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Contrast-Enhanced Ultrasound to Quantify Perfusion in a Machine-Perfused Pig Liver

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Abstract

This paper introduces a non-invasive, contrast-enhanced ultrasound (CEUS) infusion method to quantify the health of viable donor livers. The method uses the infusion of microbubbles and their destruction and subsequent replenishment to measure the perfusion rate in the liver microvasculature. The proposed method improves on the previous parameter extraction approaches applied to the flash-replenishment technique by addressing the effects of the microbubble mixing within the perfusate bath and destruction rate. By doing so, the tissue perfusion rate can be extracted from the data even though the microbubble concentration is not constant throughout image acquisition. The measured changes in the tissue perfusion rate showed that CEUS infusion is a viable biomarker for assessing liver health.

I. INTRODUCTION

End-stage liver disease claims up to 60,000 lives per year in the United States [1]. The only treatment for end-stage liver disease is liver transplantation, but only 28% of wait-listed patients receive transplants due to a severe shortage of viable donor organs. This shortage could be dramatically reduced by rendering marginal organs transplantable. An estimated 6,000 livers per year are damaged by ischemia and could be resuscitated for transplantation [2]. Normothermic machine perfusion (NMP) has been shown to have numerous benefits,

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including preservation time extension and marginal graft recovery. However, subsequent transplantation requires quantitative methods to accurately assess graft readiness.

The absolute level of adenosine triphosphate (ATP) content is recognized as the best parameter indicating functional recovery of the liver, thus it is most commonly used to evaluate perfused liver viability [3]. Previous liver perfusion studies have demonstrated its correlation with liver viability [3], and its concentration increases during liver perfusion in animal models [4]. However, this method relies on repeat biopsy, which may lead to donor liver damage, and ATP content is not readily determined in real time. Given the lack of immediate non-invasive quantitative tools in the area, ultrasound markers of liver viability have been investigated.

II. CONTRAST-ENHANCED ULTRASOUND

Contrast-enhanced ultrasound (CEUS) methods have been used to evaluate liver blood perfusion during liver machine perfusion. One method involves administering the contrast agent as a bolus and analyzing the resulting time intensity curve (TIC) transient response characteristics, such as peak-intensity, time-to-peak, area under the curve, and mean transit time to indirectly characterize blood flow [5], [6]. Previous data has shown that this method is comparable to other liver function markers, including liver oxygen uptake, bile production, or the portal vein (PV) and hepatic artery (HA) flow rates in the perfusion system [5], [6]. However, this evaluation parameter provides no quantitative calculation of the *local* blood flow in the liver parenchyma regarding ischemic injury or perfusion benefit.

CEUS infusion methods have been developed to measure micro blood perfusion in the heart [7], kidney [8], muscle, and fatty tissues [9]. However, the value of CEUS infusion methods in the evaluation of perfused liver status has not been established. Additionally, all these infusion methods require constant contrast concentration during imaging, which is difficult to maintain in a closed NMP system. Addressing these limitations will improve applicability of the CEUS infusion method to donor liver evaluations and perhaps expand its utility in other clinical settings.

We hypothesize that an improved CEUS infusion method will provide an imaging tool for measuring the liver parenchyma blood perfusion rate. In this research, we work to develop the CEUS infusion method as a perfused graft viability biomarker using a single porcine liver, which could ultimately be used to optimize marginal graft recovery.

III. METRICS AND METHODS

A. Liver Model and Perfusion System:

The *ex vivo* perfusion system machine consists of a pump system, two tubes connected to the HA and PV, an organ chamber, and 4 L of perfusate [10] in which the liver is suspended (Figure 1). After resection from a living healthy pig, the liver was transported to the lab, during which it experienced 2-hour static cold storage (0–4°C). After cold storage, the liver was subjected to one hour of warm ischemia, followed by 3 hours of continuous subnormothermic perfusion (SNMP) at 21°C through the HA and PV.

Ultrasound measurements were performed using a GE Logiq E9 ultrasound system (GE Medical Solutions, USA) with a 9L linear array probe (9MHz, 128 elements). The right central lobe of the pig liver was scanned. Imaging was done on a liver region with a patent hepatic vein (HV), HA, PV, and homogeneous liver parenchyma. This region was kept consistent throughout the study by fixing the transducer location with a clamp (Figure 1).

B. Data Collection: ATP Measurement and CEUS Scanning

The following five time-points were defined for the experiment: baseline, 1-hour warm ischemia, 1-hour SNMP, 2-hour SNMP, and 3-hour SNMP. ATP measurements and CEUS infusion scans were performed at each time-point.

A biopsy was performed using a 14-gauge core needle at each time point just prior to CEUS scanning. The biopsy sites were selected to be as close to the ultrasound probe as possible, without altering the B-mode images. Liver tissue sample ATP content was measurement subsequently.

