UPGRADE OF THE LANGENDORFF APPARATUS USING THE INFRARED THERMO-CONTROL SYSTEM AND AN INTELLIGENT HEATER

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Abstract

Biological experiments involving isolated organs and tissues demand precise temperature monitoring and regulation. An automatic temperature control system was proposed and optimised on real isolated swine hearts and the prototype is described in this work. The traditional Langendorff apparatus consists of a heart holder, a reservoir of perfusion solution flowing to aortic cannula and a heating bath allowing passive heat transfer to the reservoir of perfusion solution. The commercial infrared camera FLIR T62101 was added to this basic set-up and used for very precise monitoring of the temperature kinetic of the organ and connected with an electronic feedback loop, which allowed real-time and precise regulation of heat transfer from the heating bath to the perfusion solution and in turn indirectly to the heart tissue. This provides real time control and active regulation of the myocardial tissue temperature. The infrared camera was tested in several modes and several variants of detection were optimised for ideal measurement of the region of interest of the ex vivo organ. The kinetics of the temperature changes and temperature stability of the tissue were recorded and calibrated by external electronic thermometers (type Pt100, inserted in tissue). The time lapse from the hang-up of the hypo termed organ (30 °C) until optimal warming (37 °C) was less than eight minutes in the final instrument prototype. The final stability of the 37 °C tissue temperature was approved; the temperature fluctuation of left ventricle tissue was characterised as 36.8 ± 0.5 °C. This upgraded traditional instrument could be used in specific preclinical and clinical transplantation and analytical projects in future.

Keywords

isolated heart, heart perfusion, tissue thermostability, thermo controller, IR thermometry

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Introduction

The use of an isolated beating heart has, over many decades, resulted in fundamental discoveries that improved an understanding of the heart's physiology and cardiovascular medicine [1]. A retrograde-perfused heart is a method that was improved by Langendorff at the end of the 19th century [2]. This method (scheme on Fig. 1) is based on the perfusion buffer flowing retrogradely down the ascending aorta. The aortic valve is closed under pressure, and the coronary arteries are thus filled via the left and right coronary ostia (Fig. 1).

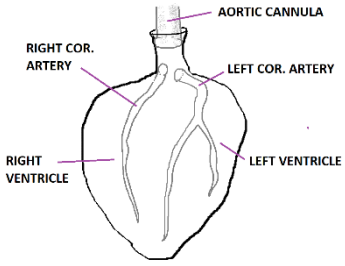

Fig. 1: Overview of heart perfusion using the Langendorff method.

The most commonly used mode for the Langendorff apparatus is called 'constant hydrostatic pressure'. The pressure is set by the height column of the fluid (the elevation of solution surface in a reservoir and hydrostatic pressure difference to the tip of the cannula in the aorta).

The *ex vivo* beating heart is an excellent model system for observing heart physiology or heart electrophysiology on the macroscopic and microscopic level, the coronary vascular function and principals of regulation, and also for simulating of medical intervention in preclinical tests [1, 2]. The perfused heart is also used for the extraction of single living cardiomyocytes from a specific site of the heart muscle or isolation of tissue entities, which are being considered by many biomedical teams [3, 4].

Experiments on isolated hearts demand a precise setting and control of the temperature of the heart tissue. Nevertheless, difference between perfusate temperature and local epicardial temperature can be several units of Celsius degrees.

We present a technological concept involving adding thermosensors to the traditional Langendorff apparatus. This upgraded system can be used for precise thermo-control of the tissue, especially the left ventricle. Our prototype was tested in experiments on pig hearts; however, the application could be easily modified for smaller or bigger isolated hearts.

Material and Methods

Basic design and redesign of the Langendorff apparatus

The basic Langendorff setup was modified. Traditional components (shown in Fig. 2) were supplemented using a Forward Looking Infrared IR (FLIR) detector camera FLIR-T62101 (FLIR Systems, Inc., Wilsonville, USA) and computer connectivity to the FLIR camera and the power supply for heating the bath (details in Fig. 3).

