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Short Communication

Unpressurized Safekeeping of Transplant Organs Using the TOP-Liver® Storage Module

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Abstract

The demand for transplant donor organs continues to grow worldwide as the level of medical care improves. This increased need is the primary driving force to develop new storage techniques for organ preservation. TOP-Liver® is an idea for a device for liver preservation conceived as a normothermic preservation system. Experimental work investigated the use of this system's storage module using cold preservation and analyzed it for use under standard preservation. Results show that organs stored in a cooling box must contact ice using standard storage methods; there is a correlation between the contact area and the median contact pressure. This experimental work demonstrates that established standard methods for organ storage can be significantly improved while serving as a starting point for future work and research concerning the relevance for transplant medicine.

Keywords: liver, storage method, preservation, pressure distribution

Introduction

The permanently increasing need and demand for donor organs for transplants is the impetus to develop new and better organ storage techniques and preservation methods. These techniques and methods can be used to make organs available to a greater number of patients [1-3]. There are many groups worldwide involved in the research of normothermic organ preservation methods [4-6], but all apparatuses are still in the developmental stages. Stainless steel bowls filled with water or some other liquid used to support the organ are generally the method of organ storage used by these storage apparatuses. Some storage chambers are only developed for use by one specific system [7]. The drawback to such methods is their inherent lack of versatility and resulting inability to be used in other preservation methods. These systems are severely limited in their crossover functions.

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TOP-Liver® is an idea for a Transportable Organ Perfusion (TOP) system for liver preservation that was conceived as a normothermic organ preservation system that simulates physiological conditions [8, 9]. This system incorporates a loop perfusion circuit with two blood pumps, an oxygenator, a dialyzer, one perfusion bag, and an organ storage chamber [10]. The unique feature of this system is that the organ is kept floating within the storage chamber [11]. Furthermore, this module possesses great versatility and can be used separately from the TOP-Liver® system, which is especially interesting for standard preservation methods. The TOP-Liver® storage module has the capacity to be used for standard cold preservation.

The standard preservation storage method is sufficient for robust organs such as hearts and kidneys, but parenchymatous organs, such as the liver, clearly need more attention.

Storage using standard cold preservation processes first requires that the organ for transplant be rinsed in a special solution and stored at a constant temperature of 4ºC after-

wards. In practice, the organ is packed in a set of three bags with the first bag containing the organ; the second containing the first bag plus the addition of a liquid in an amount sufficient enough to prevent potential pressure marks. This package is then placed in a third bag that serves as an outer layer of mechanical protection. The entire set of bags is then inserted into an ice-filled box. Our simulations and observations in the operating theatre, however, clearly indicated that the organ is constantly in contact with the ice surrounding the bags. This contact squeezes (i.e., puts pressure on) the organ and causes cold injuries.

The following experiments comparing both methods of storage provides an evaluation of the unpressurized storage method as exemplified by the use of TOP-Liver®.

Materials and Methods

Several organ models were used for these experiments. A simple yet sufficient physical model for our purposes was a water-filled balloon. We were able to document the pressure distribution of different storage surfaces effectively by using this model. Pressure distributions were repeated using livers from pigs purchased from a butcher. Additionally, livers from laboratory pigs, having an approximate body mass of 40 kg, were used to make visual comparisons as well as histological examinations. These livers were surgically extracted under actual medical conditions.

Measurement of Pressure Distribution

Pressure distribution was measured using an ultra-flexible sensor pad PX100 manufactured by Xsensor™ (Fig. 1A) in conjunction with its companion software, X3Pro V5. The sensor pad consists of 1,296 sensors with a density of 0.62 sensors per cm². The underlying principle used to determine the reaction force was based on capacitive effects. Organ measurements (obtained as organs were placed on various types of surfaces) were always conducted using the same procedure and set-up in which the sensor pad was placed between each surface and the organ model (Fig. 1B). Six different methods of surface support were tested, ranging from a hard examination table to floating storage (i.e. in water), including four different kinds of synthetic foams, each with varying degrees of hardness between the two extremes (i.e. the examination table and the floating storage) [12].

Optical Comparison

The storage chamber, which is transparent from all angles, was filled with a mixture of water and ice analogous to the cooling box used in standard cold storage. We were, therefore, able to observe the organ behaviour in the set of bags (Fig. 2A). By comparison, the TOP-Liver® chamber was filled with a 4ºC-tempered special liquid, and the organ was placed in the tube (Fig. 2B). The behaviour of the organ was observed in this case, as well.

