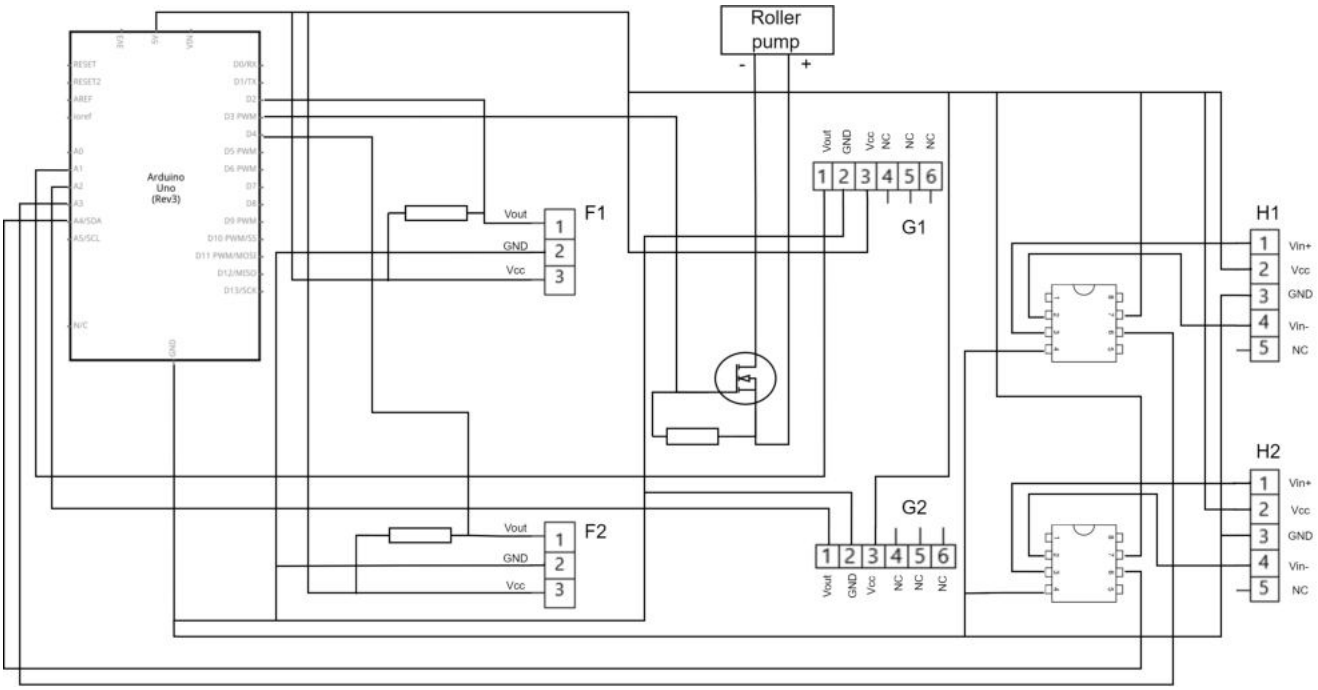


Figure 18: Electrical wiring diagram of the perfusion system

3.2 Saline Solution Perfusion and Preservation

All mentioned parameters above were monitored and recorded for evaluation of the perfusion process and the preservation quality in the relation to the selected perfusate solution, for the first case it was the saline solution.

Solution Parameters

The perfusion of the lungs, that last for 30 minutes, provided the following measured properties of the saline solution in the reservoir. The properties were been measured every 5 minutes to gain the overview about the changes in the temperature, the resistance and the conductivity that was calculated from the resistance value.

The important thing for the course of the measurement is to have a similar temperature of the solution in each perfusion cycle to ensure almost identical conditions. This must be also observed for the reason that the conductivity is sensitive to the temperature change. The saline solution was been stored at room temperature. During the first perfusion cycle, the temperature of the solution in the reservoir was moving from 22.8℃ to 23.1℃. In the case of the second perfusion, it was moving from 23.7℃ to 24℃.

The resistance of the solution was been measured also every 5 minutes of the perfusion procedure. The resistance was moving in the range from 1.26 MΩ to 1.35 MΩ during the first

	1. perfusion					
Time [min]	5	10	15	20	25	30
Temperature [℃]	23	23.1	22.9	22.9	23	22.8
Resistance [MΩ]	1.26	1.26	1.3	1.32	1.34	1.35
Conductivity [S/m]	$7.94e^{-7}$	$7.94e^{-7}$	$7.69e^{-7}$	$7.58e^{-7}$	$7.46e^{-7}$	$7.41e^{-7}$
	2. perfusion					
Time [min]	5	10	15	20	25	30
Temperature [℃]	23.5	23.5	23.6	23.8	23.8	24
Resistance [MΩ]	1.26	1.26	1.28	1.3	1.31	1.31
Conductivity [S/m]	$7.94e^{-7}$	$7.94e^{-7}$	$7.81e^{-7}$	$7.69e^{-7}$	$7.63e^{-7}$	$7.63e^{-7}$

Table 5: Measured solution parameters during the perfusion processes: Saline solution

perfusion cycle and in the range from 1.26 MΩ to 1.31 MΩ during the second perfusion.

From the measured resistance, the conductivity was then calculated. For the first perfusion, the conductivity was continuously decreased from $7.94e^{-7}$ S/m to $7.41e^{-7}$ S/m. For the second perfusion process, it was moving from $7.94e^{-7}$ S/m to $7.63e^{-7}$ S/m, table 5.

Flow and Pressure Parameters

The flow and pressure parameters were been measured during the perfusion in the perfusion system so that one flow and one pressure value was been gained in the inflow port to the lung, at the place where the solution entered the lungs (pulmonary artery). The second value of the flow and pressure was gained at the place of outflow from the lungs (left atrium). The perfusion of the lungs using the saline solution was repeated twice to see if there are some changes in the compliance between these two cycles of the perfusion. Based on the compliance which did not change, the next cycles of perfusion did not continue.

During the first perfusion process, the following values were gained. The first flow sensor provided the flow rate values increasing gradually, with the 25-75 % of values in the range from 1538.6 to 4186.4 ml/s, with a median value of 3125.4 ml/s. The second flow sensor recorded the 25-75% of values in the range from 436 to 1185.6 ml/s, the median value of 810.4 ml/s, figure 19.

The second perfusion using the saline solution gave also every second two flow values and two pressure values. The 25-75 % of flow rate values gained by the first sensor were moving in the range from 956 ml/s to 2643.4 ml/s, the median 1760.4 ml/s. The second value was been increased continuously with the values from 93.7 ml/s to 809.92 ml/s, the median is 246.69 ml/s, figure 19.

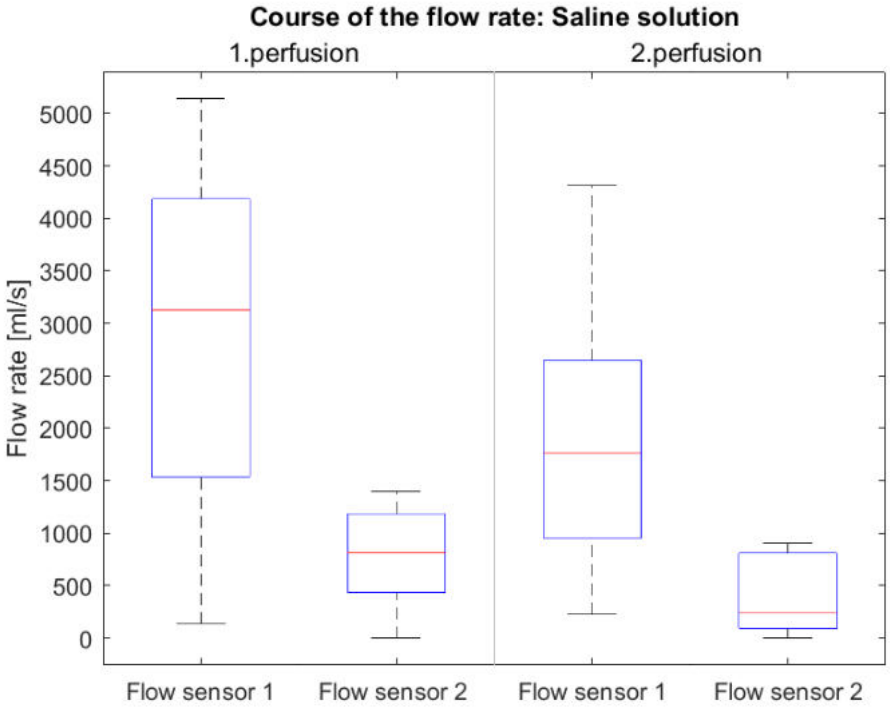


Figure 19: Flow rate course during the 1. and 2.perfusion using the saline solution

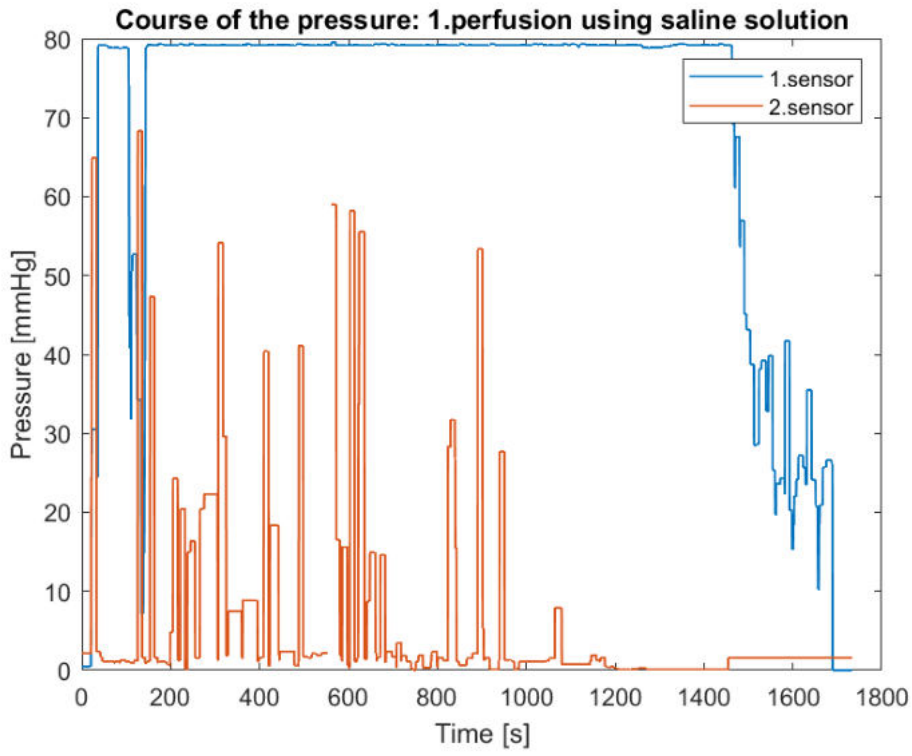


Figure 20: Pressure course during the 1.perfusion using saline solution

Pressure sensors placed in the system also recorded values at the two mentioned places. The first one gained values of 49.66 $\pm$ 31.76 mmHg, in the biggest part of the perfusion process with a maximal value of 79.26 mmHg. The pressure was almost constant, except at the beginning and the end of the measurement process. The second sensor provided values of 3.76 $\pm$ 11.77 mmHg. figure 20. The pressure values were varying a lot in a given range. The oscillation of the pressure values can be caused by the blood content in the solution that was passing through the lines in the system and by flow which sometimes gently stopped due to suction of the solution from the box where the lungs were placed back by the system circuit. The difference in pressure between the two pressure sensors was considerable.

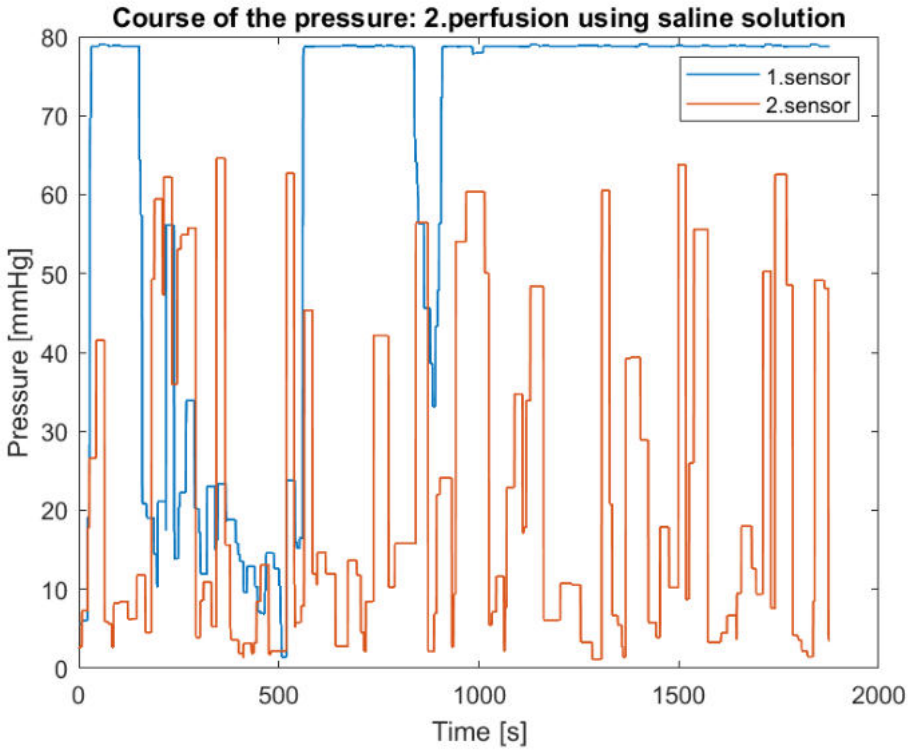


Figure 21: Pressure course during the 2.perfusion using saline solution

The pressure values recorded in the system during the second perfusion cycle oscillated more than it was in the case of the first perfusion cycle. The pressure value measured by the first pressure sensor placed in the system was moving around 41.62 $\pm$ 33.79 mmHg, the middle part with the constant maximal flow was also present. The second pressure sensor provided values of 8.77 $\pm$ 15.39 mmHg.

