High frequency ultrasound in monitoring liver suitability for transplantation

R.M. Vlad[1], G.J. Czarnota[2, 4], A. Giles[1, 3], M.D. Sherar[1, 3, 5], J.W. Hunt[1, 3] and M.C. Kolios[1, 4]

[1]Department of Medical Biophysics, University of Toronto; [2]Department of Radiation Oncology, University of Toronto, [3]Ontario Cancer Institute; [4]Department of Mathematics, Physics and Computer Science, Ryerson University; Toronto; [5]Department of Oncology, University of Western Ontario, London, Ontario, Canada.

***Abstract*--Currently there are no validated clinical methods to assess liver preservation injury. In this work we use high frequency ultrasound integrated backscatter (HFUIB) to assess liver damage in different experimental models of liver ischemia. The ultimate goal of this work is to provide a non-invasive tool to assess organ suitability for transplantation.**

To examine the effects of liver ischemia at different temperatures, livers from Wistar rats are surgically excised, immersed in phosphate buffer saline (PBS) and stored at 4 and 20°C for 24h. To mimic organ preservation, livers are excised, flushed with University of Wisconsin (UW) solution and stored at 4°C for 24h. Preservation injury is simulated by not flushing livers with UW solution. Ultrasound images and corresponding radio frequency data are collected over the ischemic periods.

No significant increase in HFUIB is measured for the livers prepared using standard preservation conditions. For all other ischemia models, the HFUIB increases by 4-9 dBr demonstrating kinetics dependent on storage conditions.

HFUIB increase is associated with liver tissue injury. The results provide a possible framework for using high frequency imaging to non-invasively assess liver preservation injury.

Keywords: **high frequency ultrasound, ultrasound backscatter, liver preservation, liver ischemia, cell cytoskeleton, ATP.**

I. INTRODUCTION

It has been previously shown that high frequency ultrasound (HFU), from 20 to 60MHz, can be used to detect cellular structural changes in cells and tissue during cell death. Backscattered ultrasound increases in cell ensembles treated with the chemotherapeutic cisplatin by 9-13dBr [1, 2]. The most striking histological features in the treated cells are nuclear condensation and fragmentation in addition to cell shrinkage, features of apoptotic cell death. If the same type of cells are left at room temperature deprived by nutrients and in the absence of drug for at least 5h, an increase in high frequency integrated backscatter (HFUIB) by 6dBr is measured concomitant with cell swelling and cytoplasm vacuolization, features of oncotic cell death [2]. The backscatter ultrasound increases as well in tissue damage, specifically liver tissue in this work, thereby increasing the brightness of ultrasonic images.

In this study we examine liver ischemia in different experimental conditions using high frequency ultrasound. Tissue morphological changes are evaluated using light and electron microscopy of samples collected at representative ischemic time points.

Flushing the liver with preservation solution and storing it at 4º C is the standard technique for liver allograft preservation. However after 8-24h of cold storage, irreversible injury occurs, leading to liver transplant failure. Liver graft injury may be divided in 3 interrelated components: pre-preservation, preservation and reperfusion injury [3] in Figure 1**.** In this project we demonstrate that HFUIB increase is associated with cell structural alterations in ischemic livers.

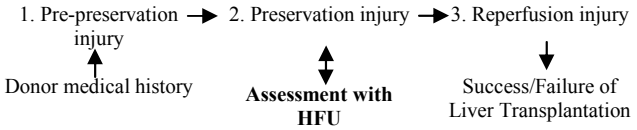

Figure 1: Components of liver graft injury.

The preservation period sets the stage for injury manifested on reperfusion and affect graft function [4]. Despite this major challenge, there are no means of predicting outcome on the basis of biochemical or other tests.

Total ischemia is produced by the cessation of blood flow resulting in the combined effects of anoxia, lack of metabolic substrates and absence of tissue perfusion. In the absence of organ preservation, total ischemia results in ion transport deregulation, cell swelling and finally death of affected region in a period varying from minutes to hours.

We hypothesize that the detected variations of backscattered ultrasound in liver ischemia are related to changes in cell size and physical properties following cell alterations manifested upon adenosine triphosphate (ATP) depletion [5].

II. MATERIALS AND METHODS

Organs from Wistar rats (n=10) are surgically excised, immersed in phosphate buffer saline (PBS) and left to decay at room temperature for 10 hours. In standard preservation experiments, organs from Wistar rats (n=2), are surgically excised, flushed with University of Wisconsin (UW) solution and stored at 4ºC for 24 hours. To simulate preservation injury the organs (n=2) are excised and stored in UW solution at 4ºC, without flushing the organ, for the same period of time. Samples are fixed for Hematoxylin & Eosin (H&E) and Electron Microscopy (EM) staining at representative time

points. High-resolution images and the corresponding raw radio frequency data are collected over the ischemic period. The VS-40B ultrasound imager, VisualSonics Inc. (www.visualsonics.com), employs a 40MHz, f/3 transducer, with a penetration depth in liver tissue of ~3mm. Radio frequency signals associated with the captured image are stored digitally at a sampling rate of 500 MHz.