Histological Examination

Hepatectomies were performed by a surgeon specializing in transplantology using actual medical standards. Two livers were immediately perfused after hepatectomies using a Custodiol solution and then preserving them for 14 hours at a temperature of 4ºC. The warm ischemic time was virtually non-existent. The conventional standard storage method, in which a cooling box is filled with a mixture of water and ice, was used in the first case. By contrast, the TOP-Liver® storage chamber, in which the organ was maintained in a floating state in TOP-Liver® tube, was used in the second case. Reference samples from both livers were extracted shortly after perfusion with the Custodiol solution. Experimental samples from each type of storage method used were taken after preservation and the 14-hour storage time of each organ. The sampling points were selected so that the samples were taken from areas with permanently increased contact pressure (i.e., the lower area of the organ in standard storage); from portions without any contact pressure (i.e. upper organ area in standard storage); and from areas with reduced contact pressure (i.e. lower and upper areas in TOP-Liver® storage method). In total, ten samples were extracted. After extraction, the samples were fixed in a solution of formalin. Routine histological examinations (e.g. PAS, glycogen distribution or diameter of the vessels) were used to analyze the samples since the mechanism of tissue injuries resulting from storage were unknown.

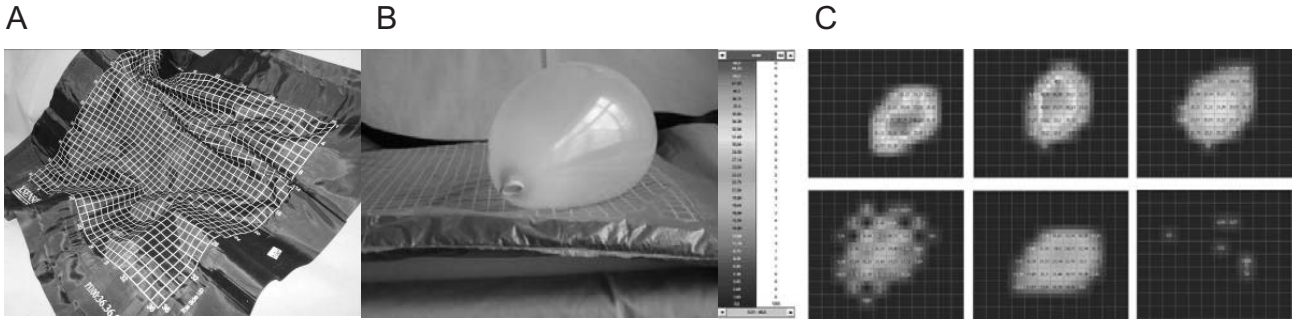

Fig. 1A: ultra-flexible sensor pad, PX100; B: reaction force measurement, surface and phantom with the sensor pad located between them; C: pressure distribution, top left hard surface, bottom right floating storage.

Table 1. Storage pressure with the use of the phantom organ.

Storage method	max p (mbar)	avg. p (mbar)
1 hard	50	31
2	45	30
3	39	25
4	42	21
5	30	17
6 floating	13	8

Results

Physical Tests

Pressure distribution was diagrammed using the X3Pro V5 software. Fig. 1C clearly shows that the pressure distribution profiles are easily distinguishable from one another [12]. As expected, the maximum pressure of 50 mbar only appeared in combination with the hard surface of the examination table. The softer the surface, the more homogeneous the pressure distribution. In the case of floating storage, the pressure was distributed over such a large area that it was nearly impossible to obtain a measurement. The numerical values are presented in Table 1.

A

B

Fig. 2 A: Liver in a set of bags with contact to ice, standard storage; B: Floating liver in the TOP Liver® storage chamber.

Optical Comparison

Liver storage in a standard cooling box resulted in organ contact with the ice. In Figs. 4A and B, the imprint of the organ shape is clearly recognizable and visible as a result of the 14-hour storage period. The ice melted at those points where the organ was located closest to it and formed a pattern. In contrast, the floating storage provides a homogeneous pressure distribution throughout the entire organ as shown in Fig. 4C. Since a liquid was used in the latter storage method, no cold spots were detected in this case.