The pressure gained during the second saline perfusion by the first sensor was clearly more constant than the values by the second sensor. It may have been affected by several things, the change in flow during the pathway of the solution through the lungs and the change in the composition of the perfusate solution based on the concentration of the ions and blood in it.

Compliance Parameters

The compliance parameter of the lungs, table 6, has been measured a couple of times, before the perfusion process, after the first perfusion, after the second perfusion, after 24 hours of storage, after 48 hours of storage, and after 72 hours of storage. To see more significant changes, the checking of the preservation has been continued after 120 hours of the storage and the last measurement of compliance was been performed after 144 hours of storage. After 144 hours (6 days) of the lung's preservation and storage, the tissue of the lungs started to be necrotic, so the preservation was terminated.

	Compliance [ml/mbar]
Before perfusion	58 ± 1
After 1.perfusion	52 ± 1
After 2.perfusion	50 ± 2
After 24 hours	45 ± 1
After 48 hours	48 ± 2
After 72 hours	44 ± 2
After 120 hours	41 ± 2
After 144 hours	39 ± 1

Table 6: Measured compliance values for lungs perfused using the saline solution

In the beginning, the static compliance of the lungs reached the value of 58 ± 1 ml/mbar. After the first perfusion, it decreased to the value of 52 ± 1 ml/mbar, and after the second perfusion, it stayed almost similar. As the storage of the lungs came, the compliance decreased again to the value of 45 ± 1 ml/mbar. With the time of the storage (24h, 48h, 72h,...) the static compliance was moving around 39-44 ml/mbar.

Even though the compliance still reported relevant values, on 6th day of the storage of the lungs the necrosis was present and so the storage did not continue. The lungs thus remained viable for 120 hours, 5 days.

Appearance of the Lungs

Visual lung assessment is one of the most decisive factors in determining the quality of perfusion or the quality of preservation and storage. In the figure 22, the process of changes in the lungs' appearance with the time is displayed.

As shown on the figure 22 (c), one part of the right lungs was not clearly flushed from the blood. With the time of the storage, the lungs began to be redder and redder in some parts, especially the surroundings of the not flushed part. In the end, the red parts started to develop and the color started to be darker. After 144 hours, these parts became dark, figure 22 (h).

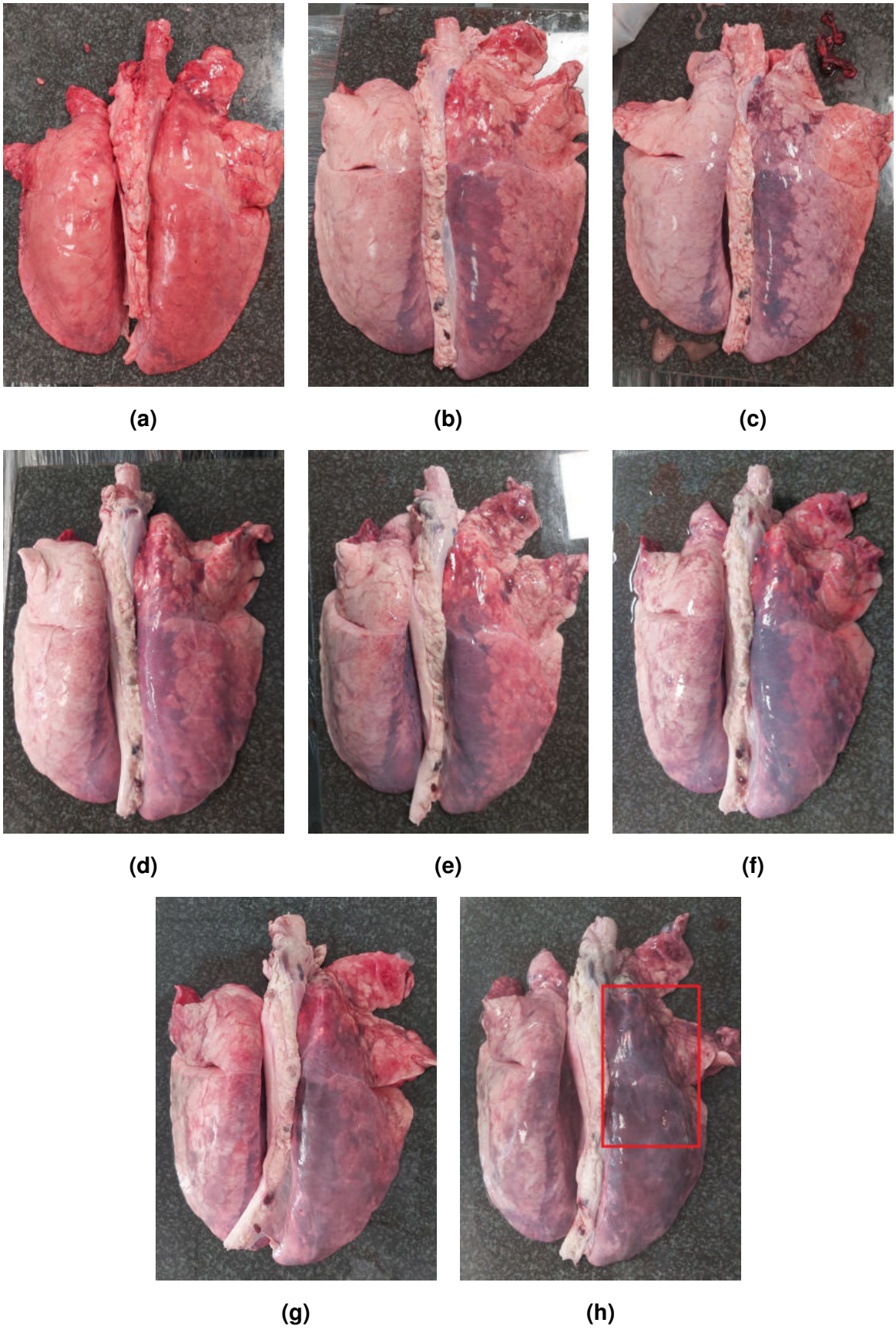


Figure 22: Process of the lung's appearance with the time of the storage using the saline solution: **(a)** before perfusion **(b)** after 1.perfusion **(c)** after 2.perfusion **(d)** after 24 hours **(e)** after 48 hours **(f)** after 72 hours **(g)** after 120 hours **(h)** after 144 hours, necrosis of the tissue

Dark places indicated the necrosis that affected the lungs' tissue. The consistency of the lungs' tissue became also change as well as the volume of the lungs' lobes. The size of the lungs gradually shrunk and the tissue became drier.

3.3 Ringer's Solution Perfusion and Preservation

Second lungs were been perfused in the same way as the previous ones were, but the solution used in the system was replaced by the Ringer's solution. All measured parameters of the solution, lungs and the perfusion system were measured in the same procedure.

Solution Parameters

The perfusion of the lungs lasted for 30 minutes and the properties were been measured every 5 minutes. The multimeters provided the measured properties of the Ringer's solution in the reservoir. The obtained temperature, the resistance and the conductivity are shown in the table 7:

	1. perfusion					
Time [min]	5	10	15	20	25	30
Temperature [℃]	23.9	24	24	24	24.1	24.1
Resistance [MΩ]	1.33	1.43	1.45	1.47	1.56	1.57
Conductivity [S/m]	7.52e^{-7}	6.99e^{-7}	6.89e^{-7}	6.80e^{-7}	6.41e^{-7}	6.37e^{-7}
	2. perfusion					
Time [min]	5	10	15	20	25	30
Temperature [℃]	23.5	23.7	23.7	23.8	23.8	23.8
Resistance [MΩ]	0.917	0.948	0.967	0.976	0.98	1.002
Conductivity [S/m]	1.09e^{-6}	1.05e^{-6}	1.03e^{-6}	1.02e^{-6}	1.02e^{-6}	9.98e^{-7}

Table 7: Measured solution parameters during the perfusion processes: Ringer's solution

The Ringer's solution was been stored at room temperature as well. During the first perfusion cycle, the temperature of the solution in the reservoir was moving from 23.9℃ to 24.1℃. In the case of the second perfusion procedure, it was moved from 23.5℃ to 23.8℃. As the temperature difference is at a maximum of 0.5℃, the conditions in which the perfusion occurred were almost similar.

The resistance of the Ringer's solution was been measured in the range from 1.33 MΩ to 1.57 MΩ during the first perfusion and in the range from 0.917 MΩ to 1.002 MΩ during the second perfusion.

From the measured resistance, the conductivity was then calculated. For the first perfusion, the conductivity gradually decreased from 7.52e^{-7} S/m until 6.37e^{-7} S/m. For the second perfusion cycle, the calculated conductivity was moved from 1.09e^{-6} S/m until 9.98e^{-7} S/m.

Flow and Pressure Parameters

The flow and pressure parameters were been measured during the perfusion in the perfusion system and were placed in the same places as it was in the case of the perfusion using the saline solution. The perfusion of the lungs using the Ringer's solution was repeated also twice to see if there are some changes in the compliance between these two cycles of the perfusion. Based on the compliance which did not change a lot, the next cycles of perfusion did not continue and the lungs were been stored.

During the first perfusion process, the 25-75 % flow rate values gained by the first sensor were moving in the range from 2861.2 ml/s to 5439.3 ml/s, the median 4088.8 ml/s. The 25-75 % of second values were continuously increased from 856.03 ml/s to 2600.9 ml/s, the median is 1728.47 ml/s, figure 23.

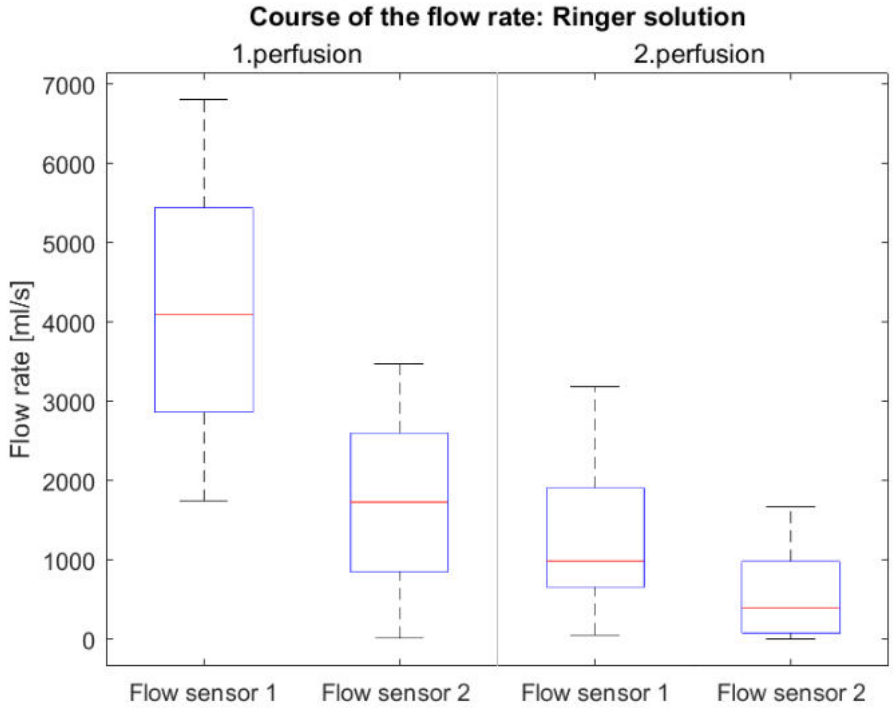


Figure 23: Flow rate course during the 1. and 2.perfusion using Ringer's solution

The second perfusion using Ringer's solution provided also every second two flow values. The 25-75 % of the first flow rate values were moving in the range from 660.2 ml/s until 1903.9 ml/s with a median of 985.2 ml/s. The 25-75 % of the second sensor values were moved gradually from 78.6 ml/s to 980.3 ml/s, the median of 399.2 ml/s, figure 23.

