# Portable Normothermic Perfusion System for laboratory test of Microwave Devices

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***Abstract -- The need to keep livers as long as possible awaiting transplantation and to conduct laboratory tests to assess the differences between perfused and non-perfused organ using a microwave thermal ablation tool, required to implement a previous perfusion system normothermic. This required the implementation of new components necessary for the survival of the organ and for the transportability of the structure, necessarily increasing its dimensions. The new perfusion system allowed to perform thermoablation tests on ex vivo pig liver using two non-internally cooled tools (14 gauge, 17 gauge) fed at 120 W. The results showed that in the case of a perfused organ its surface temperature near the tool tends to stabilize, unlike what happens in the non-perfused organ. Moreover it is observed that the ablation area undergoes a reduction of about 30% in the radius and of about 55% in the volume passing from non-active perfusion to active perfusion.***

***Keywords - Perfusion System, Ex-vivo Tissues, Liver Transplant, Thermal ablation , Microwave***

## I. INTRODUCTION

The liver has important regenerative characteristics, such as to allow the removal of some of its parts without compromising its functioning. However, situations may arise in which the organ is so compromised as to require replacement by transplant. This procedure is based on the resection of a part of the donor's liver to be implanted in the patient to regenerate itself completely.

This intervention, a highly successful therapy for the last three decades, has become one of the main treatments for late-stage liver diseases, with over 11,000 transplants performed annually worldwide. As a result, the procedure has become the victim of its own success: more and more patients require transplantation, but the quantity of available organs has remained practically constant. The main source of organs comes from donors with heart still active, although in the presence of brain death (heart-beating donor, HBD), from which the organs can be removed with a minimum interruption of oxygenation before cooling and its conservation [1]. However, the increase in request has led to the need for additional sources: both organ transplants from living donors (elderly, obese) and marginal use of organs from cadavers have entered clinical practice (patients without a heartbeat) [2], [3], [4]. They are known to be more inclined to malfunctions [5], [6], [7], [8], causing up to 40% of them to be previously rejected as a potential cause of problems in the event of a transplant [9]. In recent years, the increasing use of these organs has been accompanied by a growing interest in strategies to optimize conditions during the storage period, allowing better results to be achieved: a prolonged graft survival, a decrease in malfunctions and a consequent lower need for second transplants. The standard procedure involves a “static cold storage” of the organ taken, according to the principle of slowing down the metabolism and reducing cellular swelling thanks to the composition of the solution used for storage. However, this method can cause various damage to the liver in the later stages of the procedure, from storage to perfusion before grafting [1], [10].

Since the mid-twentieth century, the effectiveness of normothermic perfusion has been demonstrated following tests on various organs, which has allowed them to be kept alive for several days thanks to adequate oxygenation [11]. The main difference between cold storage preservation and normothermic preservation is that the metabolism of the organ is maintained through the combination of a continuous circulation of metabolic substrates for ATP regeneration and product removal waste [1] . As concerns the liver, over the years equipment such as Liver Assist, or perfusion systems for the maintenance of the organ while awaiting transplantation, have been designed and are currently on the market [12]. They allow a real regeneration of the hepatic tissue as they provide all the components necessary to develop their own functions, reproducing as much as possible the physiological environment, even if the organ is partially damaged [1].

With reference to the anatomy of the organ, the system includes two entrances through the portal vein and the hepatic artery, and two exits represent from a suprahepatic inferior vena cava and intrahepatic inferior vena cava. The circuit consists of a main tank in which the fluid temperature is kept constant at 38 ° C and from which three branches originate: the first to the venous reservoir; the second which gives the fluid the physiological characteristics towards the hepatic artery, through an oxygenator; the third towards the portal vein.

The perfusor shown is developed starting from a normothermic perfusion system born from the need to perfuse a swine liver with physiological heparinized to

conduct experimental microwave thermoablation tests without the need to access the enclosure.

## II. OBJECTIVES

The objectives of a system for liver perfusion are mainly to keep the organ alive for as long as possible before transplantation, an improvement in liver function and an increase in survival of recipient patient.