Histological Examination

No apoptosis occurred in any samples. Under standard storage, a greater degree of tumidity exists on the underside of the organ compared to its top side with an overall difference of 1-2 degrees. The tumidity present in the TOP-Liver® storage method is evenly distributed on both the underside and top side of the organ. There is no change in the glycogen distribution when comparing the reference samples to the experimental samples used in the TOP-Liver® storage method. Furthermore, a comparison between reference samples and experimental samples relative to the diameter change of vessels indicated that the relative variance in the TOP-Liver® method is less than that of the standard method used. The diameter changes resulting from the influence of each type of storage method used are best exhibited by the V. centralis. The results of these changes are shown in Fig. 3 and Table 1; the subsequent corresponding variances are 0.008 under the TOP-Liver® method and 0.016 under the standard method, respectively.

Discussion

The results of the experiments with the phantom organ clearly display the correlation between the contact area and the median contact pressure. As a result of this experiment, we were able to narrow down the experiments on animals to two extreme cases to (i.e. the examination table and

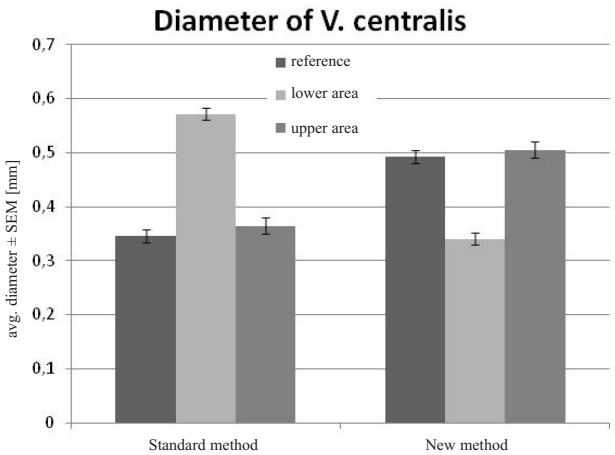

Fig. 3. Values of average diameter changes in vena centralis.

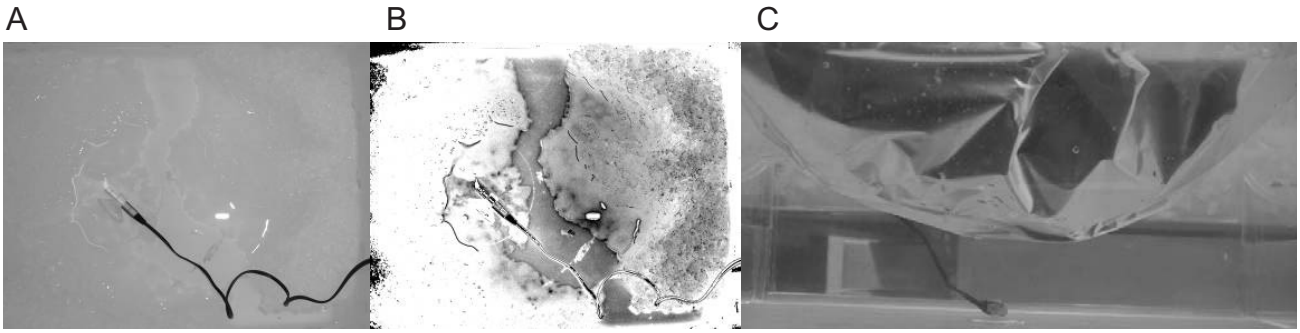

Fig. 4A) molten ice with the organ imprint (original photo), B) molten ice with the organ imprint (digitally enhanced), C) floating storage in the storage chamber.

floating storage). The visual evaluation of the organ during storage in the standard cooling box and in the TOP-Liver® storage chamber came to a clear conclusion: organs that are stored in the cooling box have indirect contact with the ice (i.e. the barrier produced from the three foil layers between the organ and the ice), but this contact, even though it is indirect, can result in hard pressure marks or cold spots on the organ. Livers floating in the TOP-Liver® storage chamber are exposed to surrounding homogeneous pressure and do not result in these same problems. There are observable differences in the organ behaviour between the standard and floating storage methods used. In the case of standard storage methods, the extracted tissue samples also show differences between the upper and lower areas not seen in the TOP-Liver® storage method used.

Conclusion

Our experiments only examined the storage module of the TOP-Liver® system employing cold preservation and analyzed this module for use in standard preservation. These demonstrated that the established standard method for organ storage can be significantly improved. Our results, however, cannot serve as definitive evaluation criteria for methods of storage because the sample size used is not statistically reliable; these experiments were solely initial in nature and serve as a starting point for future work. Systematic investigations must be conducted in order to find a correlation of measurable differences between these storage methods and to clearly articulate the relevance for transplant medicine.

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