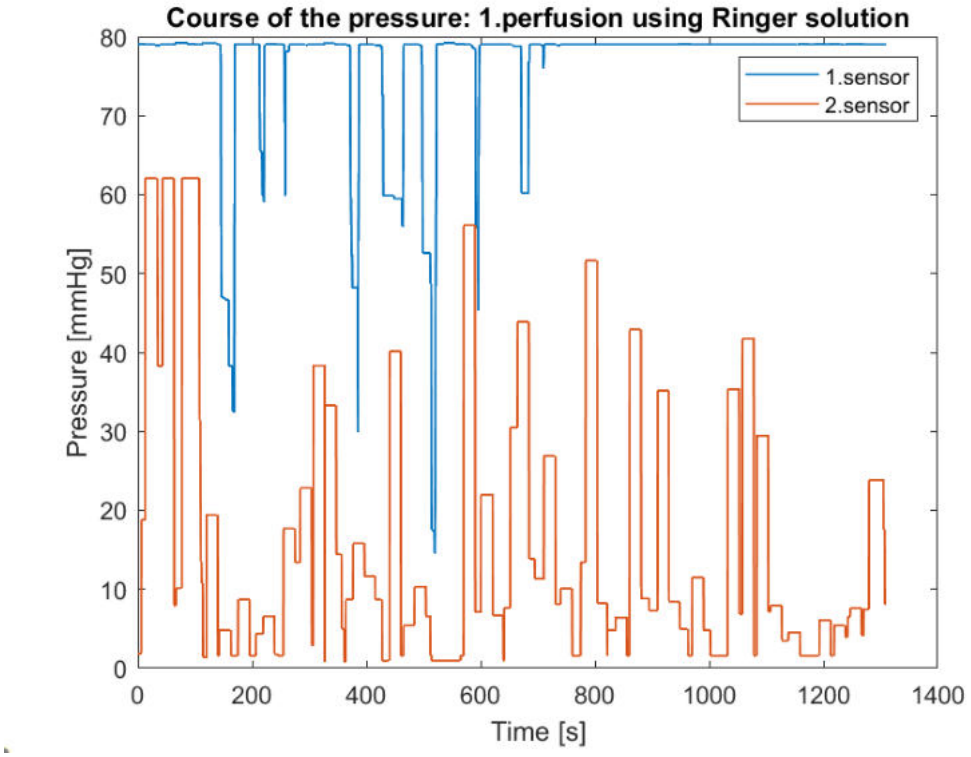


Figure 24: Pressure course during the 1.perfusion using Ringer's solution

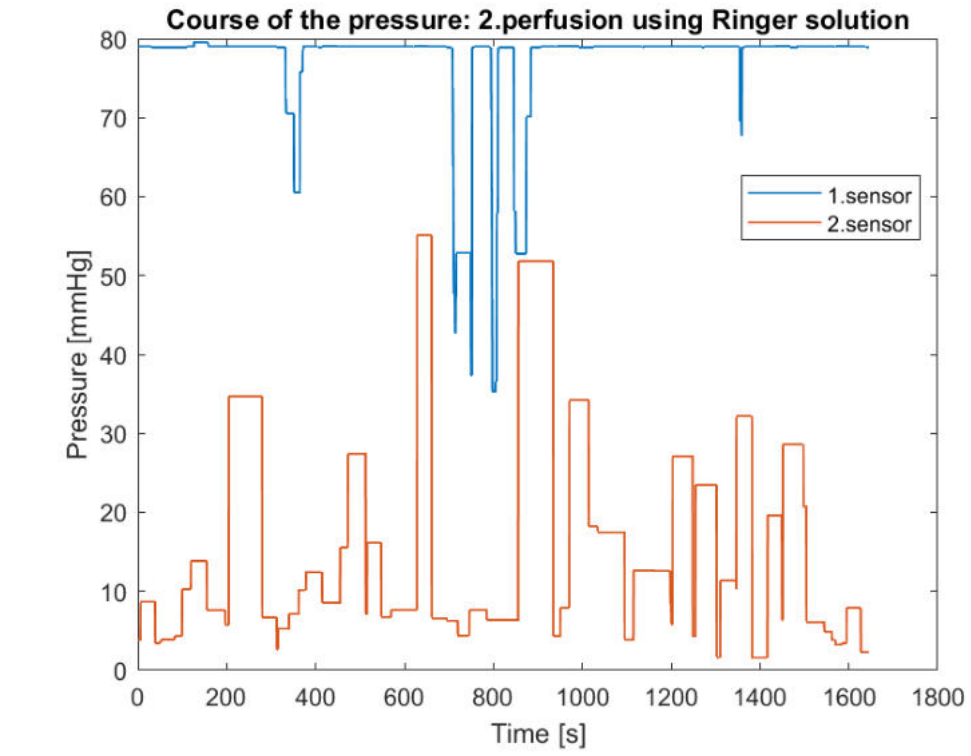


Figure 25: Pressure course during the 2.perfusion using Ringer's solution

The values from the pressure sensors during the first Ringer's perfusion varied significantly throughout the entire duration of perfusion. The values that appeared in the system were in the range of 48.96 $\pm$ 31.94 mmHg for the first sensor. Sometimes, the value decreased for a while and then increased again to the maximal value of 79.34 mmHg. The second sensor recorded more variable values with a mean of 6.54 $\pm$ 2.25 mmHg. This second pressure sensor in the system is highly dependent on the events in the lungs because is placed in the outflow direction from the measured lungs, figure 24.

The pressure values during the second perfusion again moved in the given range. The first pressure sensor recorded the values of 46.02 $\pm$ 32.2 mmHg with multiple reaching the maximal value. The pressure values provided by the second sensor in the system were varying in the range of 8.68 $\pm$ 10.43 mmHg, figure 25. In the case of the second sensor, the values significantly differed again.

Compliance Parameters

The compliance of the second lungs, table 8, was also measured several times. In this case, the compliance was measured for a longer time than in the case of the lungs perfused with the saline solution because necrosis of the lungs' tissue started later.

	Compliance [ml/mbar]		**Compliance [ml/mbar]**
Before perfusion	87 $\pm$ 2	**After 144 hours**	65 $\pm$ 2
After 1.perfusion	81 $\pm$ 2	**After 168 hours**	64 $\pm$ 2
After 2.perfusion	77 $\pm$ 2	**After 192 hours**	65 $\pm$ 2
After 24 hours	66 $\pm$ 2	**After 216 hours**	66 $\pm$ 2
After 48 hours	62 $\pm$ 2	**After 240 hours**	62 $\pm$ 2
After 72 hours	62 $\pm$ 2	**After 288 hours**	64 $\pm$ 2
After 120 hours	65 $\pm$ 2		

Table 8: Measured compliance values for lungs perfused using the Ringer's solution

In the beginning, before any process, the static compliance of the lungs reached the value of 87 $\pm$ 2 ml/mbar. After the first perfusion, it decreased to the value of around 81 $\pm$ 2 ml/mbar, and after the second perfusion, it stayed at the value of 77 $\pm$ 2 ml/mbar. As the storage of the lungs came, the compliance decreased again and so to a value of 66 $\pm$ 2 ml/mbar after 24 hours. With the time of the storage (48h, 72h, 120 hours...) the static compliance was moving still around 65 $\pm$ 2 ml/mbar.

During the compliance measurement on the 12th day (after 288 hours) of the storage, the necrosis of the tissue was revealed. Therefore the storage of the lungs did not continue. The lungs thus remained viable for 240 hours, 10 days.

Appearance of the Lungs

The visual assessment was also applied to the lungs perfused using Ringer's solution. The process of the lungs' appearance is shown in the figure 26, figure 27. Besides the color of the lungs changing with the time of the perfusion and the following storage, the consistency of the lungs' tissue and the size of the lobes changed as well.

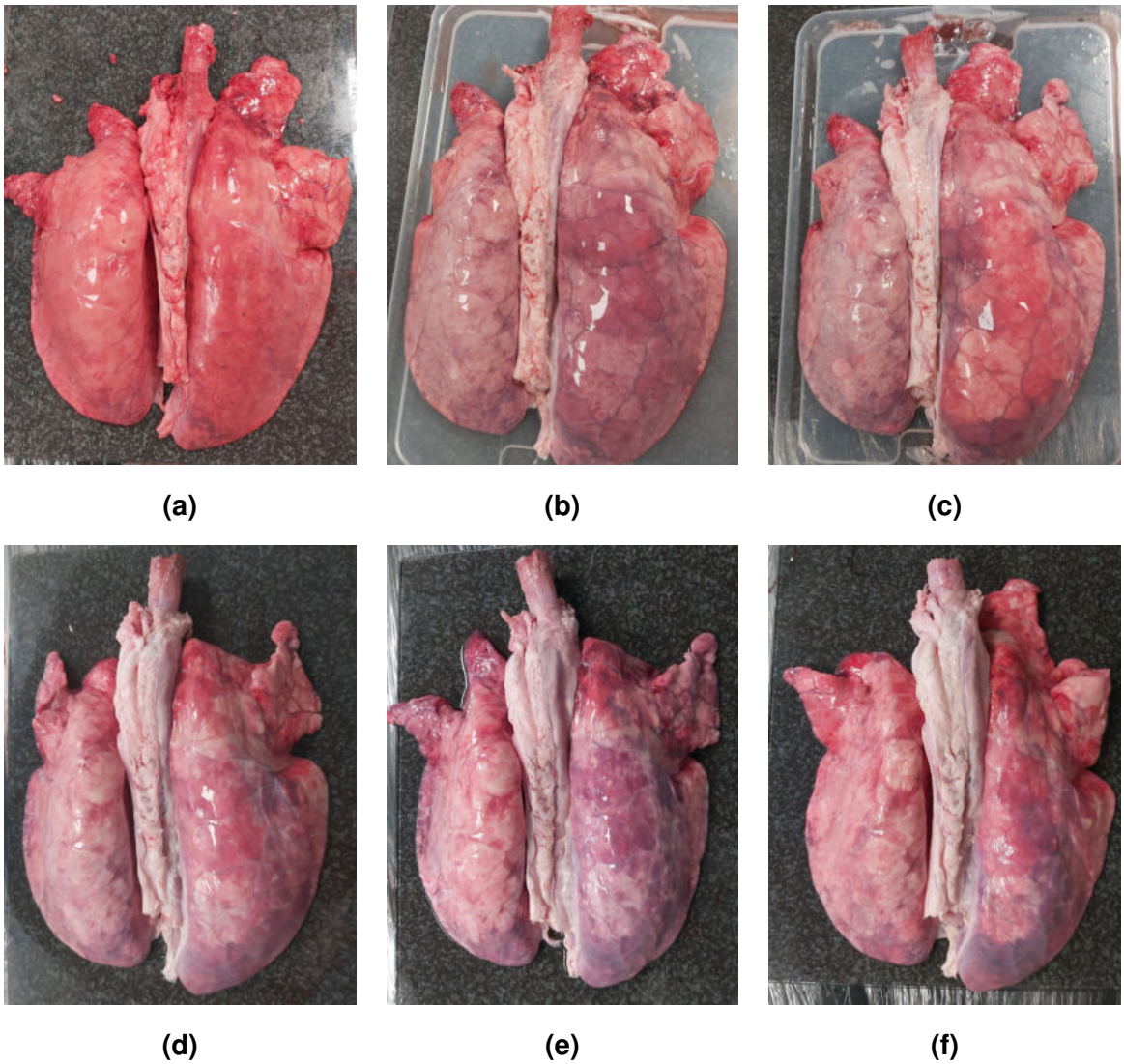
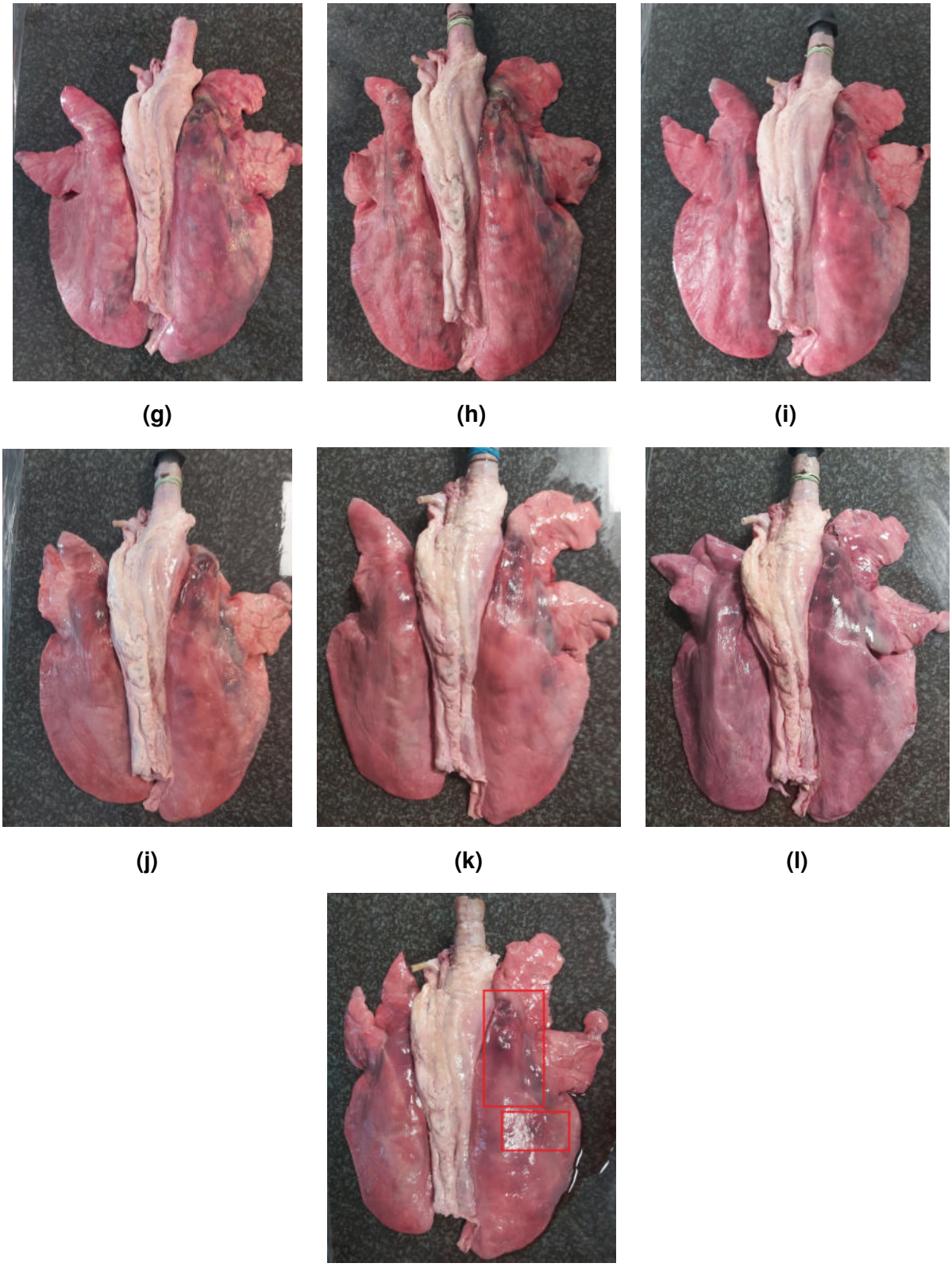