In the liver it's possible to study the phenomena that involve the interactions between the cellular component of the hepatocytes and the parenchymal cell component, including the cells of the bile ducts, the connective cells and those of the capillary endothelium. Thanks to the maintenance of an adequate blood perfusion it's possible to control or study physiological parameters such as blood pressure and perfusion speed.

The main objective of the developed normothermal perfusor is to demonstrate the successful perfusion of the liver by the infrared thermal-camera (FLIR A320G) and to evaluate the effect of perfusion during microwave thermal thermoablation test [14].

Due to intrinsic limits of the previous perfusor system [13] it is also necessary to implement the device by adding a suitable heater and an oxygenation circuit to evaluate a possible future use for organ transport even if today the perfusor has a strictly research purpose. For the choice of the components, a study was carried out on the desired flow rates, pressures and temperatures and a market survey that allowed the selection of components with a high quality - price ratio.

So that, it has been necessary to introduce the following improvements:

- Integrate an organ oxygenation circuit inside the system. This requires the addition of two components, such as an oxygen concentrator and an oxygenator.
- The introduction of a heating system that does not damage the erythrocytes.
- Identify a system able to guarantee a constant flow rate in portal vein equal to the physiological value of 900 ml/min – 1200 ml/min.
- Identify a fluid with rheological characteristics like blood.
- Plan a battery to be integrated into the system to ensure perfusion during transport.

It was therefore essential to modify the configuration of the system, necessarily going to increase its size to allow the introduction of the new components and relocating the present constituent elements. In addition, the liver housing inside the device has been shifted to the upper part, for both practical and hygienic reasons.

The new structure is made entirely in aluminum and plexiglass, making it extremely light.

## III. MATERIALS AND METHODS

### A. *Perfusor Structure*

The addition of the new components required the enlargement of the structure. For practical reasons the structure has been designed on two levels: the upper part houses the organ, while in the lower part are housed the various components of the perfusion circuit, such as the oxygenation circuit, the two centrifugal pumps, a peristaltic pump and the heating system with its 5-liter capacity reservoir. The main reservoir sizes are of 200x100x150 mm and 5 mm thick with a capacity of 1.5 l, are arranged. To make the structure light and therefore more easily transportable, it is made entirely of aluminum, while the 2 mm thick plexiglass panels make the various components visually accessible. The presence of four self-locking wheels further facilitates transport.

The structure has a size of 900x500x500 mm, of which the upper part of 200x500x500 mm, and the lower part of 500x500x500 mm. The liver case consists of a plexiglass reservoir measuring 150x500x300 mm and 5 mm thick.

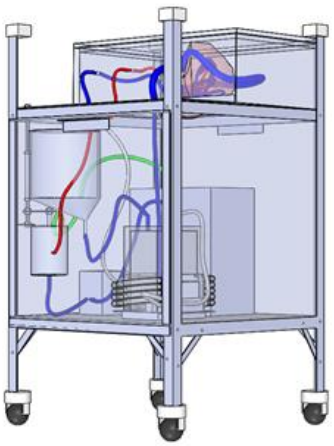

*Figure 1: Perfusion Structure.*

### B. *Oxygenation Circuit*

To keep the liver alive, it is necessary to integrate a circuit for the oxygenation of the blood inlet in hepatic artery with which it is perfused into the system. This requires the addition of two components, such as an oxygen concentrator and an oxygenator. The first step was to conduct a bibliographic analysis to identify the components necessary for the realization of a normothermal perfusion device for the liver. A market analysis was therefore performed in order to select suitable components for this purpose, at a reasonable price, such as:

Oxygen concentrator: SimplyGo PHILIPS - RESPIRONICS;

Oxygenator: Affinity Pixie - MEDTRONIC.

The chosen oxygen concentrator is a portable device capable of delivering both pulsed and continuous flow oxygen (from 0.5 l / min to 2 l / min), a mandatory mode

for the perfusion of taken organs. The device has the following operating characteristics:
Weight included battery 4.5 kg;
Operating temperature 5 - 40 ° C;
Battery life 3 h (pulsed flow) and 0.9 h (continuous flow at 2 l / min);
Internal antibacterial filter;
Lithium ion battery 14.4 VDC 6.600 mAh (Li-Ion), 0.7 kg;
Charging time about 2-3 h (completely discharged battery).

