# Prolonged Ex Vivo Animation of Tumor-bearing Liver as the Ideal Tumor Model System

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## Introduction

- Currently available pre-clinical liver tumor model systems (i.e. cell lines, mouse models, or patient-derived xenografts) lack the appropriate clinical predictive power
  - Largely due to the absence of human stromal components and immune system, and their interactions with tumor cells
- Ex vivo liver perfusion has been performed in the transplant setting since 2005
  - Currently most literature has indicated a maximum viability time of 6 hours at normothermic temperatures (37 °C) and 12 hours at cold perfusion (21 °C) in whole livers
- Use organ perfusion systems to create a new tumor model of the human liver as a site of metastatic disease.

## Objectives

- To optimize methods for ex vivo perfusion of resected, tumor-bearing liver specimens
- To prolong viability of liver specimen
  - Reduce liver lymphedema and improve lymphatic drainage through mechanical compressions
- To explore applications of the tumor model system

## Methods

- Specimen Preparation
  - *Porcine Liver specimens.* Whole porcine livers were resected to obtain the left liver segment for perfusion.
  - *Human liver specimens.* Specimens were taken from patients with primary or metastatic tumors of left liver segments and underwent clinically indicated resection.
  - The portal vein, hepatic artery and bile duct of specimens were cannulated.

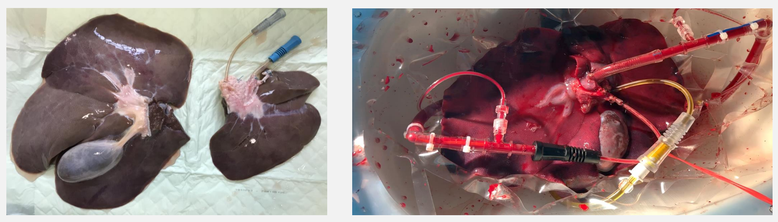

**Figure 1: (a)** Resection of the whole porcine liver to yield left liver segment specimen. **(b)** Perfusion of liver specimen in the LiverAssist.

- Liver Assist perfusion machine (OrganAssist, Netherlands)
  - Consists of two pump units, a thermo unit, a rotary pump, an oxygenator and perfusion reservoir
  - System pumps oxygenated perfusate continuously through the portal vein (non-pulsatile) and hepatic artery (pulsatile) of the liver specimen

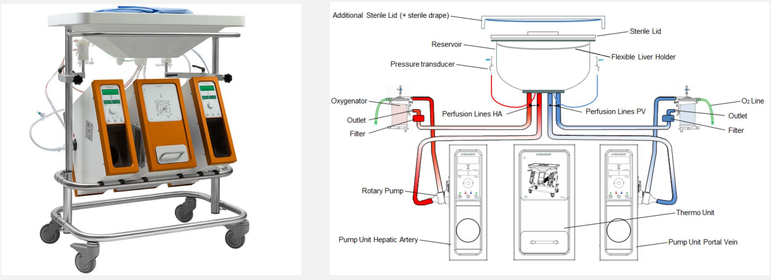


**Figure 2: (a)** LiverAssist machine. **(b)** Schematic of the perfusion of liver specimen in the LiverAssist.

- Perfusate Composition
  - Consists of cell free oxygen carrier (HBOC), packed red blood cells (pRBCs), fresh frozen plasma, albumin, modified parenteral nutritial, multivitamins, trace elements, insulin, metronidazole, sodium bicarbonate, heparin, bile salts, etc. Oxygen is added to circulating perfusate by LiverAssist oxygenator.

## Liver Compression Device

- Preliminary testing showed that perfusion flow rates are maintained initially, but then significantly decrease as perfusion continues. This correlates with the declining function and viability of the specimen.