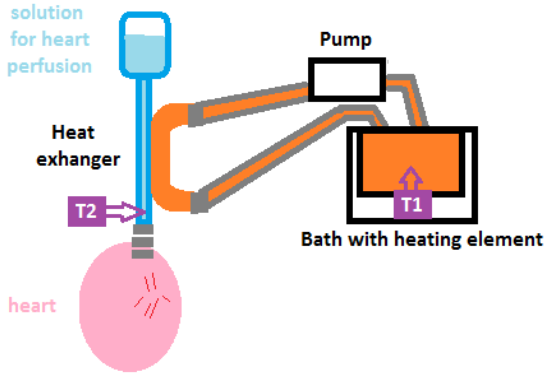

Fig. 2: Components of the basic Langendorff setup. T1 is temperature of the thermostat bath, T2 is temperature of the perfusion solution near the entrance to the aorta.

The computer was equipped with Universal Serial Bus (USB version 3.1, 10 Gbit/s) designed to high-speed upload of data packets from the FLIR camera. The utility created on MATLAB platform (MathWorks, Natick, Massachusetts, USA) was able to identify the temperature of the left ventricle (the central field of the heart – 320×256 pixels) and record it in the form of a data matrix in real time. The utility was set to compute the minimal, maximal and mean value of the temperature points on the 320×256 matrix and command the power supply of the heating elements in the bath in real time (see Fig. 3 and Fig. 4).

The illustrative visualisation of the data matrix (left ventricle) is presented in the form of a pseudocolour frame, as shown in Fig. 5. After carrying out a basic test of functionality with respect to the setting of the camera and computing system, three important engineering tasks had to be resolved: (i) testing of an effective time lap between detecting the tissue temperature (ΔT_{IR} respectively) and change in the temperature of the perfusion fluids (point with 'T1 thermometer', as shown in Fig. 3); (ii) testing of the time laps between detecting the tissue temperature and sufficient compensation of temperature T2 and the real temperature of the left ventricle; (iii) assessing the temperature measured by the IR camera (a comparison with contact needle thermometers).

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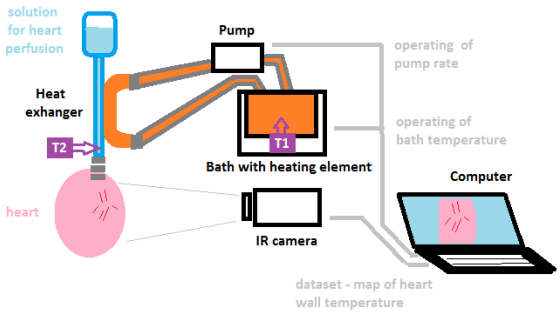

Fig. 3: Langendorff setup modified by the IR camera and computer for electronic regulation of the heating elements of the bath. Automatic screening of heart tissue is followed through image processing using a computer and the operation of the heating elements (respectively the pump rate). The repletion of the camera scan and commanding of the heating element is 1 Hz. For each small area of 4×4 pixels the average temperature T_{IR} *and difference* $\Delta T_{IR} = 37 - T_{IR}$ *(in degrees of Celsius) are computed. The mass of the tested experimental pig heart was 0.35 kg, the heart tissue was obtained during slaughter processing of the pigs.*

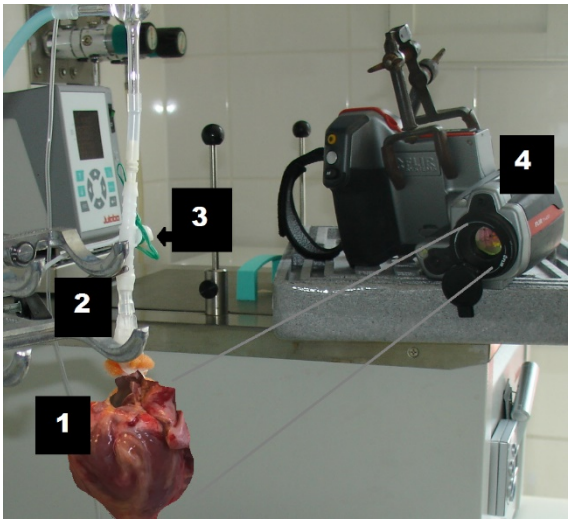

Fig. 4: The scheme of geometrical arrangement of components: 1 – isolated heart, 2 – holder and cannula, 3 – thermometer used for the fluids introduced into the aorta, 4 – IR camera (focused on the left ventricle).