*Figure 2: Oxygen Concentrator.*

The Affinity Pixie Oxygenation System delivers performance and versatility for neonates, infants and small children requiring cardiopulmonary bypass at flow rates up to 2.0 L/min. It is composed of an oxygenator and a venous reservoir.
This pediatric hollow fiber oxygenator, which is a microporous disposable device with hollow fibers for gaseous exchange with plasmaresistent polypropylene fibers and integral PET heat exchanger. It presents the following characteristics:
Max gas / blood ratio 2: 1
Oxygenator membrane surface 0.67 m2
Recommended blood flow rate 0.1-2.0 l / min
Max water pressure 206 kPa (1550 mmHg)
Max nominal blood pressure 100 kPa (750 mmHg)

The venous reservoir is a disposable device for blood collection and storage during extracorporeal circulation. Its characteristics are:
Tank capacity 1200 ml
Recommended blood flow rate 0.1-2.0 l / min
Min operating level 20 ml
Max nominal pressure +20 mmHg / -100 mmHg.

*Figure 3: Oxygenator.*

To obtain a pulsed flow typical of hepatic artery flow, a peristaltic pump is used which takes the fluid from the heating circuit reservoir and sends it to the oxygenator inlet. The peristaltic pump is upstream of the oxygenator to prevent the presence of lumps and air bubbles entering the organ. Despite its location in the oxygenation circuit, fed at 5V, it is possible to reproduce the physiological values of pressure and flow rate.

### *C. Heating System*

The fluid in the reservoir circulates in the pipes passing through the system thanks to a recirculation pump, fed at 5V. A thermostat powered by 12V 10A, detects the temperature of the fluid, through a probe immersed in the reservoir and in contact with the wall, allowing the activation of the plates to guarantee a temperature of 38-39 ° C.
The thermostat has the following characteristics:
Measuring range: -40 / 120 ° C;
Measurement accuracy: 0.1 ° C;
Measurement error: + 0.5 ° C;
Operation temperature: -20/70 ° C.
The heating system is composed of two Joule effect plates made of PTC aluminum, secured by M3 screws to milled aluminum plates to allow for the housing of 304 stainless steel, cheap and compatible (ASME Publication, 1980). The plates have been made of aluminum by virtue of their characteristics in terms of lightness, thermal conductivity, economy and ease of processing. Hemocompatible silicone tubes are mounted by interference on steel tubes of the same diameter to ensure continuity of flow.
Two Joule Effect PTC Aluminum Plates have the following characteristics:
Power: 60W;
Voltage: 12 V;
Temperature: 180 + 10 ° C.

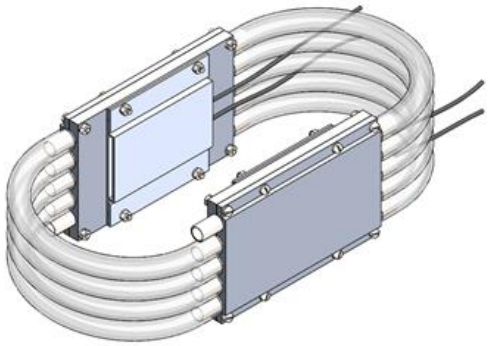

*Figure 4: Heating System.*

### D. Constant Flow Rate in the Portal Vein

It has been adopted a centrifugal pump fed at 5V located in the heating system reservoir to guarantee a constant flow rate in the portal vein, given the presence of losses neglected in the previous configuration. Tests have been carried out to assess the most suitable voltage to guarantee values that best match the physiological values of flow and pressure (tests performed at 3V, 4.5V, 5V, 6V, 7.5V, 9V, 12V).
It is reliable, biocompatible, low cost and not re-sterilizable. Its characteristics are:

- Voltage DC: 12 V;
- Power: 4.8 W;
- Max flow rate: 240 l / h;
- Operation temperature: 0-60 ° C;
- Max lifting high: 3 m.