The CEUS scanning protocol was: 1) 25 mg Lumason® (contrast agent in the shape of microbubbles) was suspended in 5 mL 0.9% saline and slowly injected into the perfusate in the organ chamber over the course of 5–10 seconds. 2) Real-time CEUS infusion data was recorded for a specific region of interest (ROI) for a total of 4 minutes after injecting the contrast agent into the perfusate. 3) Within the 4 minute window, once the concentration within the ROI reached a steady state value (determined visually by monitoring the contrast intensity on the ultrasound monitor), multiple high energy microbubble destruction “flash” pulses were performed at intervals of 30–40 seconds.

The flash pulses had a high mechanical index of 1.6 in order to destroy the microbubbles, and the imaging between flashes had a low mechanical index of 0.16. The flash pulses locally destroyed the microbubbles within the acoustic window, and the interval between flashes allowed for local microbubble replenishment through reperfusion. This rate of replenishment is representative of the rate of local blood flow in the liver parenchyma.

Microbubbles destruction was done between each data collection time-point, to mitigate contrast accumulation effects. High mechanical index imaging was used to destroy as many of the remaining microbubbles in the perfusate as possible.

C. Contrast Replenishment Curves

To obtain the contrast replenishment curves, a ROI was operator selected for each time-point. The ROIs were required to include homogeneous liver parenchyma and exclude visible blood vessels (left panel of Figure 2). Due to slight movement of the liver within the organ chamber during perfusion, the ROIs were slightly different at each time point, but were drawn to be as close in size, shape, and location, as possible between time-points to minimize variation. Representative contrast replenishment curves for a single time-point are shown in Figure 2.

At each consecutive time-point, there was a slight increase in the baseline intensity value of the contrast. This was due to the accumulation of microbubbles throughout the experiment

due to their incomplete destruction between each time-point. In order to mitigate this effect, the curves were intensity shifted, to start at zero intensity, by subtracting the starting intensity value. There were multiple flash-replenishments performed at each time point. Each replenishment curve was time-shifted to a common time origin, with 0-seconds corresponding to the start of each flash-replenishment cycle.

D. Curve Fitting

For each time-point, all replenishment curves were fit using two methods. Both methods were based on the work by Wei et al. [7], who used an exponential function to fit the replenishment curves:

$$y = A(1 - e^{\beta t}). \quad (1)$$

The first curve fitting method used the equation introduced by Schlosser et al. [8], who modified the Wei method by adding a correction factor, s :

$$y = s + A(1 - e^{\beta t}). \quad (2)$$

The second method was based on the equation from Wei et al., combined with a novel normalization method (referred to as the improved method).

In equations (1) and (2), A represents the cross-sectional area of the vessel (visible as the amplitude of the image intensity), β the blood velocity (visible as the replenishment of the microbubbles), and s the correction factor. $A \cdot \beta$ represents the volumetric blood flow (indicative of the perfusion rate) through the ROI [7].

In the Schlosser method, the s correction factor was added because the intensity within the ROI does not return to baseline levels after the flash, and the replenishment curve starts at a higher intensity with each consecutive flash (Figure 2). This was due to the fact that the concentration of the microbubbles that was pumped in to the liver changed throughout the image acquisition process. This can be attributed to the finite amount of time that is needed for the contrast agent to travel from the injection site in the bath to the pump's intake. As a result, immediately following the injection, the perfusate that is pumped into the liver is largely devoid of microbubbles; this concentration gradually increases over time as more microbubbles travel to the intake port within the bath, until the homogenous mixing and equilibrium concentration are reached.

After each flash-replenishment curve was fit using the Schlosser method, the s value was subtracted from each curve for comparison. Figure 3 shows that the curves successively decreased in magnitude, which was due to the destruction of the microbubbles. Microsphere destruction resulted from the high mechanical index flashes and mechanical losses within the perfusion pump. The decreasing magnitudes demonstrated that the microbubble destruction rate was not accounted for in the curve fitting, which meant any computation of perfusion rate would be inaccurate.

The two competing factors, increasing microbubble concentration from mixing and decreasing microbubble concentration from destruction, made it difficult to calculate the perfusion rate using the Schlosser method. The increasing baseline intensity and microbubble destruction were experimental aspects that could not be avoided, and therefore we developed a novel normalization method to extract the perfusion rate.

E. Curve Fitting: Improved Method

The intensity curves are shaped by three main factors: 1) liver tissue perfusion rate (**PR**) 2) microbubble mixing rate (**MR**) throughout the perfusate 3) microbubble destruction rate (**DR**), from both the flashes and the perfusion system. In order to isolate the PR from the raw curves shown in Figure 2, it was assumed the PR was fairly constant during CEUS acquisition. This is reasonable given the short time frame of acquisition (minutes) compared to the overall perfusion time (hours). As stated previously, the increase in microbubble concentration due to mixing can be seen in the increasing baseline intensity of each consecutive flash. The effect of microbubble destruction could not be directly observed in the curves but would have affected the baseline intensity values. The intensity values immediately following the flash (the hollow circles, Figure 4) were fit to a curve (dotted lines, Figure 4), which represented the balance between the microbubble mixing and destruction rates.