Figure 26: Lung's appearance with the time (Ringer's solution): **(a)** before perfusion **(b)** after 1.perfusion **(c)** after 2.perfusion **(d)** after 24 hours **(e)** after 48 hours **(f)** after 72 hours



(g) **(h)** **(i)**

(j) **(k)** **(l)**

(m)

Figure 27: Lung's appearance with the time (Ringer's solution): **(g)** after 120 hours **(h)** after 144 hours **(i)** after 168 hours **(j)** after 192 hours **(k)** after 216 hours **(l)** after 240 hours **(m)** after 288 hours, necrosis present

As shown in the figure 26 (c), these lungs were flushed from the blood better than it was in the case of the saline solution perfusion. With the time of the storage, the lungs began to be redder and redder in some parts, especially on the middle lobe of the right lung. The red parts started to develop more and more and after 120 hours, figure 27 (g), the lungs were whole red. The darker parts, indicators of the necrosis, were first visible after 240 hours of storage, figure 27 (l). During the measurement after 288 hours, there were clearly visible dark areas in the middle and lower lobe of the lungs. The storage of the lungs finished on this day.

Same as in the first case, the size and the consistency of the lungs changed. All lobes of the lungs shrunk and the tissue became drier.

3.4 Histofix Perfusion and Preservation

The third perfusion was the perfusion with Histofix in the place of the perfusate solution. The perfusion using Histofix was tested on two lungs, one lungs were perfused for 25 minutes and one for 40 minutes to see whether the perfusion time has any effect on lung preservation. Due to the solution besides other components containing methanol and formaldehyde, the perfusion process took place in the lab with a fume hood to prevent the avoid possible adverse effects on the human being while working with it.

The measurement procedure using Histofix brought one major change in the comparison to other solutions. Due to the limited amount of Histofix, the same solution was used in both perfusion cycles for the same lungs. All measured parameters of the solution, lungs, and the perfusion system were measured in the same procedure.

3.4.1 Histofix Perfusion: 25 minutes

Solution Parameters

The perfusion of the lungs lasted for 25 minutes and the properties of the solution were measured every 5 minutes. The obtained temperature, the resistance and the conductivity are shown in the table 9:

	1.perfusion				
Time [min]	5	10	15	20	25
Temperature [℃]	23.3	23.3	23.2	23.1	23.1
Resistance [kΩ]	178.6	187.9	194.7	199.5	205.6
Conductivity [S/m]	$5.59e^{-6}$	$5.32e^{-6}$	$5.14e^{-6}$	$5.01e^{-6}$	$4.86e^{-6}$
	2.perfusion				
Time [min]	5	10	15	20	25
Temperature [℃]	22.9	22.9	22.9	23	23.1
Resistance [kΩ]	235.6	242.7	260.4	273.9	301.9
Conductivity [S/m]	$4.24e^{-6}$	$4.12e^{-6}$	$3.84e^{-6}$	$3.65e^{-6}$	$3.31e^{-6}$

Table 9: Measured solution parameters during the perfusion processes: Histofix 25 minutes

During the first perfusion cycle, the temperature of the solution in the reservoir was moving from 23.1℃ to 23.3℃. In the case of the second perfusion procedure, it was moved from 22.9℃ to 23.1℃. As the temperature difference is at a maximum of 0.2℃, the conditions in which the perfusion occurred were almost similar and these differences did not influence the measurement process.

The resistance of Histofix differed from other solutions. The resistance was in the range from 178.6 kΩ to 205.6 kΩ during the first perfusion and in the range from 235.6 kΩ to 301.9 kΩ during the second perfusion.

From the measured resistance, the conductivity was then calculated. For the first perfusion, the conductivity was continuously decreased with the maximal value of $5.59e^{-6}$ S/m and a minimal value of $4.86e^{-6}$ S/m. For the second perfusion cycle, the calculated conductivity was moving from $4.24e^{-6}$ S/m to $3.31e^{-6}$ S/m with decreasing tendency.

Flow and Pressure Parameters

The flow and pressure parameters were again measured in the same places as they were in two previous cases. The perfusion was repeated twice.

During the first perfusion process, the 25-75 % of flow rate values gained by the first sensor were moving in the range from 1231.1 ml/s to 1810.5 ml/s, the median 1522.4 ml/s. The 25-75 % second values were continuously increased from 95.6 ml/s to 344.4 ml/s, the median is 216.8 ml/s, figure 28.

In the second perfusion process, the 25-75 % flow rate values gained by the first sensor were moving in the range from 380.5 ml/s to 1264.6 ml/s, the median 826 ml/s. The 25-75 % of values from the second sensor were continuously increased from 169.2.1 ml/s to 717.8 ml/s, the median was 531.6 ml/s, figure 28.

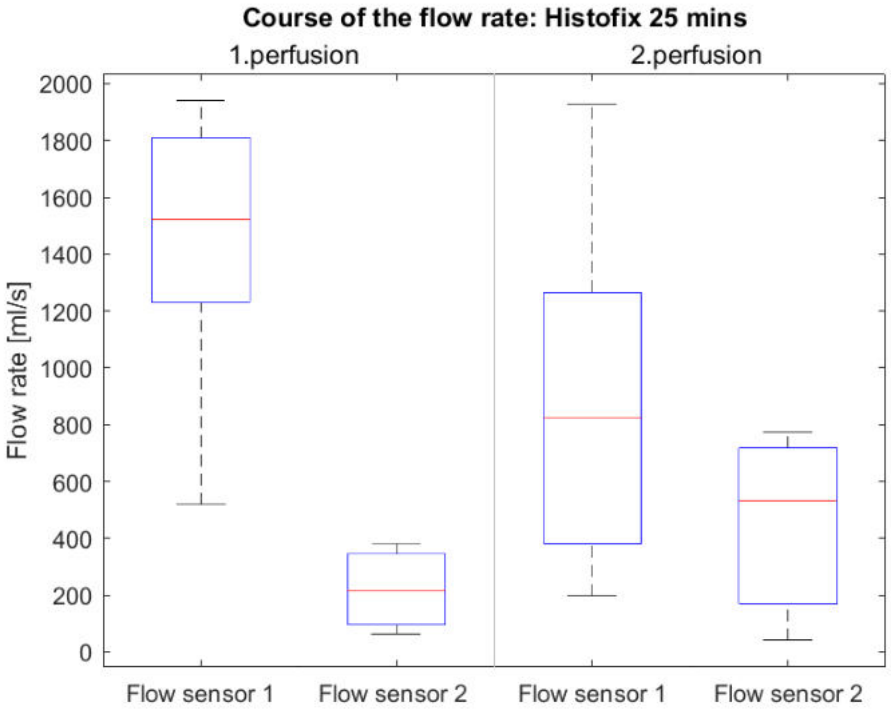


Figure 28: Flow rate course during the 1. and 2.perfusion using Histofix for 25 minutes

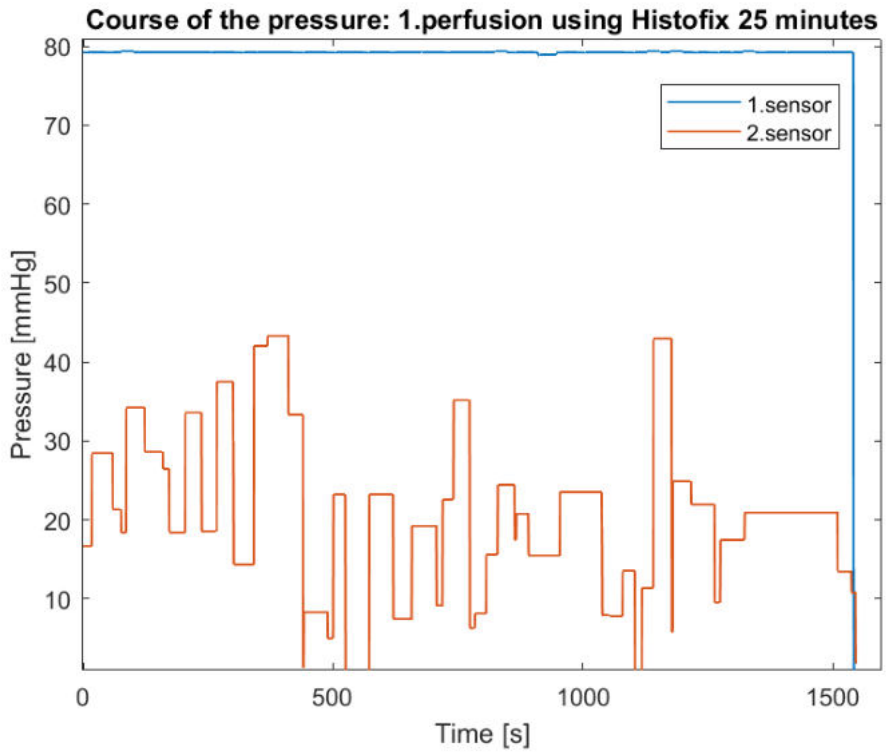


Figure 29: Pressure course during the 1.perfusion using Histofix for 25 minutes

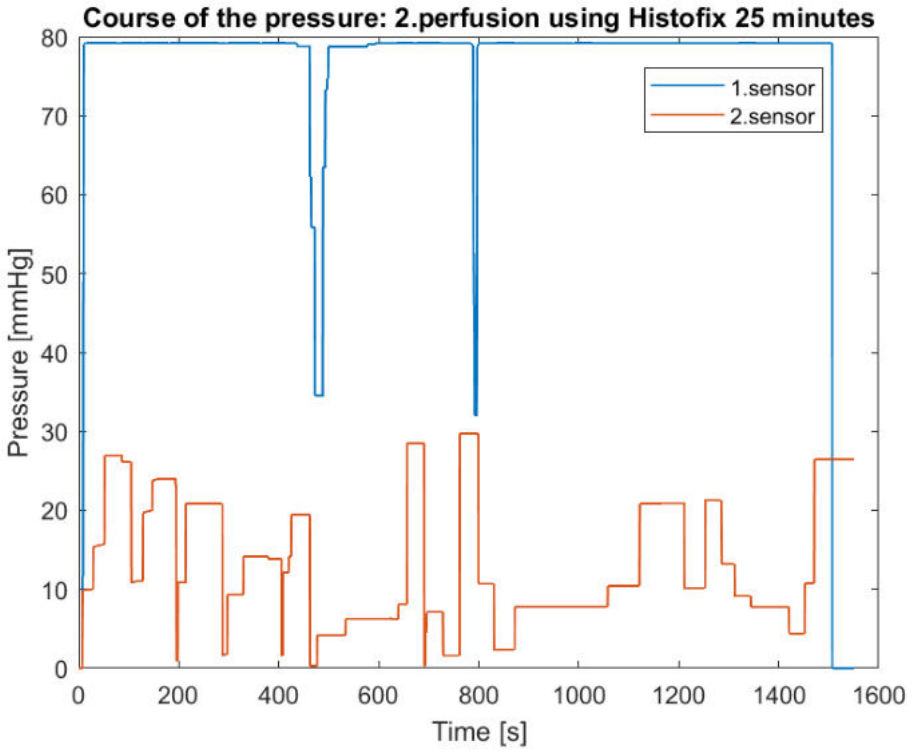


Figure 30: Pressure course during the 2.perfusion using Histofix for 25 minutes

The pressure values that appeared in the system during the first perfusion cycle were almost constant from the beginning of the cycle until the end. The pressure value only gently oscillated around the maximal value of 79.65 mmHg. The second sensor recorded more variable values with a mean of 13.99 $\pm$ 10.62 mmHg. The values from the second pressure sensor were gently higher than it was in the previous cases, figure 29.