### E. Non - Newtonian Fluid

In order to evaluate the functioning of the system avoiding the use of blood, a fluid was identified that was able to simulate its behavior. The blood has a dynamic viscosity of about 3 cP. To make a fluid capable of reproducing the rheological properties of blood in terms of viscosity and density, it is possible to use distilled water, glycerol and cornstarch. Since the blood has a physiological temperature of about 37 °C, the percentages of the constituent elements of the fluid must therefore be determined.
With a capillary viscometer, it is possible to measure the kinematic viscosity [m2⁄s], from which it is possible to calculate the dynamic viscosity [cP]. It was estimated that at 37 °C the fluid with a viscosity as close as possible to the blood is composed of:
70% distilled water; 30% vegetable glycerol. Dynamic viscosity $\mu = 3.52 \pm 0.01$ cP
70% distilled water; 30% vegetable glycerol; 1% cornstarch. Dynamic viscosity $\mu = 3.52 \pm 0.15$ cP.

Therefore, since about 3.5 L of fluid is circulated in the system in question, it is possible to simulate blood using 2450 mL of distilled water and 1050 mL of glycerol (and 35 mL of cornstarch).

### F. Electronic and battery components

A lithium ion battery has been designed for the perfusion system to be autonomous during the organ transport phase. The choice of these cells rather than those of nickel-cadmium or nickel-metal-hydride depends on the highest level of safety, energy density, efficiency, occupied space and costs.
A 6s-3p configuration was chosen to power all the components of the perfusor. The wiring diagram for battery management and component operation was printed on a dedicated PCB which controls: the Arduino Nano 5V 16MHz board, the five Hall effect current sensors - ACS712 20 A, the 16x2 5V LCD display to display the battery charge level, the 22 kΩ + 4.7 kΩ voltage divider, the buzzer and the 10k trimmer contrast display regulator. Each component to be powered was then connected to the PCB using the appropriate DC-DC power converter.
The batteries chosen are the Samsung INR18650 - 35E 3500mAh, with a diameter of (18,55 ± 0.1) mm, a high of (62,25 ± 0,1) mm, and a weight of (48 ± 1) g. Their features are:

- Nominal Capacity: 3500 mAh;
- Minimum Capacity: 3350 mAh;
- Nominal Tension: 3,6 – 3,7 V;
- Charging Voltage: 4,2 V;
- Discharge Current: 8 A;
- Max Discharge Current:13 A;
- Charging Current: 0,6 C 2000 mA;
- Final Discharge Voltage: 2,65 V
- Pluspol: FlatTop
- Chemistry: LiNiCoAlO2.

The system is housed in a case to allow transport even separately from the perfusion device.

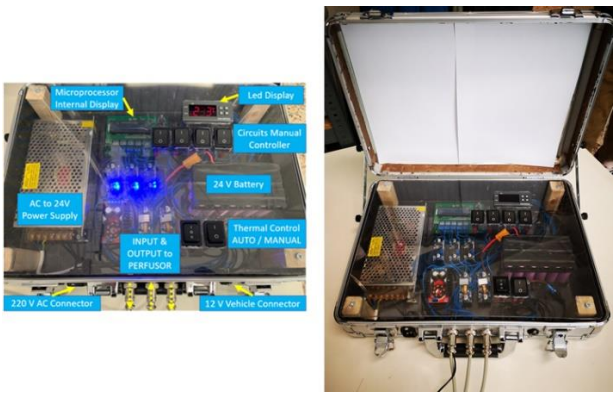


*Figure 5: Electronic Components.*

### G. *Starter Protocol*

As a first step it is necessary to fill the main reservoir with the perfusion fluid to then activate centrifugal pump of the heater system. Therefore the circuit preheating phase starts. It is necessary to carry out a gradual heating of the blood to avoid the onset of too high thermal divergences in the heat exchanger, as with instantaneous ΔT too high between the wall and the adjacent layer of fluid meatus the formation of clots may arise: this it would cause a loss of efficiency of the exchanger and of the amount of blood useful for perfusion. This phase ends upon reaching the physiological temperature value (38 ° C) set and monitored through the RP Thermocouple and the RA Thermocouple and is kept constant until the completion of the cannulation and deposit phase of the explanted organ.

### H. *Thermal Ablation Tests*

Two TATO[1] thermal ablation systems no-internally cooled 14-gauge and 17-gauge applicators (NIC) were tested. The experiment was carried out on fresh ex vivo swine livers recovered from animals in the food chain: this would make it possible to obviate important ethical implications and also reduce the economic burden compared to the most common in vivo studies.
The liver was taken and immediately connected to the perfusion apparatus by means of flexible tubes sutured to the vessels.