In this study, there were two experimental conditions, which required two different curve fitting methods. The first was pre-perfusion, which included the baseline and 1-hour warm ischemia time points. The second was post-perfusion, which included the 3-hour consecutive perfusion. The microbubble MR was coupled with the PR in both pre- and post-perfusion conditions. The MR was directly affected by the pump speed, which in turn, was dictated by the PR. In the pre-perfusion condition, there were low pump circulation speeds, and thus slow mixing of microbubbles. Additionally, the PR was likely low due to the cold storage period. These two factors led to the slow increase in baseline intensity, as seen in the roughly linear balance between the MR and DR (dotted line in the pre-perfusion panel of Figure 4). Because the intensity contributions from MR and DR were almost constant throughout the image acquisition process, the PR could be isolated by simply subtracting the MR-DR (dotted line) from the original intensity curves (solid line).

In the post-perfusion condition, the combined MR-DR rate were found to be non-linear. This is most likely because after perfusion, the MR was dramatically increased due to higher machine pump speeds, thus causing the microbubbles to approach their equilibrium concentration at a faster rate. As the image acquisition progressed, the MR rate decreased. This was represented by the exponential shape of the increase in baseline intensity (dotted line in post-perfusion panel of Figure 4). Because the MR-DR balance was not consistent throughout imaging, the same subtraction normalization method from the pre-perfusion condition could not be used. The changing MR-DR balance significantly affected the shape of the intensity curves, thus the intensity curves had to be scaled according to the MR-DR balance. The MR-DR exponential fit was used to baseline normalize the original intensity curves - scaled to go from 0 to 1 and then multiplied by the original intensity curve (solid

lines, Figure 4). The normalized intensity curves are shown in the right panels of Figure 4, and show consistent intensity magnitudes with each consecutive flash.

After baseline intensity normalization, mean A , β , and $A \cdot \beta \pm SD$ values were calculated for each time point using both the Schlosser and the improved method. All image and data analysis was performed using MATLAB® version 2016 and Microsoft® Excel 2016.

IV. RESULTS

Figure 5 shows the mean PRs (solid line) calculated from multiple replenishment curves for each time point. The results indicate that the rate remains relatively constant throughout the warm ischemia period and increases significantly after 1-hour perfusion. The mean PR has a similar trend to the ATP level (dotted line) at each time-point. Cross-correlation between PR and ATP was highest at 0 lag, indicating that the CEUS method is capable of detecting changes in liver health over time.

The PR decreases from 2 to 3-hour mark, which is potentially a marker of decreased liver health, which can occur even during machine perfusion. Additionally, the 1-hour warm ischemia in the experiment no doubt subjected the liver to significant damage that could not be fully reversed by machine perfusion. The PR increase after 1 hour of warm ischemia is unexplained but may relate to vasodilation. Notably, ATP concentration follows a similar trend.

To determine if the increase in flow rate occurred as a result of increased vessel cross sectional area (A) or increased blood velocity (β), it is necessary to look at the values separately. Figure 6 shows mean A and β at each time point. A remains relatively constant throughout while β has a sharp increase after 1 hour of perfusion. The stable cross-sectional area is consistent with our expectations. The vessel size does not change much throughout the experiment because the liver is not drained of blood at any point; there is no significant vessel collapse or expansion. The shape of the velocity curve is consistent with the shape of the overall PR, indicating that the increase in blood velocity is likely the root cause of the increase in PR post-perfusion.

V. CONCLUSION

In this work, we addressed limitations of existing CEUS methods and developed an improved approach to quantify the perfusion quantitation in the *ex vivo* machine-perfused liver. This approach demonstrated measurable changes after warm liver ischemia and offers potential for the evaluation of perfusion in the machine-perfused pre-transplant liver. Other potential applications of this methodology include the evaluation of organ perfusion and tumor perfusion measurements. Further investigation of applicability of this approach in other contexts is warranted.

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Figure 1. Experimental setup.

The pig liver is submerged the perfusion bath within the organ chamber. Gray and black arrows indicate the hepatic artery and portal vein connections, respectively.

Figure 2. ROI selection and intensity curve for a single time point.

Left: Exported video from GE Logiq E9 machine (representative frame shown), showing the contrast setting. Blue outline is the selected ROI. Right: Intensity curve of selected ROI, showing multiple flash-replenishments.

Figure 3. Normalization using the Schlosser method.

The steady state intensity value decreases for each consecutive flash-replenishment curve.

Figure 4. Curve normalization using the improved method.

Original intensity curves shown by the solid lines in the left panels, curves fitting the post-flash intensity values (representing the mixing-destruction rate) are shown by the dotted lines, and the normalized curves are shown by the solid lines in the right panels. Pre-perfusion: The diffusion-destruction rate appears linear. Post-perfusion: The diffusion-destruction rate appears exponential.

Figure 5. Mean flow rate ($A \cdot \beta$) overlaid with ATP results.

Averages and standard deviations calculated from multiple flashes at each time point. Both metrics show an increase in liver health as perfusion time is increased, ending with a slight decrease, indicating degradation in liver quality. Cross-correlation between flow rate and ATP shown in box.

Figure 6. Mean A overlaid with mean β results at each time point.

The tissue blood volume (A) is fairly constant throughout while the blood velocity (β) dramatically increases after 1hour perfusion.