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Preservation Solution for Liver Tissue Samples from the Wistar Strain of Rattus Norvegicus in Cote d'Ivoire

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Abstract: ***Tissue samples must be frozen in liquid nitrogen for cell biology analysis. In Côte d'Ivoire, tissue samples are mainly transported in tanks containing liquid nitrogen. However, liquid nitrogen is a difficult and dangerous product to handle and represents a significant investment. Faced with this problem, it is necessary to prepare a preservation solution in order to determine its capacity to preserve the cellular characteristics of the tissues. A preservation solution called KKA solution was prepared. Five Wistar rats (laboratory rats) were used as experimental animals. These healthy rats were sacrificed, followed by the removal of liver organs. Each rat liver organ was cut into small pieces corresponding to 10 mg. The liver tissue samples were separated into groups to assess cell viability. Proportion tests were used for comparison of the sample groups. The KKA solution preserves more than 80% of liver cells for 24 hours at +4°C. KKA solution protects the cellular characteristics of healthy rat liver tissue for 24 hours at +4°C.***

Keywords: Cell viability, preservation solution, liver tissue.

1. Introduction

Cell preservation is a very complex technique in the field of cell biology. During surgery, the ischaemic time occurs, which is defined as the time of cessation of blood supply to a tissue or organ. This interruption of the flow in the vascular network leads to the cessation of nutrient supply and a fall in the partial pressure of oxygen in the vascularised areas, causing an alteration in cellular metabolism [1]. One of the techniques used to preserve cells is cryopreservation (preservation at very low temperature) which blocks all extracellular and intracellular metabolism in order to maintain cellular integrity for future use [1]. In Côte d'Ivoire, the transport of tissue samples from a collection area to a cell biology laboratory is mostly done in liquid nitrogen tanks. However, liquid nitrogen is difficult and dangerous to handle and represents a significant investment [2]. This often leads to difficulties in transporting tissue samples and increases transport costs as samples must be transferred under perfect conditions.

In recent years, a large number of appropriate preservation solutions have been developed to maintain the integrity of tissues and cells, in particular solutions for tissue and organ transplantation. These include the University of Wisconsin (UW) solution [3], the Euro - Collins solution [4], the histidine - tryptophan - ketoglutarate (HTK) solution [5], the Stanford solution [6], and the St. Thomas solution [7]. The UW solution is the most widely used of these solutions for its effectiveness in preserving cells. Thus, an alternative preservation solution to UW solution that is easy to prepare, stable and inexpensive is needed to improve the preservation quality of tissues during transport of tissue samples.

Thus, the preparation of a preservative solution to determine its ability to preserve the cellular characteristics of the tissue is necessary. In this experimental study, liver tissue from the Wistar strain of Rattus norvegicus (laboratory rat) was used.

2. Materials and Methods

Materials for the preparation of the preservation solution

The preservation solution named KKA solution was prepared using the following chemicals: Raffinose pentahydrate (17.83 g/L), KH2PO4 (1 mL/L), Glycerol (35.83 mL/L), Polyethylene glycol (PEG) (500 µL/L), Adenosine (1.34 g/L), Allopurinol (0.136 g/ L), Glutamate (1.47 g/L), NaCl (1.2 g/L), KCl (8.95 g/L), MgSO4 (1.23g/L). The pH of the KKA solution was 7. The KKA solution is a modification of the UW solution, which is the most commonly used solution for organ transplantation. The chemicals used for the preparation of the KKA preservation solution are manufactured by Sigma - Aldrich.

Materials for the biological test

Materials and reagents for cell viability assessment

The equipment for studying cell viability consists of microscope, oven (Binder, Germany), microbiological safety station type II (Baker company), centrifuge (Denley BS400, England), Malassez cell of 0.0025 mm2 and 0.200 mm depth (MARIENFELD, Germany), coverslip, gown, sterile gloves (Delta Plus Group, France), waterproof garbage bags, 15ml tubes, 2ml Eppendorf tubes (Eppendorf AG, 22331 Hamburg, Germany), absorbent paper, 0.4% trypan blue for

cell staining, MEM medium (2% SVF, 1% Antibiotic, 1% HEPES, 1% L - Glutamine), 0.25% trypsin EDTA and PBS.

Animal model used

Five 16 - week - old Wistar Rattus norvegicus of both sexes were used in our study. These rats were bred at the Animal Resource Management Unit of the Pasteur Institute of Côte d'Ivoire from August to December 2020.