During the second perfusion cycle, the pressure values from the first sensor placed in the system showed a mean value of 57.83 $\pm$ 18.87 mmHg with the most often value at the maximum throughout the measurement. The second sensor provided values of 8.1 $\pm$ 7.02 mmHg with a less constant course, figure 30.

Compliance Parameters

The compliance of the lungs perfused with Histofix for the time unit of 25 minutes, table **??**, was measured during perfusion and also during the entire storage period every 24 hours until necrosis set in, table 10.

Before the perfusion process, the static compliance of the lungs reached the value of 43 $\pm$ 2 ml/mbar. After the first perfusion, it decreased to the value of 25 $\pm$ 2 ml/mbar, and after the second perfusion, it stayed at the value of 23 $\pm$ 2 ml/mbar. With the storage of the lungs, the compliance decreased to the value of 22 $\pm$ 1 ml/mbar after 24 hours. With the time of the storage (48h, 72h, 120 hours...) the static compliance was moving still around 20 $\pm$ 2 ml/mbar.

	Compliance [ml/mbar]		Compliance [ml/mbar]
Before perfusion	43 ± 2	**After 144 hours**	21 ± 2
After 1.perfusion	25 ± 2	**After 168 hours**	20 ± 1
After 2.perfusion	23 ± 2	**After 192 hours**	19 ± 1
After 24 hours	22 ± 1	**After 216 hours**	18 ± 1
After 48 hours	22 ± 1	**After 240 hours**	20 ± 1
After 72 hours	20 ± 2	**After 264 hours**	19 ± 1
After 96 hours	20 ± 1		

Table 10: Measured compliance values for lungs perfused using Histofix: Lungs perfused 25 minutes

Appearance of the Lungs

The process of the lungs' appearance is shown in the figure 32, figure 33. As in the other cases, the color of the lungs changed with the time of the perfusion and storage, and the consistency of the lungs' tissue and the size of the lobes changed as well. The tissue of the lungs perfused with Histofix remained firmer.

As shown in the figure 31 (a), these lungs were bloody more than other lungs. Therefore the flushing from the blood was more complicated. With the time of the storage, the lungs began to be redder as usual and it began from the right lung. From day 3 after storage, figure 32 (f), the lungs seemed to be identical and no significant changes were present.

(a) **(b)** **(c)**

Figure 31: Lung's appearance with the time (Histofix 25 minutes): **(a)** before perfusion **(b)** after 1.perfusion **(c)** after 2.perfusion

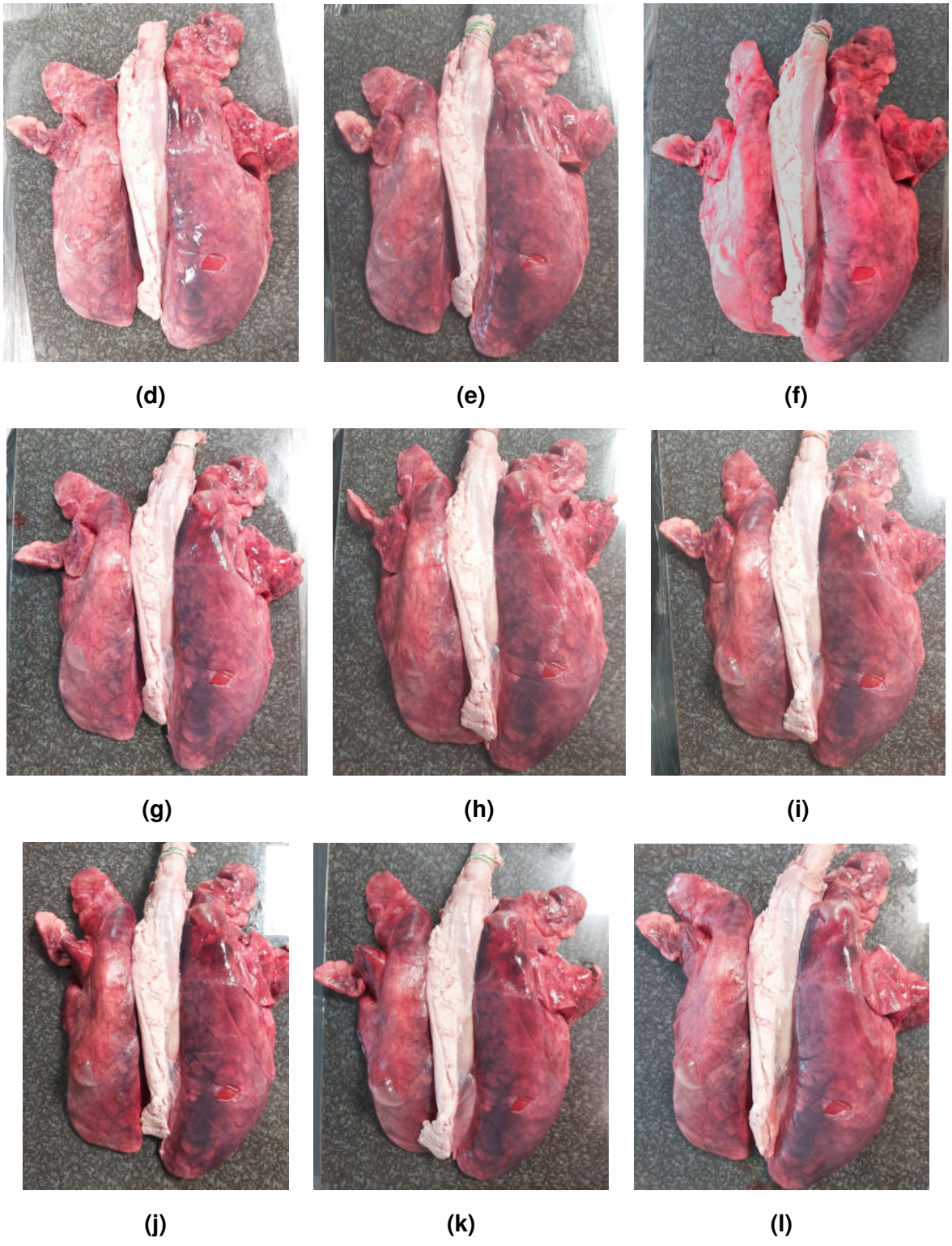


Figure 32: Lung's appearance with the time (Histofix 25 minutes): **(d)** after 24 hours **(e)** after 48 hours **(f)** after 72 hours **(g)** after 96 hours **(h)** after 144 hours **(i)** after 168 hours **(j)** after 192 hours **(k)** after 216 hours **(l)** after 240 hours

(m)

Figure 33: Lung's appearance with the time (Histofix 25 minutes):
(m) after 264 hours

Same as in all previous cases, the size and the consistency of the lungs changed. All lobes of the lungs shrunk and the tissue became drier. There was also one difference between the lungs perfused using saline or Ringer's solution, the tissue of the lungs perfused with Histofix remained firmer.

The biggest change in the appearance of the lungs occurred after 192 hours (8 days) of the storage. Since this day, the right lung darkened significantly, figure 32 (j).

3.4.2 Histofix Perfusion: 40 minutes

Solution Parameters

The perfusion of the lungs last for 40 minutes and the solution properties were been measured every 5 minutes. The obtained temperature, the resistance, and the conductivity are shown in the table 11.

During the first perfusion cycle, the temperature of the solution in the reservoir was moving from 22.7℃ to 22.9℃. In the case of the second perfusion procedure, it was moved from 22.8℃ to 23.1℃. As the temperature difference is minimal, the perfusion took place without a bigger impact on the measurement procedure.

The resistance was in the range from 1745.9 kΩ to 233.2 kΩ during the first perfusion and in the range from 248.9 kΩ to 337.5 kΩ during the second perfusion.

The calculated conductivity for the first perfusion gradually decreased with starting value of 6.85e^{-6} S/m until 4.14e^{-6} S/m. For the second perfusion cycle, the calculated conductivity was moved from 4.02e^{-6} S/m to 2.96e^{-6} S/m.

	1.perfusion							
Time [min]	5	10	15	20	25	30	35	40
Temperature [℃]	22.7	22.7	22.8	22.8	22.9	22.9	22.9	22.9
Resistance [kΩ]	145.9	155.3	167.8	188.6	203.7	227.9	233.2	241.5
Conductivity [S/m]	$6.85e^{-6}$	$6.44e^{-6}$	$5.96e^{-6}$	$5.30e^{-6}$	$4.91e^{-6}$	$4.39e^{-6}$	$4.29e^{-6}$	$4.14e^{-6}$
	2.perfusion							
Time [min]	5	10	15	20	25	30	35	40
Temperature [℃]	22.8	22.8	22.9	22.9	23	23.1	23.1	23.1
Resistance [kΩ]	248.9	261.5	277.4	286.2	306.3	321.9	328.3	337.5
Conductivity [S/m]	$4.02e^{-6}$	$3.82e^{-6}$	$3.60e^{-6}$	$3.49e^{-6}$	$3.26e^{-6}$	$3.11e^{-6}$	$3.05e^{-6}$	$2.96e^{-6}$

Table 11: Measured solution parameters during the perfusion processes: Histofix 40 minutes

Flow and Pressure Parameters

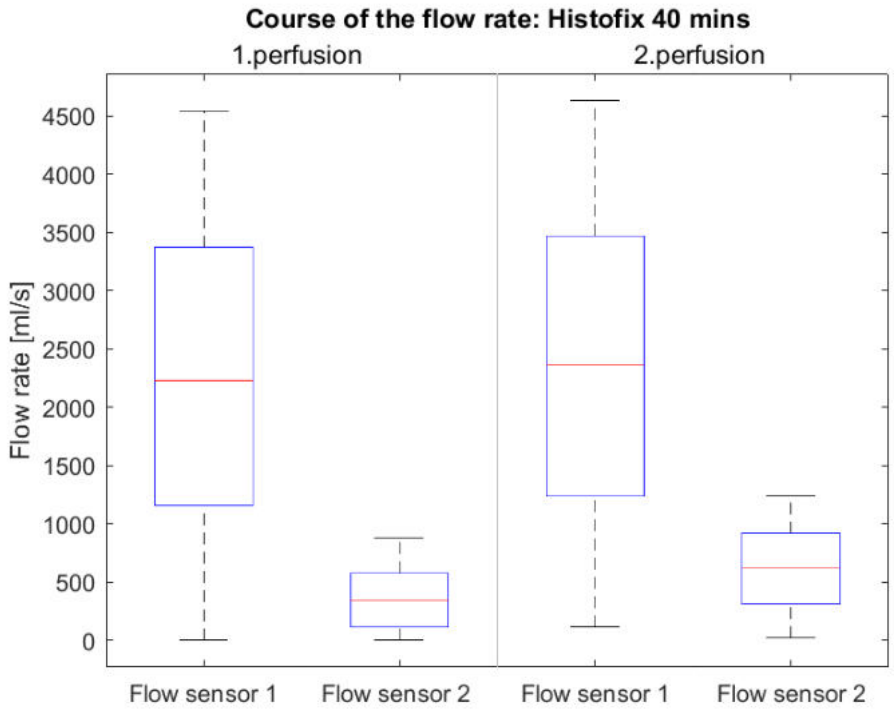


Figure 34: Flow rate course during the 1. and 2.perfusion using Histofix for 40 minutes

The first perfusion process provided the 25-75 % of flow rate values by the first sensor in the range from 1154.4 ml/s to 3373.8 ml/s, the median 2228.8 ml/s. The 25-75 of the % second values were gradually increased from 110.6 ml/s to 573.4 ml/s, the median is 344.8 ml/s.

During the second perfusion cycle, the first flow sensor provided 25-75 % of values in the

range from 1233.6 ml/s to 3472.3 ml/s, the median 2359.6 ml/s. The 25-75 % of second values were continuously increased from 308.2 ml/s to 919.8 ml/s, the median is 614.8 ml/s.

The flow rate measured during the first and second perfusion was very similar in this case as is shown in the figure 34.