For the calculation of normalized backscatter power we use a pulse echo reflected from a flat quartz situated at the transducer focus. To compensate for different attenuation coefficients of PBS and UW solution and greater attenuation at lower temperature, livers immersed in PBS/UW solution at 4 or 20ºC are calibrated with a reference taken from the flat quartz immersed in the same solution and temperature as the organ.

III. RESULTS

In order to demonstrate the potential of high frequency ultrasound to detect tissue structural changes at various conditions, three different liver ischemic conditions are imaged: warm ischemia, preservation and simulated preservation injury.

The purpose of warm ischemia experiments is to provide an understanding of how cell structural changes relate to the backscattered ultrasound variations during sever tissue injury. In these experiments the excised rat livers are immersed in PBS and left to decay at room temperature(20ºC) for up to 10 hours. As after 6-10 hours of warm ischemia the liver tissue becomes necrotic imaging the organs beyond 10 hours does not have clinical relevance due to advanced liver tissue necrosis.

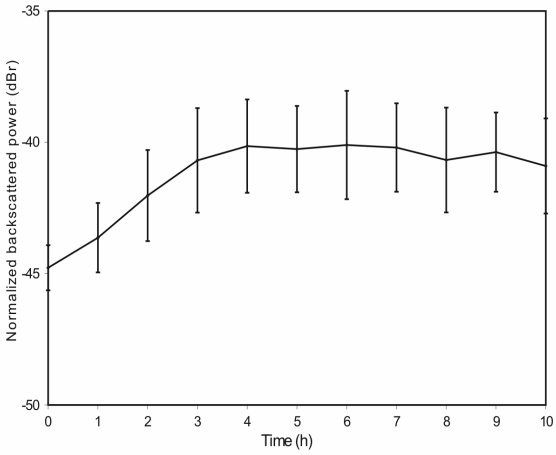

Figure 2: Normalized backscattered power averaged for 6 different livers during 1 to 10 h liver ischemia (20°C).

For n=6 of the 10 organs analyzed in warm ischemia experiments, we measure a maximum increase in the backscattered ultrasound within the interval of 4 to 6 hours. For these livers, it is possible to average HFUIB values measured for each liver at the same time point in Figure 2. The maximum increase of backscatter ultrasound is measured at 1, 2, 3 and 8 hours for the other four analyzed livers ranging from **4.5** to **7.6dBr**. The averaged maximum increase of HFUIB is **~6dB**r for all n=10 livers immersed in PBS and left to undergo cell death at 20°C. Ultrasonic images and H&E histology of one representative liver warm ischemia are shown in Figure 3.