Methods

Type of study

This is an experimental study on the assessment of cell viability of laboratory rat liver tissue samples.

Aliquoting and storage of liver tissue

Rats were sacrificed by isoflurane inhalation in accordance with the guidelines of the Canadian Council on Animal Care. In particular, this study received approval from the National Health and Life Sciences Ethics Committee under the number: 043 - 21/MSHP/CNESVS - km. Each liver tissue obtained corresponds to 10 mg. For preservation, the liver tissue was divided into six groups, defined as follows:

Group 1: tissue placed in 1 ml of KKA solution and kept at room temperature for 6, 12 and 24 hours;

Group 2: Tissue placed in 1 ml of KKA solution and maintained at +4°C for 6, 12 and 24 hours;

Group 3: Tissue kept at room temperature for 6, 12 and 24 hours without any solution.

Group 4: Tissue kept at +4°C for 6, 12 and 24 hours without any solution;

Group 5: Tissue not stored and used for cell viability analysis within minutes of organ removal (control group).

A total of 13 pieces of liver tissue were collected from each rat to assess cell viability.

Assessment of cell viability

Cell isolation

Lysis: Each 10 mg piece of liver tissue is placed in a 15 ml tube containing 5 ml of the enzyme trypsin EDTA. After homogenisation, the tube containing the liver pieces and the trypsin EDTA is placed in an incubator at 37°C for a few minutes.

Extraction of liver cells: Remove the tube from the incubator. Using a sterile pipette, aspirate and discard the liver several times to allow it to disintegrate. Then add a few drops of 2% MEM solution to inhibit the action of the EDTA trypsin. Then centrifuge for 5 minutes at 1000rpm.

Wash the liver cells: Check for the presence of pellet (cells at the bottom of the tube) and remove the supernatant. Then recover the pellet and add 2.5 ml of 2% MEM medium using a new sterile pipette and centrifuge for 5 minutes at 1000rpm. Repeat the same experiment twice.

Obtaining the cell solution: Add 2.5 ml of 2% MEM medium to the pellet and vortex to obtain a cell solution.

Reading the concentration and viability of liver cells under the microscope

Add diluted 0.2% trypan blue to the cell solution. The mixture is then placed on the counting slide (Malassez cell) and observed under the light microscope.

The cells stained with trypan blue as well as the unstained cells are counted.

The cells that are stained with trypan blue are dead and those that are not stained with trypan blue are alive. The viability (i. e. the number of living cells in relation to the total number of cells) and the cell concentration are thus obtained from the same sample.