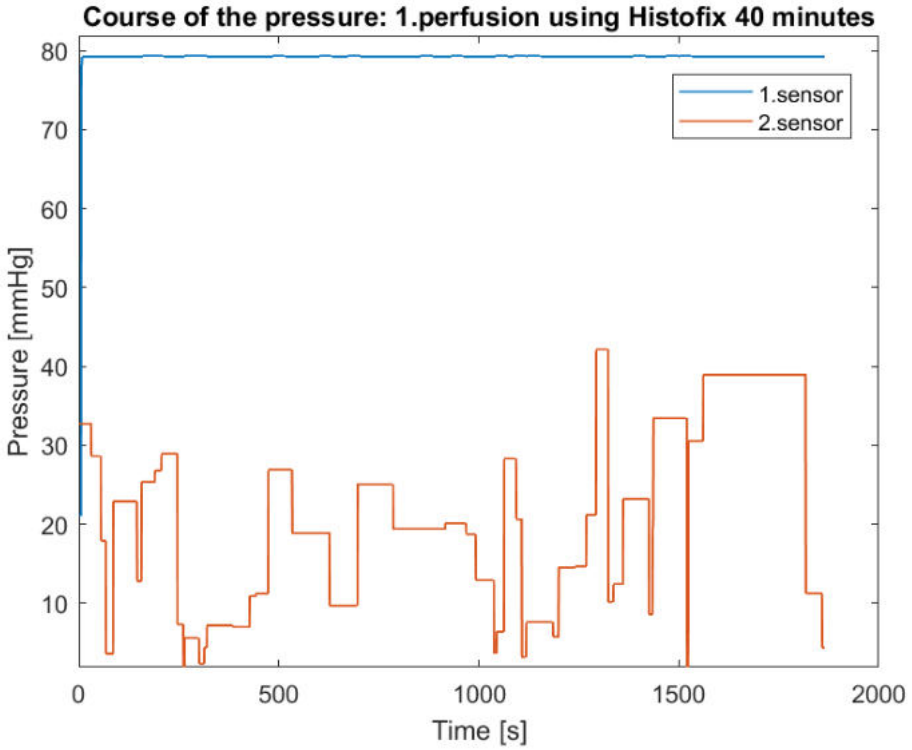


Figure 35: Pressure course during the 1.perfusion using Histofix for 40 minutes

The pressure values that appeared in the system during the first perfusion cycle were in the range of 62.58 $\pm$ 17.37 mmHg for the first sensor. As is shown in the figure 35, the pressure was moving around the maximal value throughout the entire measurement cycle. The second sensor recorded more variable values with a mean of 15.98 $\pm$ 12.09 mmHg. The values from the second pressure sensor were gently higher than it was during the perfusion using saline or Ringer's solutions, figure 35. In this case, the pressure value quite often fell to the same lower value and then rose to the same upper value.

The values of the pressure during the second perfusion cycle were similar to those during the first perfusion, oscillating around a maximal value of 79.24 mmHg. The second sensor recorded more variable values with a mean of 13.58 $\pm$ 9.46 mmHg. Also in this case the second pressure sensor values were gently higher than it was in the previous cases, figure 36.

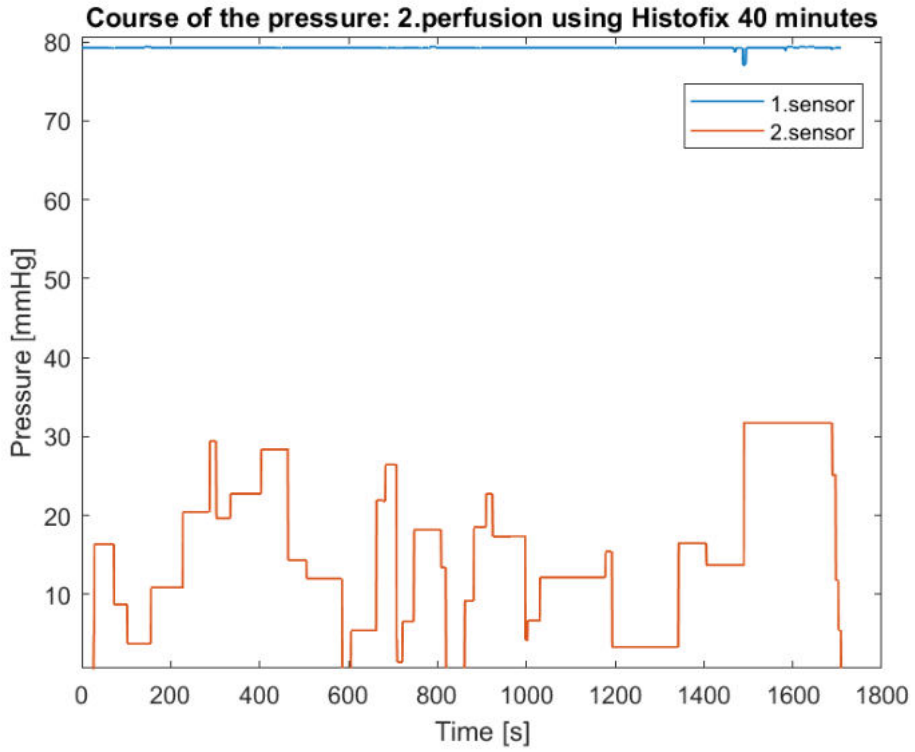


Figure 36: Pressure course during the 2.perfusion using Histofix for 40 minutes

Compliance Parameters

The compliance of the lungs perfused with Histofix for the time unit of 40 minutes, table 12, was measured during perfusion and also during the entire storage period every 24 hours.

	Compliance [ml/mbar]		**Compliance [ml/mbar]**
Before perfusion	41 $\pm$ 1	**After 144 hours**	20 $\pm$ 1
After 1.perfusion	32 $\pm$ 1	**After 168 hours**	20 $\pm$ 2
After 2.perfusion	30 $\pm$ 1	**After 192 hours**	19 $\pm$ 2
After 24 hours	27 $\pm$ 1	**After 216 hours**	19 $\pm$ 2
After 48 hours	24 $\pm$ 1	**After 240 hours**	18 $\pm$ 1
After 72 hours	22 $\pm$ 1	**After 264 hours**	19 $\pm$ 1
After 96 hours	20 $\pm$ 1		

Table 12: Measured compliance values for lungs perfused using Histofix: Lungs perfused 40 minutes

The static compliance of the lungs reached the value of 41 $\pm$ 1 ml/mbar at the beginning. After the first perfusion, it decreased to the value of 32 $\pm$ 1 ml/mbar. After the second perfusion, it stayed at the value of 30 $\pm$ 1 ml/mbar. With the storage of the lungs, the compliance decreased

to the value of 27 $\pm$ 1 ml/mbar after 24 hours. With the time of the storage (48h, 72h, 120 hours...) the static compliance was moving still around 20 $\pm$ 1 ml/mbar.

Appearance of the Lungs

The process of the lungs' appearance has also very similar stages, figure 37, figure 38. As is shown in the figure 37 (a), the lungs were from the beginning darker in some parts. The upper and middle lobes of the left lung and the left side of the right lung along the entire length showed signs of susceptibility to necrosis from the beginning. The perfusion thus turned out according to the possibilities.

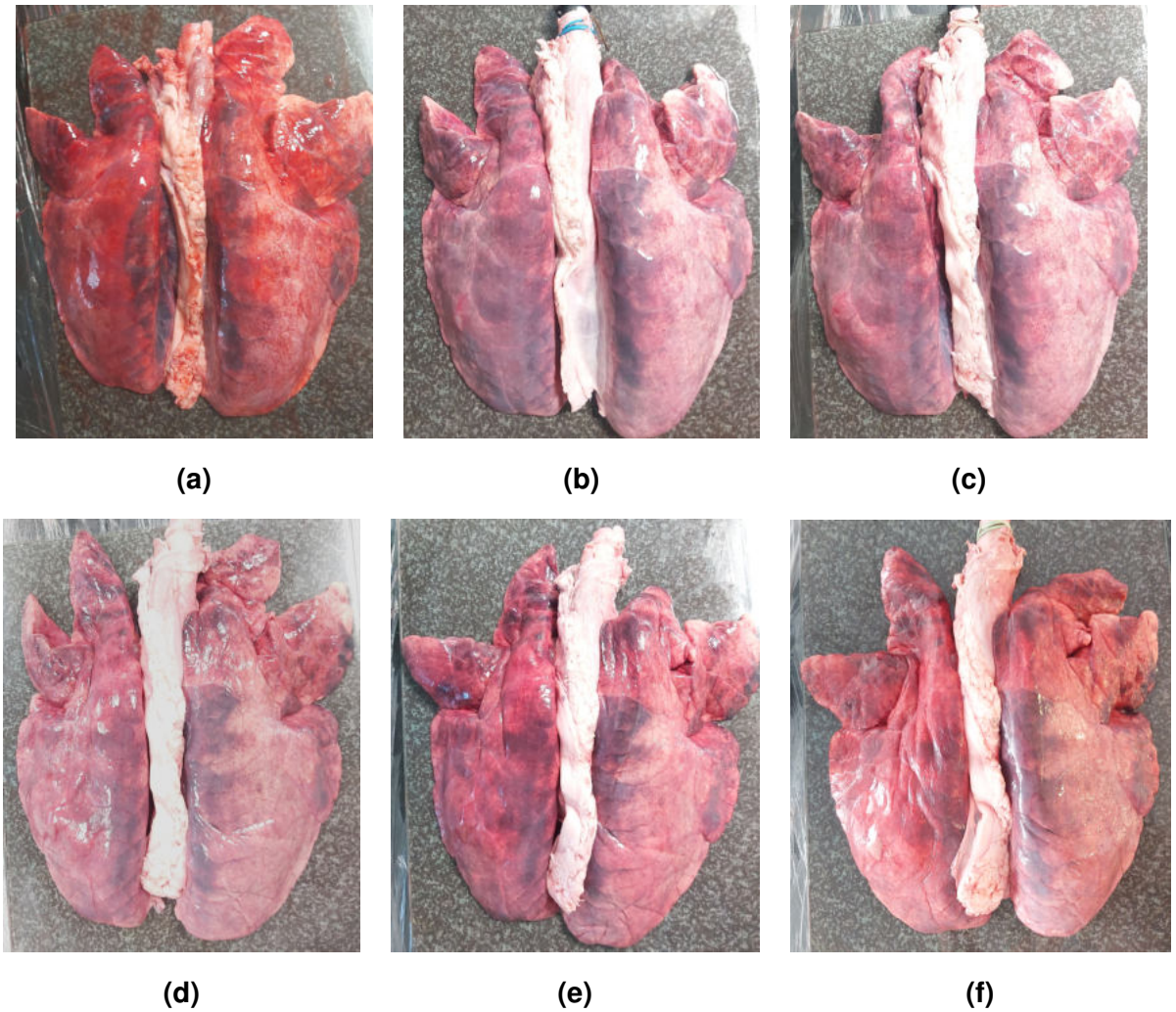


Figure 37: Lung's appearance with the time (Histofix 40 minutes): **(a)** before perfusion **(b)** after 1.perfusion **(c)** after 2.perfusion **(d)** after 24 hours **(e)** after 48 hours **(f)** after 72 hours

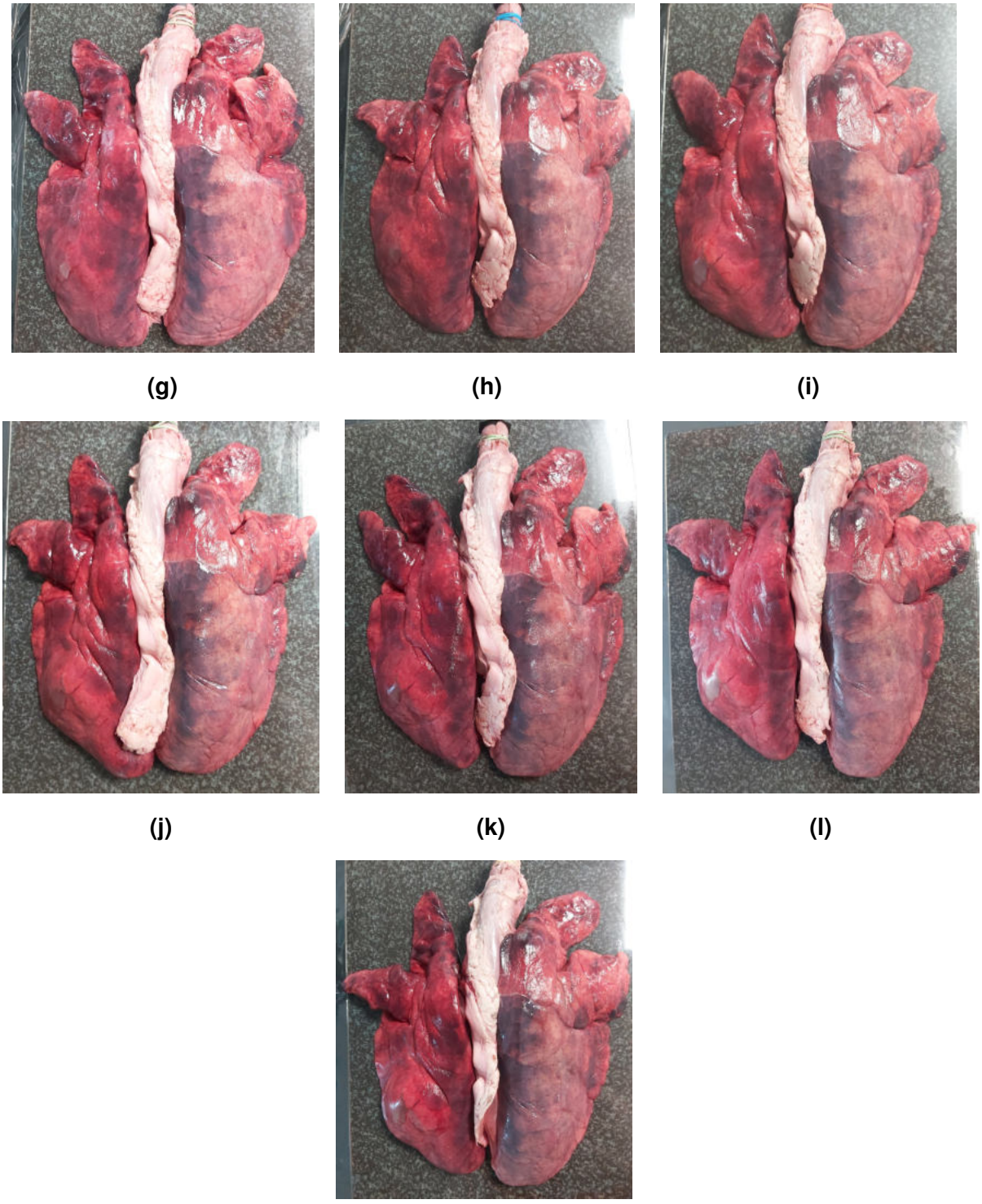


Figure 38: Lung's appearance with the time (Histofix 40 minutes): **(g)** after 96 hours **(h)** after 144 hours **(i)** after 168 hours **(j)** after 192 hours **(k)** after 216 hours **(l)** after 240 hours **(m)** after 264 hours