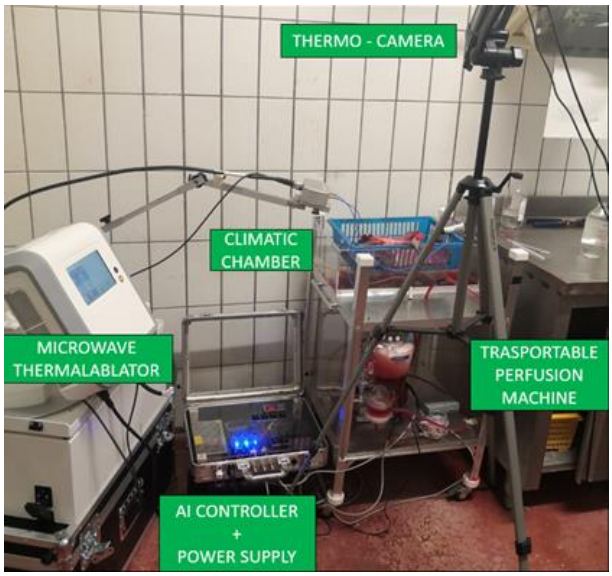


*Figure 6: Setup System.*

The tests were conducted on an ex vivo organ, perfused and not perfused, in order to evaluate the coagulation divergences. The tool is powered at 120 W for 10 minutes.

## IV. RESULTS

Plotting on a time-temperature graph the thermal trend acquired by the thermal-camera during the tests it's clear that:
when perfusion is active, the surface temperature of the liver near the tool tends to stabilize reaching lower temperature peaks than in the case in which the perfusion does not is active;
when perfusion is not active, the temperature continues to grow over time without stabilizing itself following an almost linear trend.

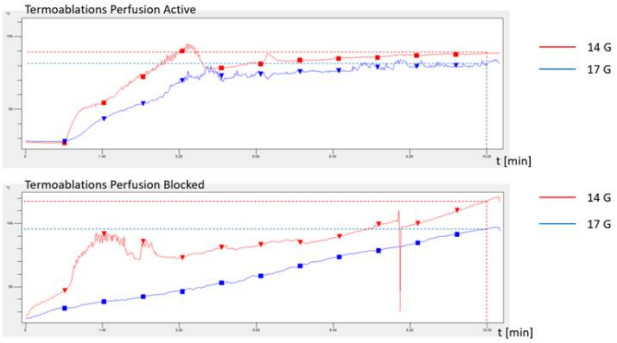


*Figure 7: Tests Thermical Trend*

The preliminary results obtained by comparing ablation performed in the non-perfused and perfused state show a reduction of about 30% in the radius and about 55% in the volume passing from one to the other, regardless of the size of the tool.

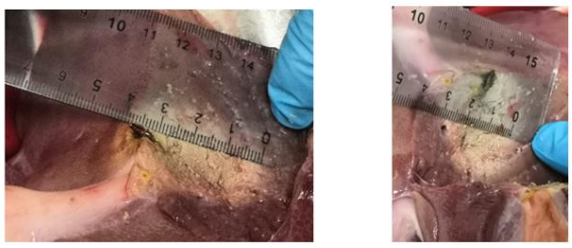

*Figure 8: 14 Gauge Non-Perfused Liver.*

[1] TATO (Thermal Ablation Treatments for Oncology) is a multi-applicator system developed from a scientific/industrial collaboration between the University of Florence and the biomedical society.