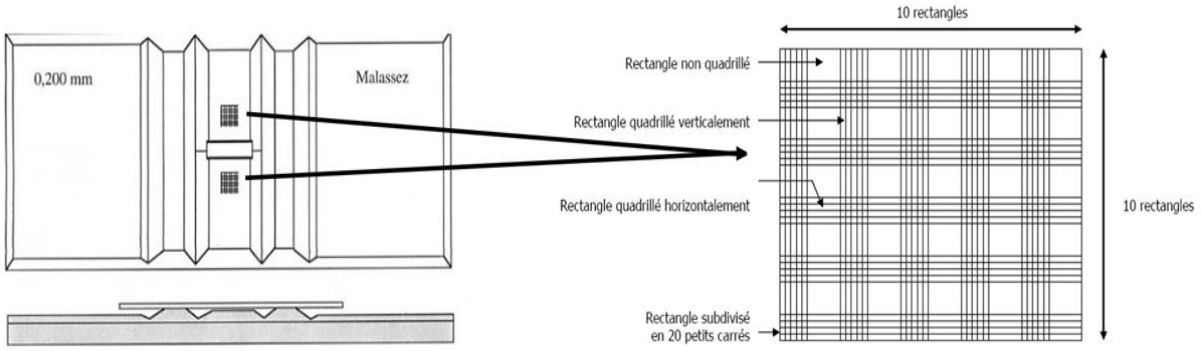

Figure 1: Glass blade with its grid

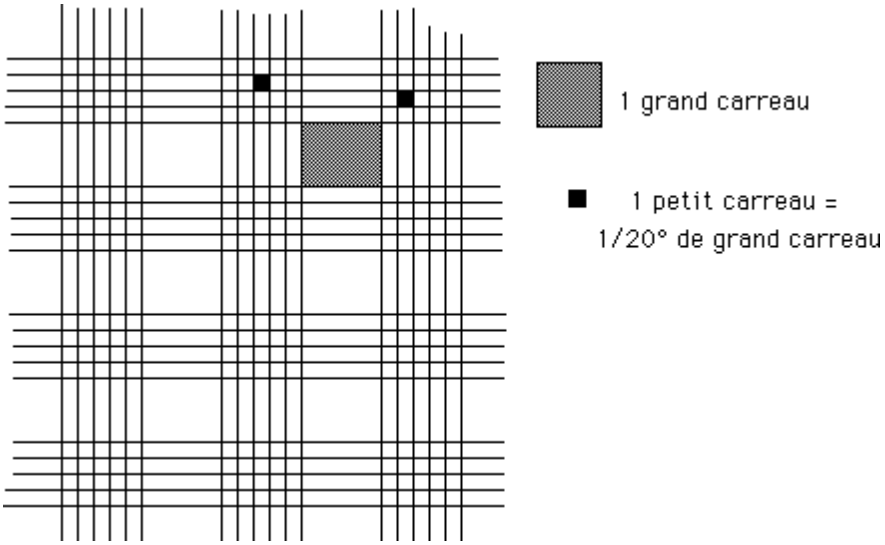

Figure 2: Diagram of a portion of the lamella

Calculations:
% Viability = (Number of live cells / Number of live and dead cells) x 100

Statistical analysis
Statistical analyses were performed using Excel software. Proportion tests were used for comparison of sample groups. If the P - value was less than 0.05, the difference was considered significant.

3. Results

A total of 65 liver tissue samples were analysed. The cell viability of groups 1 to 4 was compared to that of group 5 (control group). The results obtained showed that the liver tissue samples stored at room temperature and at +4°C with KKA solution had a cell viability of 80% or higher at 6 hours. This is almost similar to the cell viability of the control group (>80%). Then at 12 hours of storage, a 30% reduction in cell viability was observed for samples stored at room temperature with or without the solution. In contrast, samples stored at +4°C with or without solution maintained almost the same number of live cells between 6 hours, 12 hours and 24 hours of storage. At 24 hours of storage, no live cells were observed in the samples stored at room temperature with or without solution (Figure 4).