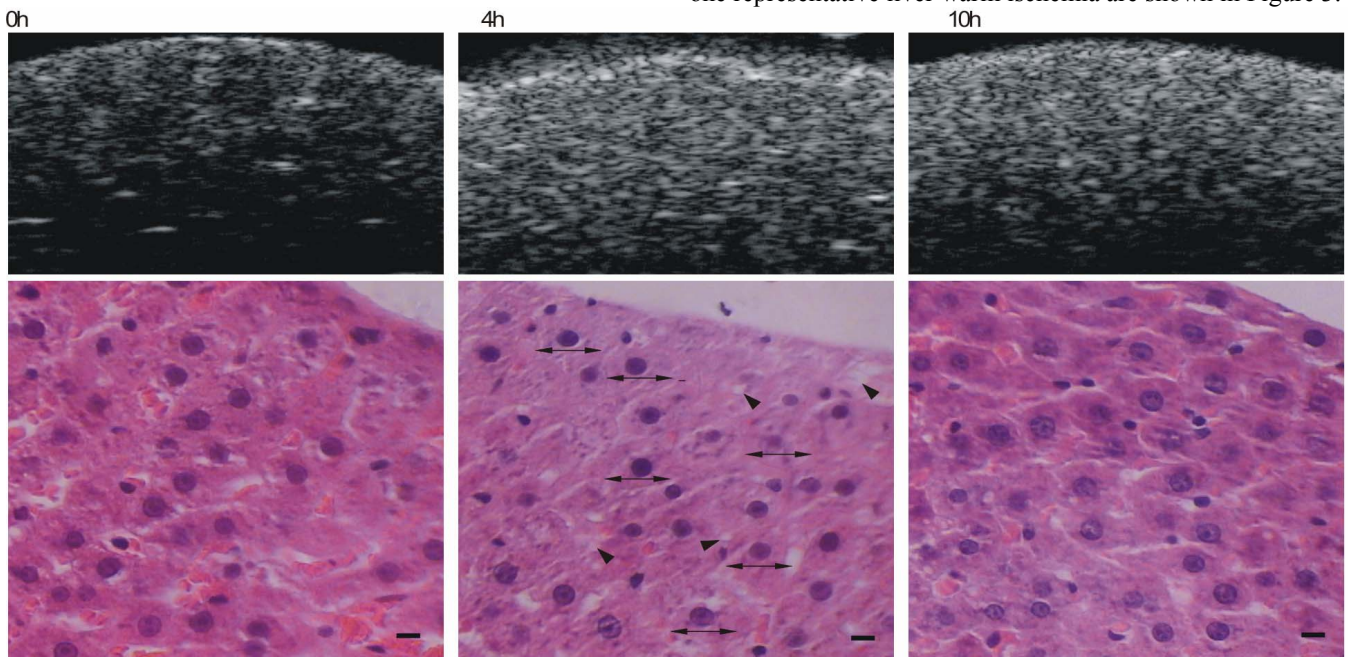

Figure 3: Ultrasonic images (2.5 x 6mm) demonstrate an increase in brightness from 0 to 10h. Hematoxylin & Eosin staining of liver ischemia (20ºC) illustrate liver gradual damage from 0 to 10 h. (0h) Control with normal morphology. At 4h, cytoplasm vacuolization (►) and cell swelling (↔). At 10h tissue morphology indicates cell necrosis. The scale bar is 10 μm.

Individual cells cannot be resolved at the operating wavelength of high frequency ultrasound scanner but we demonstrate in this study that structural changes in tissue injury can result in significant changes in high frequency integrated backscatter.

In the preservation experiments, excised organs are flushed with UW solution, stored at 4°C and imaged on an ice bath to maintain a constant temperature during scanning. The tissue temperature is continuously measured during scanning with a digital thermometer.

For the organs prepared after standard preservation conditions the maximum increase in HFUIB is ~2.5dBr close to maximum standard deviation of ± 2.1dBr in Figure 4. To simulate injury, livers are not flushed but stored in UW solution at 4°C. The ultrasound backscatter increases in preservation injury as shown in Figure 4 and correlates with structural damage observed in liver histology after 24 h in Figure 5.

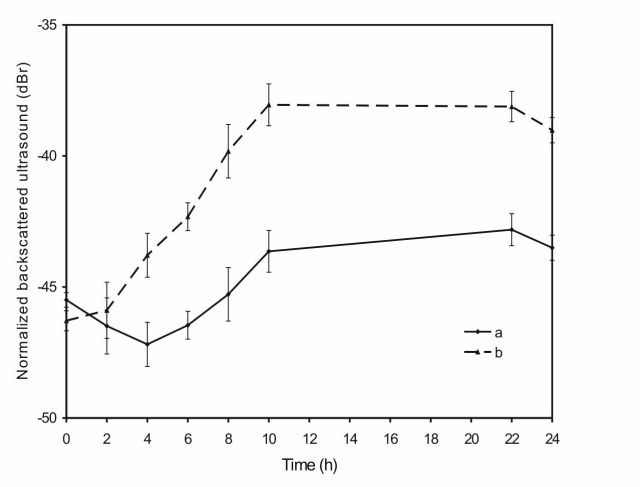

Figure 4: Normalized backscatter power (NBP) represented as a function of storage period, for livers**:** (a) preserved in standard preservation conditions; (b) not flushed but stored in UW solution at 4ºC. The error bars represent the NBP variations at different locations on the same liver. They also count for NBP variations with temperature changes during data acquisition. The standard deviations are calculated at one time point for each liver and averaged over the number of livers analyzed.

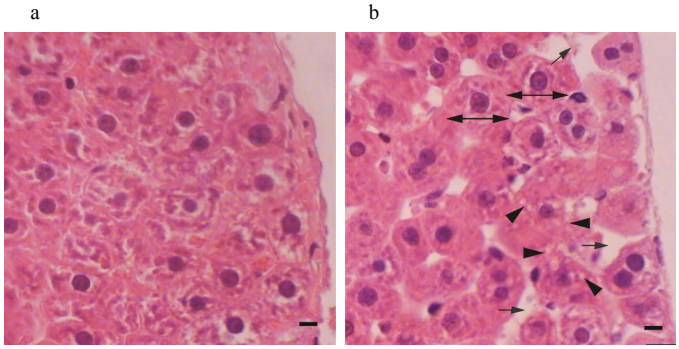

Figure 5: Hematoxylin & Eosin staining of livers collected at 24 h. **(a)** prepared in standard preservation conditions with cell morphology close to normal; **(b)** simulated preservation injury, presenting cytoplasm vacuolization (►), cell shape changes (↔) and larger sinusoidal spaces (→).The scale bar is 10 μm**.**

The histology of the livers prepared after standard preservation conditions show morphology still close to normal in Figure 3 at 0h, whereas liver histology for the preservation injury shows cytoplasm vacuolization and disrupted cell morphology.