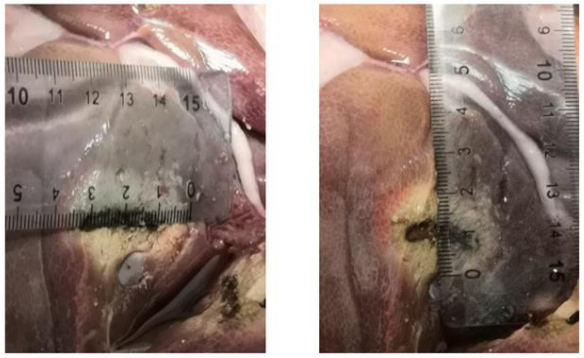

*Figure 9: 14 Gauge Perfused Liver.*

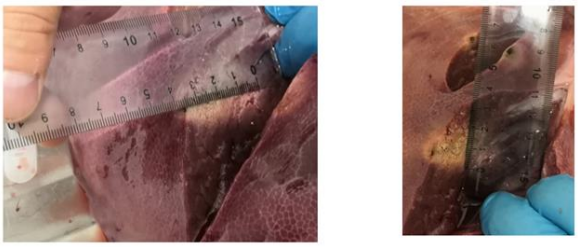

*Figure 10: 17 Gauge Non-Perfused Liver.*

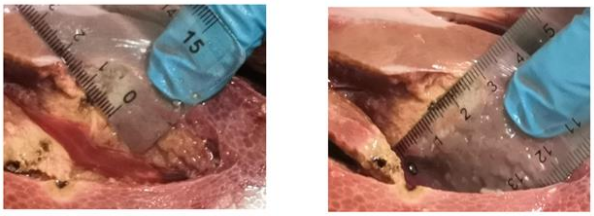

*Figure 11: 17 Gauge Perfused Liver.*

The availability of a thermoablation model based on an ex vivo perfused liver could lead to a more detailed investigation into the effect of heat dissipation in peripheral and central sites of vessels. This model could also be used to emulate laparotomic surgical ablation, which is rapid and economical, allowing the surgeon to rotate the liver within its anatomical environment, manually protect the heat-sensitive organs (intestine) and easily insert grouped applicators to treat large non-spherical ablations. The perfusion device used to ensure blood flow within the explanted swine liver makes it possible to easily modify the perfusion rate of the hepatic parenchyma by emulating fibrosis, cirrhosis or steatosis in order to study the influence on volume and on ablation size. However, the preliminary results obtained with this study are too limited to obtain statistical relevance: the final validation of the proposed approach will therefore require a further and more in-depth experimental campaign.

## V. Discussion

The plates are designed to avoid haemolysis of red blood cells due to the effects of thermal osmosis, a phenomenon comparable to a force capable of guiding the moleculars water transport in cellular structures. Maintaining small values of ΔT through the cell membrane can generate high values of stationary ΔP (if ΔT = 0.01 ° C, ΔP = 1.3 atm). The extent of thermal osmosis depends on the transfer energy of the water molecules, Q*, from the outside of the membrane inside the cell $\left(\Delta P = -\frac{Q*}{VT}\Delta T, V = Molar\ Volume\right)$. The induced ΔP can determine the swelling of the cells and causes its rupture if the ΔP is large and associated with a rapid T. For times less than 2 μs, it results:

- If ΔT = 0.05 ° C → No visible damage;
- If ΔT = 0.5 ° C → Hemolysis.

The designed system is such that the instantaneous ΔT between fluid and plates is less than 0.5 ° C.

Our main objective was to create a compact and transportable system able to preserve the physiological behavior of the liver once it was removed, to allow the implantation in a receiving patient.

The experimental tests performed allowed to verify the stability of the system in terms of pressure, flow rate and temperature. Experimental tests were performed using a simplified liver model made through 3D printing and with the sole function of heat exchanger.

The subdivision of the device into three distinct modules allows the possible replacement of the individual components in the event of need.

The positioning of the liver in the upper part allows better accessibility and guarantees greater sterility of the organ.

The aluminum and plexiglass structure ensure that the device is light and easily transportable even thanks to the wheels.

## VI. Conclusion

We have designed a light and easily transportable device capable of perfusing an explanted liver with a fluid with characteristics in terms of viscosity similar to those of blood.

The tests carried out using a mockup instead of a real liver made it possible to verify, using electronic sensors, the correspondence with the physiological values in terms of pressure, exposure and temperature.

However, it was not possible to conduct in vivo validation tests on laboratory animal guinea pigs as it requires a prior analysis of reliability and criticality of the system. This would also allow an optimization of the electronic part by implementing safety devices and monitoring of the variables.

This device would also allow testing with energy surgical tools to evaluate the coagulation efficacy on perfused ex vivo tissue and could be used for cell culture processes on a 3D scaffold.

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