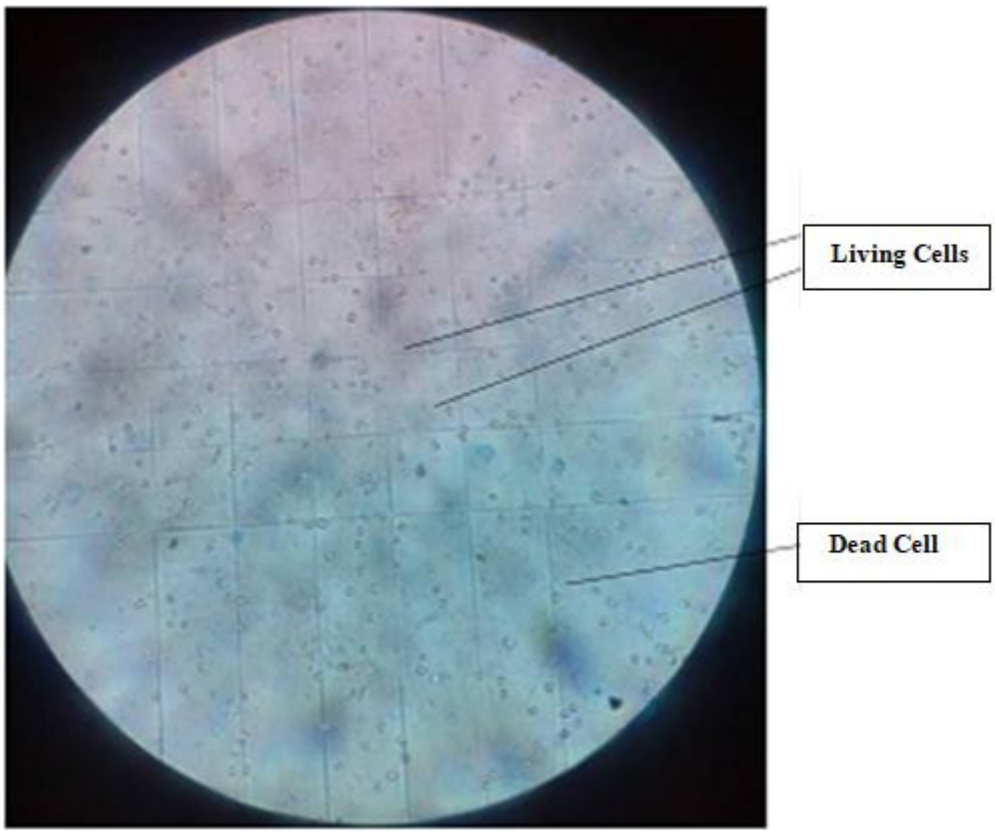

Figure 3: Live and dead cells on the Malassez haematometer

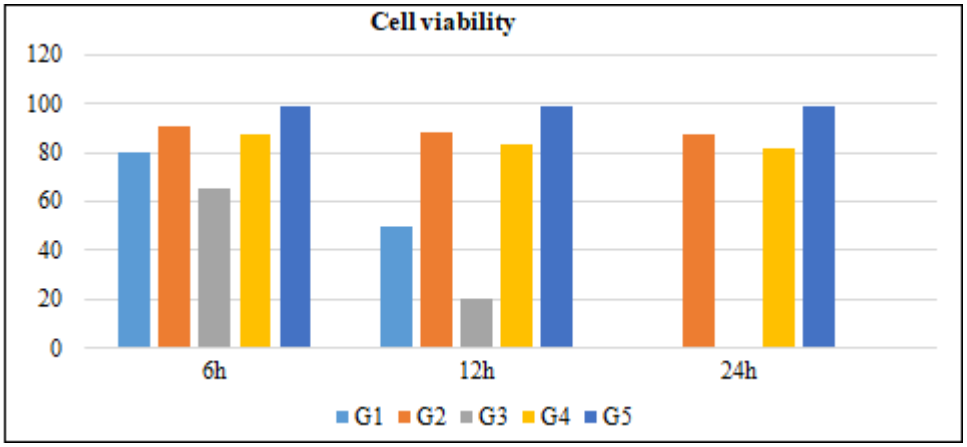

Figure 4: Cell viability of liver tissue samples in %.

4. Discussion

Liver biopsy samples are an important tool in diagnosis and research in cell and molecular biology. The transport of liver biopsies from the collection site to the laboratory is a real problem in the diagnosis and research of liver diseases. In Côte d'Ivoire, tissue transport is done using liquid nitrogen. Although this has several advantages in terms of conservation, it represents an important investment and is very difficult to handle. In this study, a less expensive and easy to prepare alternative to liquid nitrogen was studied. This is a solution with the ability to protect liver cells from liver tissue until they reach a diagnostic laboratory.

The results of this study showed that storage at +4°C in KKA solution is best suited for the preservation of liver cells for 24 h compared to storage at room temperature. These results are almost similar to a study by Straatsburg *et al* in 2002. This study compared the effects of Celsior, University of Wisconsin (UW) and histidine - tryptophan - ketoglutarate (HTK) storage solutions on liver cell death. Rat livers were stored at +4°C for 0, 8, 16 or 24 hours in Celsior, UW or HTK and reperfused for 90 minutes [8]. The results of this study show that Celsior and UW solutions are equally effective in preventing rat liver cell death after 0 - 16 h of storage at +4°C as HTK solution which is less effective. Furthermore, rat livers were better preserved in the UW solution after 24 h of storage at +4°C [8]. Similarly, the study by Hermann Janssen et al in 2003 also showed the effectiveness of UW and Celsior solutions in preserving liver cells at +4°C for up to 48 hours [9].

The KKA solution (modified UW solution) shows almost the same results as the UW solution for the ability to protect liver cells from Wistar rats for 24 hours at +4°C.

5. Conclusion

In our study, we developed a solution to preserve liver cells for a period at least long enough to reach a cell biology laboratory. It was shown that the KKA solution could preserve the cellular characteristics of liver tissues at room temperature for 6 hours and at +4°C for 24 hours. In most countries, a period of 24 hours would be sufficient for the transport of a sample from the collection site to a cell biology laboratory.

Competing Interests
The authors have not declared any competing interests.

Authors' Contributions
AKK performed the isolation of liver cells from liver tissue, assessed cell viability, analysed the data and wrote the paper. LNA provided technical assistance and valuable comments on the manuscript. MD provided valuable scientific and critical advice.

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