IV. DISCUSSION

Cell structural changes such as cell swelling, cytoplasm vacuolization and nuclear alteration are observed in H&E staining in Figures 3 and 5 and represent consequences of nutrients deprivations including ATP and oxygen in tissue ischemia [5]. They correlate with the increase of HFUIB in warm ischemia (20ºC) and preservation injury experiments.

To test reproducibility of the technique and the dynamic of increase in HFUIB a greater number of organs (n=10) is used in warm ischemia experiments. The standard deviation of maximum increase in HFUIB is ± 1.07dBr.

Cell ischemic death is dependent on temperature, the rate of anaerobic glycolysis and availability of stored glycogen [5]. The temperature is 20°C during warm ischemia experiments. Thus, the variation between the periods (from 1 to 8 hours), at which the maximum increase in HFUIB is measured is likely to be related to the availability of stored glycogen in the analyzed livers.

An important principle of hypothermic organ storage is to slow those processes that require ATP and accumulate injury after ATP depletion occurs [6]. Since low temperatures slow cell injury, the maximum increase of HFUIB is measured after 10 hours at 4°C and 4-6h at 20°C in Figures 2 and 4.

The composition of UW solution is designed to minimize hypothermic induced cell swelling [7] and thus to prevent injury produced by ATP depletion during preservation period. For livers prepared in standard preservation conditions and flushed with preservation solution, after 24h, the tissue morphology is still close to normal and HFUIB does not show significant variations. For the livers that are not flushed, the protective effect of the solution is at most limited to the cells on the liver surface. HFUIB increases after 2-4 hours of cold storage likely due to cell structural alteration in an ATP deprived environment in Figures 4 and 5.

Changes in ultrasound backscatter are due to the changes in size, density and compressibility of the ultrasound scatterers [8], all of which may be modified during these experiments. However, we have not yet identified the nature of increase in ultrasound backscatter. Further research in our lab will concentrate on determining whether there is a predominant scattering structure or the ultrasound backscatter increase is caused by a complex of cell structural changes.

Cell swelling, cytoplasm vacuolization and cytoskeleton alteration have been reported as the most common consequences of ischemic injury [5]. These alterations possibly increase mechanical stiffness in the cell exposed to stress [9]. Scanning acoustic microscopy studies [10] on cell cytoskeleton isolated components measured variations of elasticity and viscoelaticity modulus during cell cytoskeleton polymerization and disruption. These findings suggest that the cell mechanical properties depend on the state of the cell cytoskeleton and its

interaction with associated proteins. Cell swelling and cytoplasm vacuolization [5] are the most striking cell injuries observable in the H&E staining collected from liver samples. These are consequences of cell cytoskeleton disruption [5] and therefore may potentially cause variations of acoustic parameters in liver ischemia experiments.

V. CONCLUSIONS

Ultrasound backscatter variations in liver preservation injury correlate with cell swelling and cytoplasm vacuolization that are consequences of ATP depletion in liver ischemia. Therefore, the high frequency ultrasound has the potential to monitor the liver damage during preservation period, prior to transplant.

To use this technique in assessing organ damage during preservation we have to understand which specific changes in tissue induce ultrasound backscatter variations and whether they are related to reversible or irreversible changes. Cell swelling and ionic imbalance could be important factors leading directly to cell death if ischemia is maintained beyond 8-24 hours [5]. On the other hand, cell swelling could cause microvascular compression and perfusion defects which could further exacerbate anoxia or prevent reperfusion of ischemic areas and thereby potentate parenchymal cell injury [5].

In these experiments we are limited to a maximum penetration depth of 3mm in liver for the 40MHz transducer. Preliminary results show that with frequencies of 20MHz we measure similar variations of the HFUIB, thereby extending the technique utility. To assess preservation injury with this technique we have to demonstrate that data collected from the liver surface are representative for the injury of the entire organ.

ACKNOWLEDGEMENTS

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Corresponding author:
R.M. Vlad, e-mail:vroxana@uhnres.utoronto.ca