Results

Time lap between the IR camera's detection of T_{LV} and readjusting T1

The stability of the temperature of the water in the bath is the main limiting factor for regulating the speed of the temperature of the perfusing solution (blood substitute - Tyrod solution) entering the aorta. This thermodynamic fact was immediately obvious at the beginning of the experiment. The bath contains two litres of distilled water, and the heating elements have maximal output P_{max} = 2 kW. We tested the several programming formulas for commanding the heating elements, the optimised software setting for the activation of output P of the heating elements was adjusted to the following formula:

IF $\mathbf{\Delta T_{IR}} \geq 3$ THEN set $\mathbf{P} = 1 \times P_{max}$
IF $3 \geq \mathbf{\Delta T_{IR}} \geq 0$ THEN set $\mathbf{P} = 0.5 \times P_{max}$

This setting gives the following time laps (Table 1) for stabilising the temperature of the bath (final 37 °C).

Table 1: Stabilisation of T1.

Starting $\mathbf{T_{IR}}$	Final $\mathbf{T_{IR}}$	Time (s)
30 °C	37 °C	138
32 °C	37 °C	69
34 °C	37 °C	35
36 °C	37 °C	8

These time laps are sufficient for stabilising the heart tissue temperature for large set of biological applications.

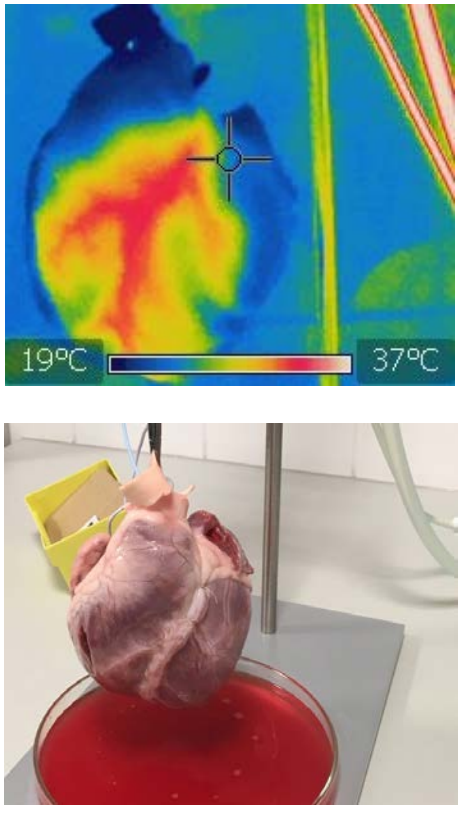

Fig. 5: Typical frame from the IR camera visualised by the MATLAB utility (A). The corresponding photo of the left ventricle (B).

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Quantification of the time lap between the IR camera's detection of T_{LV} and readjusting of T2

The effective transfer of heat from the bath to the fluids in the proximity of the aorta is another problem. A primary setting involving operating the heat elements has been found to be insufficient for transferring the heat quickly. An operation also had to be carried out for the pump rate: the pump was upgraded through an electronical connection to the computer, where the motor of the pump was operated by this optimised direction:

IF $\mathbf{\Delta T_{IR}} \geq 3$ THEN set $\mathbf{V} = 1 \times V_{max}$
IF $3 \geq \mathbf{\Delta T_{IR}} \geq 0$ THEN set $\mathbf{V} = 0.5 \times V_{max}$

(where the maximal output V_{max} is the maximal pump output 0.3 litres per second; V is the actual pump output).

This setting gives the following time laps for stabilising the temperature **T2** and $\mathbf{T_{IR}}$ (Table 2; for this purpose measured as the average temperature in the central 2×2 cm of the left ventricle).

*Table 2: Stabilisation of **T2** and $\mathbf{T_{IR}}$.*

Starting $\mathbf{T_{IR}}$	Time t_{T2} (s)	Time t_{TIR} (s)
30 °C	246	382
32 °C	97	199
34 °C	52	85
36 °C	17	32

Assessment of the accuracy of the temperature measurement using the IR camera

The evaluation of the accuracy for the temperature measurement using the IR camera can be split into two steps. First step (calibration of FLIR camera system itself); the parameters of reflectance in the IR camera setup was adjusted to the moistened surface of the skin model (heated body) by a FLIR service technician. Second step (checking of detection validity on real cadaver); the correlation between the real pig tissue temperature (cadaver preheated in thermostatic bath, control needle temperature at depth of 3 mm) and temperature value from display of IR camera was checked using. Thermometer (Pt1000, type VOLTCRAFT TPT-206 VC-8603650) connected to Voltage-meter and PC was used (Fig 6A). In order to minimize the influences of the cable resistance and its temperature dependant fluctuations, the 3-wire circuit was used for connecting of Pt100 sensors (Fig 6B).