The change of the color with the perfusion and the time of the storage was also present. Besides the color, the consistency of the lungs' tissue and the size of the lobes changed as well. The tissue of the lungs perfused with Histofix was firmer as well in this case. After 48 hours of storage, figure 37 (e), there was a large increase in red areas in the lungs. From this day, the lungs remained similar in terms of appearance with an obvious better preserved right lung.

4 Discussion

The development and validation of the perfusion system provided promising results. The benefit of the perfusion system is considerable and it could find its place in the field of animal organ preservation procedure.

Comparison of the Perfusion Properties

The properties of the perfusate solution varied during perfusion depending on the solution used in the system but also depending on the time of perfusion. The temperature of the perfusate solution was moved in all four lungs' perfusion processes in the same ranges which ensured almost identical perfusion conditions for all lungs and thus no unwanted influence on the perfusion course. The resistance as a default value for conductivity calculation was changed with the time with the increasing tendency. The calculated conductivity as the inverse value of the resistance then provided decreasing values with the time of the perfusion. The drop in the solution's conductivity with the time was in accordance with the fact that the solution contained fewer and fewer ions that formed the conductivity of the solution. These ions were been already transferred to the lung tissue. In the case of the saline perfusion, the conductivity was moved around 7.669e$^{-7}\pm$ 2.297e^{-8} during the first perfusion and 7.7745e$^{-7}\pm$ 1.418e^{-8} for the second one. In the case of the Ringer's perfusion, it was 6.832e$^{-7}\pm$ 4.229e^{-8} for the first perfusion and 1.037e$^{-6}\pm$ 3.226e^{-8} for the second perfusion. In Histofix perfusion that last for 25 minutes, the first perfusion reached value of 5.184e$^{-6}\pm$ 2.831e^{-7}, the second perfusion the value of 3.832e$^{-6}\pm$ 3.725e^{-7}. The perfusion using Histofix for 40 minutes it was 5.449e$^{-6}\pm$ 9.986e^{-7} for the first perfusion cycle and 3.466e$^{-6}\pm$ 3.821e^{-7} for the second cycle. The difference in the conductivity at the beginning of the two cycles of the same solutions was caused by the difference in the temperature of the solution because the temperature is one of the most influential things on conductivity.

The flow rate of the solution through the system at two different points had still the same property. Whether it was the perfusion using saline, Ringer's solution, or Histofix, the flow rate of the solution recorded by the first sensor placed in the system was higher than the one measured by the second flow sensor. For the perfusion using saline solution, the first perfusion provided the value of 1867.5 $\pm$ 1083.7 ml/s by the first sensor and the value of 504 $\pm$ 339.43 ml/s by the second sensor. The second perfusion provided a value of 1176.6 $\pm$ 511.58 ml/s by the first sensor and a value of 411.2 $\pm$ 304.48 ml/s by the second one. It is possible to determine that the two perfusion cycles provided similar values of the flow rate whether during the first or second cycle.

The perfusion using Ringer's solution provided values of 3292.8 $\pm$ 901.36 ml/s and 1136.6 $\pm$ 665.59 ml/s during the first perfusion cycle. The second perfusion cycle provided values of 1228.7 $\pm$ 672.92 ml/s and 492.92 $\pm$ 459.84 ml/s. Compared with the flow rate measured during perfusion with saline, the measured values of the first perfusion using the Ringer's solution were higher. The second perfusion values were similar to the saline perfusion.

The Histofix perfusions, for 25 and 40 minutes, provided different values. The perfusion that lasted for 25 minutes recorded values of 1414.9 $\pm$ 345.65 ml/s by the first sensor and values of 201.42 $\pm$ 108.84 ml/s by the second sensor during the first perfusion. The second perfusion values gained by the first sensor were 526.05 $\pm$ 310.97 ml/s and by the second sensor 289.09 $\pm$ 229.49 ml/s. The perfusion that last 40 minutes recorded values of 2238.9 $\pm$ 1316.8 ml/s and 359.89 $\pm$ 270.67 ml/s for the first perfusion cycle and the values of 2356.9 $\pm$ 1296.6 ml/s and 617.37 $\pm$ 350.52 ml/s for the second perfusion cycle. The flow rate values between cycles of perfusion and two different perfusions differed only slightly. In general, the measured values of the Histofix perfusion in the comparison to the previous two measurements were very similar to each other and still confirmed the rule of the higher flow rate measured by the first sensor than by the second.

In general, the flow rate measured by the first sensor was 2.5 to 3 times higher than the flow rate by the second sensor. The differences in the flow rate between the two measured points were influenced by the fact that while through the first flow sensor passed the solution directly from the reservoir without any tissue placed in the way to this sensor, the second flow sensor was the one placed directly after the solution passed through the lungs tissue. This passage could change the properties of the perfusate solution and thus also the flow values.

In all perfusions, using saline solution, Ringer's solution, and Histofix, the first pressure sensor provided higher values. These values oscillated around the maximal value of 79 $\pm$ 0.86 mmHg. In some cases, perfusion using Histofix for 25 and 40, perfusion using Ringer's solution, and the first perfusion using saline, this trend was more or less confirmed. The difference that was observable was the pressure course for the second saline solution perfusion. In this case, the pressure value was not been constant in the first half of the perfusion cycle and oscillated between maximal value and minimum value of 0 mmHg very often. This could be caused by numerous air bubbles in the system from the beginning of the perfusion cycle which disappeared with the time of perfusion.

The second pressure sensor recorded values several times lower. For the saline solution perfusion the pressure values were moved around 3.76 $\pm$ 11.77 mmHg, 8.77 $\pm$ 15.39 mmHg by the first sensor and 2.25 $\pm$ 6.54 mmHg, 8.68 $\pm$ 10.43 mmHg for Ringer's solution. For both Histofix perfusion processes, the detected pressure by the second sensor was higher. The values of 13.99 $\pm$ 10.62 mmHg and 8.1 $\pm$ 7.02 mmHg were recorded during the first and second perfusion cycles of the perfusion and lasted for 25 minutes. The mean pressure was 15.98 $\pm$ 12.09 mmHg and 13.58 $\pm$ 9.46 mmHg for the perfusion using Histofix for 40 minutes. The second sensor provided in all cases the oscillation of values that was caused by the pulsatile passage of the solution through the perfusion system and the fact that the pressure detected

by this sensor was influenced by the lungs tissue passage.

Comparison of the Compliance Drop by the Perfusate Solution

The compliance drop was present in all cases. Sometimes, the compliance decreased by half of the initial compliance, other times by less than a half.

The compliance of the lungs was the crucial factor for the assessment of the mechanical ability of the lungs and whether they can be reused for the relevant measurement longer. The initial compliance of 58 ml/mbar for the lungs perfused using saline solution, 88 ml/mbar for the lungs perfused using Ringer's solution, 43 ml/mbar for the lungs perfused with Histofix for 25 minutes, and 41 ml/mbar for the lungs perfused with Histofix for 40 minutes was undergoing changes with the perfusion cycles and the time of the storage.

As is shown in the figure 39, the drop in the compliance was in every single case. For all lungs, major changes in compliance were terminated after only 24 hours of storage. After one day, the compliance value remained at the approximately same value. Compliance for the lungs perfused and preserved usually decreased by around 20 ml/mbar in total. For the lungs perfused with saline and Ringer's solution, the drop of 20 ml/mbar represented only a one-third decrease while in the lungs perfused with Histofix it was the drop by half of the initial compliance.

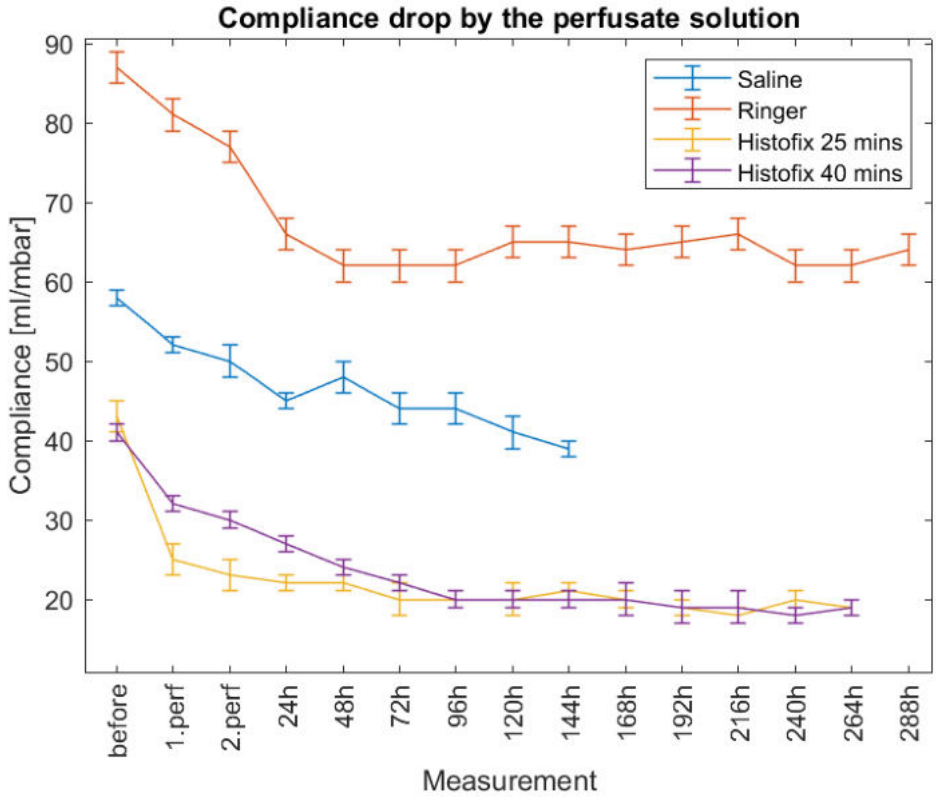


Figure 39: Compliance drop by the perfusate solution

Comparison of the Lungs Storage Duration

The last and very important evaluation of the lungs' preservation success was performed by the visual assessment of the lungs. The duration of the storage for the lungs was varied depending on the used perfusate solution. The flushing from the blood turned out pretty well in all lungs. In the case of the lungs perfused with saline solution, just one part of the right lung was not flushed at all due to the presence of the coagulated blood in this area. The lungs flushed using Ringer's solution were flushed better, only one part of the upper lobe of the right lung remained not flushed. The lungs flushed using Histofix for 25 minutes were bloodier than others, so the appearance of the lungs after the flushing procedure were not flushed up as much as the previous lungs but were flushed out evenly. The last lungs, lungs perfused by Histofix for 40 minutes were from the beginning darker in some areas. These areas were not been flushed from the blood at all.