The heart was immersed in the bath for 20 minutes at temperatures of 30, 34, 37 or 38 °C in an attempt to obtain a uniform distribution of the temperatures across the tissue. The results from four independent measurements (Table 3) show a high correlation for $\mathbf{T_{IR}}$ and $\mathbf{T_{control}}$.

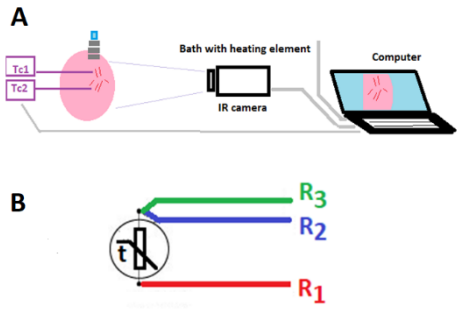

Fig. 6: (A) Control thermometers at two points of the tissue for comparison with the IR non-contact camera measurement. (B) Scheme of the 3-wire circuit for connecting of Pt100.

Table 3: Correlation of the temperatures (average value and standard deviation from 4 experiments).

Heart bath	$T_{control}$ (°C)	T_{IR} (°C)
30 °C	29.9 ±0.6	29.5 ±0.8
34 °C	33.9 ±0.4	33.8 ±0.5
37 °C	37.0 ±0.3	36.8 ±0.5
38 °C	37.9 ±0.4	37.9 ±0.6

The evaluation of the accuracy for the temperature measurement on real tissue using the non-contact IR camera is very important for all future biological experiments. The IR camera was calibrated (readjusted to the moistened surface of the organ) by calibration protocol of FLIR company. After that, the correlation between the surface temperature measured by the camera and the temperatures of the tissue (at a depth of 0–5 mm) was checked using a thermometer Pt1000. The average difference between IR measured temperature and Pt100 measured temperature was detected as 0.2 °C for target temperature 37 °C.

Discussion

The Oskar Langendorff model of isolated heart has been going through numerous modifications, over the decades the concept proved its simplicity and practicability, even facing working heart system [5].

The temperature of the heart tissue has always been the key parameter [6] and the importance has grown due to utilization of sophisticated techniques for extraction of myocytes, extraction of extracellular components or infusion of gene-delivery system [7]. Reproducible results of all these experimental methods depend on oxygen, nutrients delivery, perfusion pressure, but foremost on the stable temperature. This is usually kept by thermocouples within the bath itself, nevertheless this is applicable only when whole organ is submerged. Our methods replaced traditional contact thermometer by non-contact IR camera. The selection of the camera was based on knowledge of IR imaging,

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which has been used predominantly in the evaluation of sports injuries and the progression of therapy or in disease evaluation till today [8].

The feedback loop in many variants of historical Langendorff systems was represented by thermostats with smaller or longer response imperfection. Our methods replaced the thermostats by the software utility commanding the heating and circulating system. The final stability of temperature in our prototype is excellent in comparison with some previous variant of thermocouple system [9]. The focus on the adequate area of measured heart is the important aspect of our study, application of controlled condition will allow precise preparation of the heart tissue for experimental delivery, physiological simulation or harvesting of multiple cell types (progenitor cells, cardiomyocytes, etc.), which are commonly dissociated by thermo-responsive enzymes from extracted hearts [10].

Conclusion

Our prototype of the lab system based on the traditional Langendorff apparatus provides precise monitoring and regulation of the temperature of the isolated heart. The possibility of fast and precise thermoregulation of the perfusate and heart tissue entering the aorta was documented. The time lap from the hang-up of the hypotermed organ and optimal warming to 37 °C was less than eight minutes (the initial temperature was 30 °C). The stability of the tissue temperature was approved. Our aim for future upgrades is to serve an automatic system with control software for routine user-friendly applications for both clinical and research use.

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