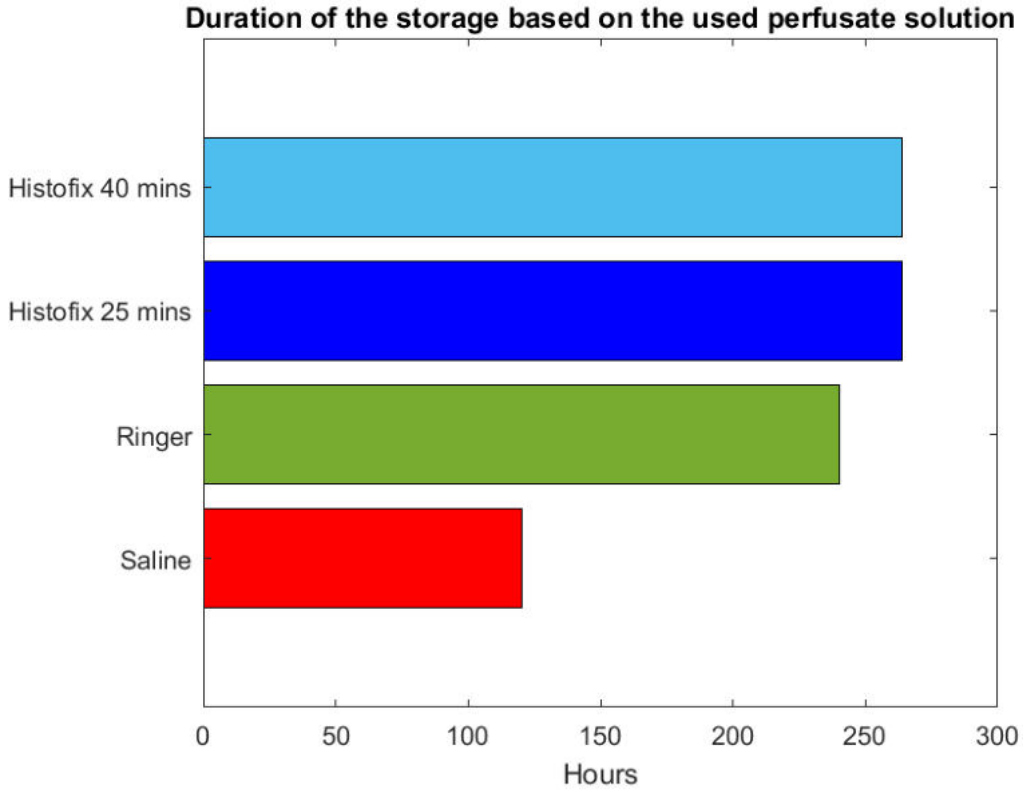


Figure 40: Duration of the lungs storage based on the used perfusate solution

In all cases, the red parts of the lungs increased with the time of the storage. After some time, the red areas changed the color and were darker. Darker parts were then marked as suspicious areas that needed to be monitored. The change of color could indicate the necrosis of the tissue. The progress of the red areas and their change to the black ones was the fastest in the case of the lungs perfused with saline solution. The change in the color of the tissue in

the case of Ringer's solution progressed slower. Both lungs perfused by Histofix, with regard to initial condition, changed their appearance the slowest. The change in the lungs appearance with the time was accompanied by the decrease of the lobe size and by the change of the tissue consistency. In the lungs perfused by the saline solution and Ringer's solution the sudden change in the size and consistency of the lungs were accompanied by an odour. The lungs perfused using Histofix showed no change in odour during storage.

The necrosis of the tissue appeared after 144 hours of the storage in the case of the lungs perfused by the saline solution and so the storage terminated on that day. So, the storage of viable lungs lasted for 120 days. In the case of the lungs perfused by Ringer's solution, the necrosis accompanied the lungs' tissue after 288 hours of storage. On that day the storage of the lungs terminated and the maximal time of the viable lungs storage reached 240 hours. The storage of the lungs perfused with the Histofix for 25 minutes does not finish yet. The lungs stayed viable without visible necrosis at least for 264 hours. The lungs that were perfused using Histofix for 40 minutes were been stored for 264 hours and the storage continued, no necrosis was present on this day, figure 40.

Comparison of the Solution Effect on the Preservation Process

In all cases, the perfusion helped the lungs to stay viable for a longer time and proposed the possibility to be stored. As was expected, saline solution showed the weakest preservation abilities. The color of the lungs as well as the consistency of the tissue changed the fastest. Development of tissue necrosis also occurred the fastest among all measurements.

Ringer's solution and Histofix revealed greater potential in terms of organ preservation. Although the change in tissue consistency, as well as the color, was relatively rapid in the lungs perfused using Ringer's solution, the solution was able to preserve the lungs so that they remained viable and without necrosis for twice as long as the saline perfused lungs.

Histofix perfused lungs were, as expected due to the composition of the solution, the lungs where some tissue areas remained firm and well preserved more than 10 days after storage. The perfusion of the lungs using Histofix was tested twice with the different times of the perfusion (25 and 40 minutes cycles) to quantify if the time of perfusion using Histofix has an impact on the quality of preservation. The lungs were preserved for 40 minutes had still on the 11th day of the storage almost the entire right lung was well preserved and firm. For comparison, the lungs perfused for 25 minutes whereas such well-preserved areas after 11 days were much fewer and were rather an exception.

Based on results from individual measurements, the saline was considered very usable for shorter-term preservation while Ringer's solution and Histofix were applicable in the longer term.

5 Conclusion

In conclusion, it can be said that both the development of the system and its validation were successful. The functionality of the perfusion system has been verified in several measurements. The ability to preserve animal organs for experimental purposes was monitored when using three different solutions, the saline solution, Ringer's solution, and Histofix. In the case of Histofix, the duration of perfusion also showed a positive impact on the quality of the preservation. The system thus developed can be a good basis for further research in the field of the perfusion and preservation of animal organs. For future projects, the subjects of improvement could be the composition of the solution as well as the perfusion system itself.

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List of Figures

List of Tables

List of Abbreviations

ATP Adenosine Triphosphate

Ci Internal Compliance

CNS Central Nervous System

CO_2 Carbone Dioxide

ERV Expiratory Reserve Volume

EVLP *Ex-Vivo* Lung Perfusion

FRC Functional residual capacity

IC Inspiratory Capacity

IRV Inspiratory Reserve Volume

LA Left Atrium

MAP Mean Arterial Pressure

O_2 Oxygen

OCS Organ Care System

ORP Oxidation Reduction Potential

PA Pulmonary Artery

PAP Pulmonary Artery Pressure

Re Expiratory Resistance

Ri Inspiratory Resistance

Rz Resistance of the Central Respiratory Element

RV Residual Volume

TLC Total Lung Capacity

TV Tidal Volume

VC Vital Capacity

A Appendix A

Measurement protocol

Materials

- Animal lungs
- Perfusate solution (Saline solution, Ringer's solution, Histofix)
- Roller pump
- Solution reservoir
- Lung container
- 2 Flow sensors BIO-TECH
- 2 Invasive pressure sensors TruWave
- 2 On-chip pressure sensors MPX
- Microcontroller Arduino
- CoolTerm serial port terminal software
- Cannulas
- Perfusor lines
- Stopcocks
- BellaVista ventilator
- Steinless steel chamber
- MeterMulti 3620 IDS (for measuring of temperature)
- 3 Boxes for lungs storage
- 3 Resealable bags

Measurement Procedure

1. Measure the compliance of the lungs.
2. Connect the lungs to the setup and place them into the lungs box in the lying position.
3. Flush the lungs using a small quantity of the Saline to clean up the lungs from the blood.
4. Fill the reservoir with the determined volume of the fresh perfusate solution:
   a) 1 L of perfusate solution for rabbit lungs
   b) 2 L of perfusate solution for porcine lungs

Perfusate Solution	**Name**	**Composition**
1. solution	Saline solution	NaCl, water
2. solution	Ringer's solution	NaCl, KCl, $CaCl_2$, $NaHCO_3$, distilled water
3. solution	Histofix	4 % formaldehyde, methanol, Na_3PO_4

5. Measure the temperature of the solution in the container using the MeterMulti 3620 IDS multimeter.
6. Turn on the roller pump on constant speed (duty of the pump: 100-150).
7. Perfuse the lungs with the solution for the time unit:
   a) 20 minutes for rabbit lungs
   b) 30 minutes for porcine lungs
8. Record flow parameters of the solution using two flow sensors. Sensors are placed in the direction to the pulmonary artery (prior) and to the left atrium (posterior) that are cannulated (could be secured by sewing).
9. Record pressure parameters of the solution using two on-chip MPX pressure sensors and two invasive pressure sensors. One from each is placed at the input point of the lungs (pulmonary artery) and one at the output point of the lungs (left atrium).
10. Continuously, measure the resistance the solution in the reservoir using the Amprobe multimeter.
11. From the resistance values, calculate the conductivity of the solution using the given relation:
$$\sigma = \frac{1}{\rho} \tag{6}$$
12. After the specified time unit, turn off the roller pump.
13. Dispose of the already used solution.

14. Evaluate differences between two measured points of flow and pressure.
15. On BellaVista ventilator, chose the adult values of ventilation (P_{insp} = 20 cmH_2O, PEEP = 5 cmH_2O, freq = 12 AZ/min).
16. Measure compliance of the lungs by placing the lungs into the enclosed stain-less steel chamber and connecting it to BellaVista ventilator.
17. Evaluate measured compliance.
18. Repeat the cycle from the step 3 (with the same type of the solution), until the differences in compliance are present.
19. When there are no differences in compliance, place the lungs to the resealable bag filled with a small quantity of the Ringer's solution, place them to the box and stored them in the fridge.
20. Repeat the measurement process from step 1 for each solution in the table (Saline, Ringer's solution, Histofix). Every time, new lungs are used.

Evaluation of preservation: Solution influence

1. Take out from the fridge the lungs stored for 24 hours.
2. Take the photo of the stored lungs.
3. Measure compliance on the BellaVista ventilator.
4. Store and measure the compliance every 24 hours.
5. Repeat the procedure while the necrosis of the tissue is not present.
6. Evaluate the visual and mechanical changes in the lungs with the time of the storage.
7. Repeat the evaluation procedure for the lungs perfused with the Saline solution, the Ringer's solution and Histofix.

B Appendix B

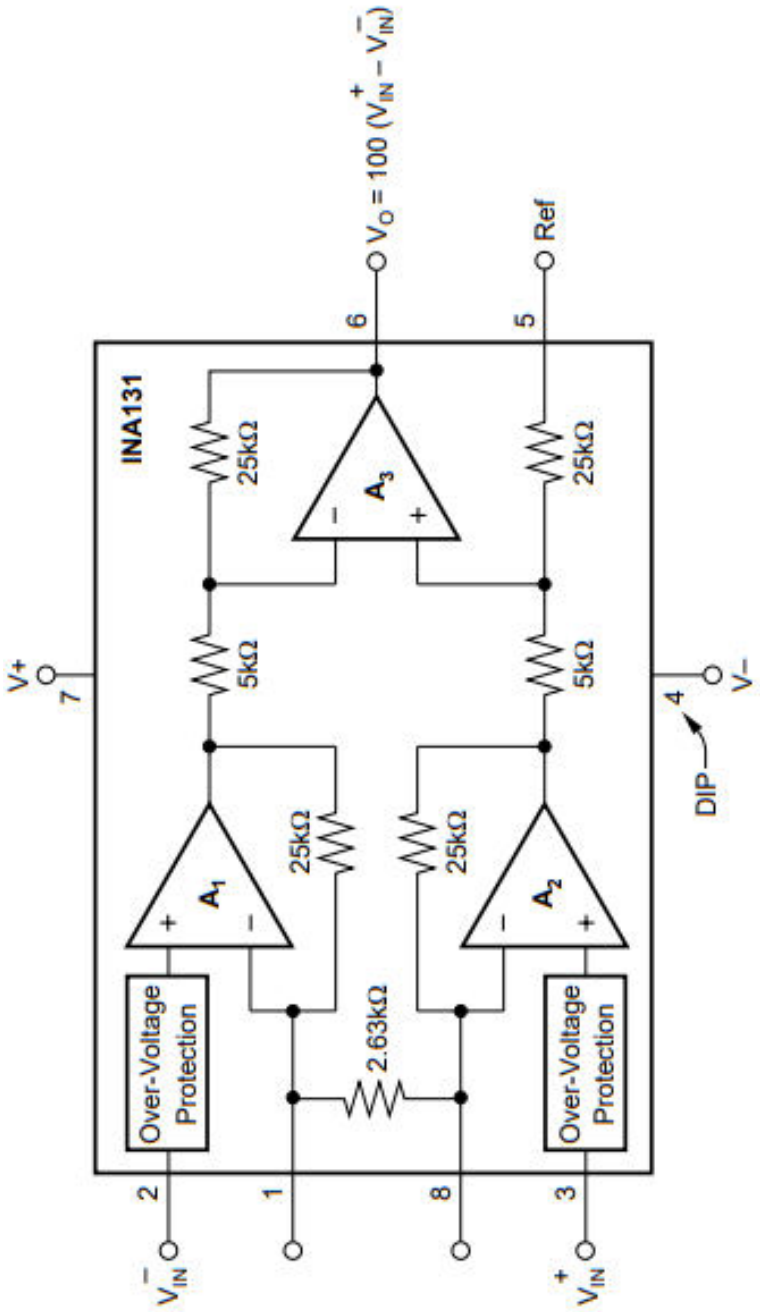

Figure 41: Instrumentation amplifier INA131 [32]