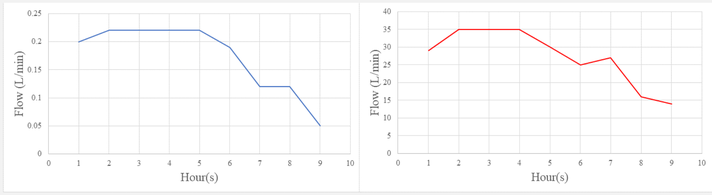


**Figure 3: (a)** Portal vein flow rates significantly decreases starting at the 5 hr time point. **(b)** Hepatic artery flow rates significantly decreases starting at the 4 hr time point

- Lymphatic flow of the liver influences tolerance of machine perfusion. The specimen is qualitatively observed to swell over the course of the perfusion due to the accumulation of lymph.
- A liver compression device was developed to mimic the natural motion of the diaphragm on the liver. A mechanical ventilator inflates and deflates a bladder at regular intervals, providing regular compression to the liver. Motion of the bladder stimulates lymph drainage and prevents lymph accumulation

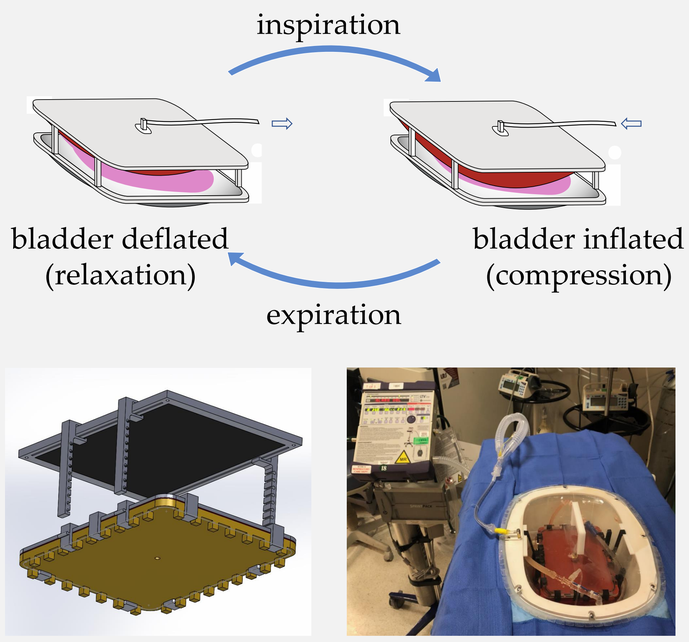


**Figure 4: (a)** Mechanism of the liver compression device. Bladder inflates and deflates at regular intervals using a mechanical ventilator. **(b)** SolidWorks figure of liver compression device. **(c)** Setup of liver compression device, mechanical ventilator, and LiverAssist perfusion machine. 0

## Results

- General and liver-specific parameters (pH, $pO_2$, glucose, lactate, urea, bile acids, etc.) as well as liver enzymes (ALP, ALT, AST) were analyzed over the course of the perfusion to evaluate liver viability. Tissue pressure was measured using a Stryker needle, and tumor architecture and immune components was evaluated using histopathologic examination from liver biopsies.

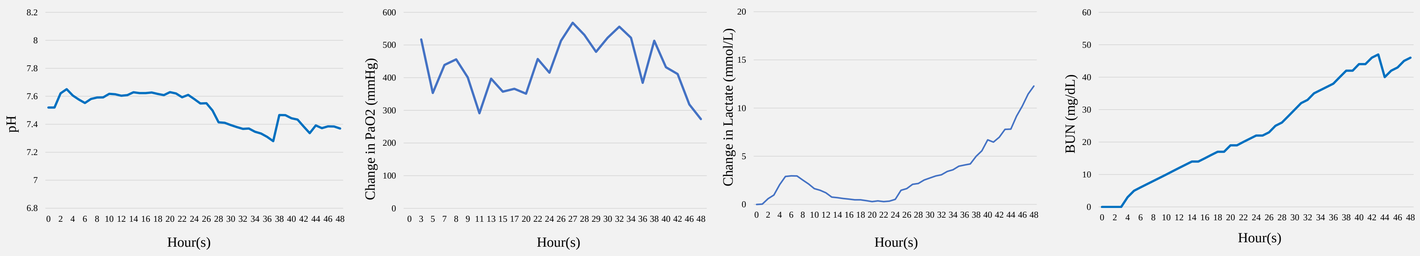


**Figure 4: Sample parameters monitoring liver specimen viability and function.** Liver exhibited regulation of pH, oxygen consumption, lactate metabolism, and secretion of urea

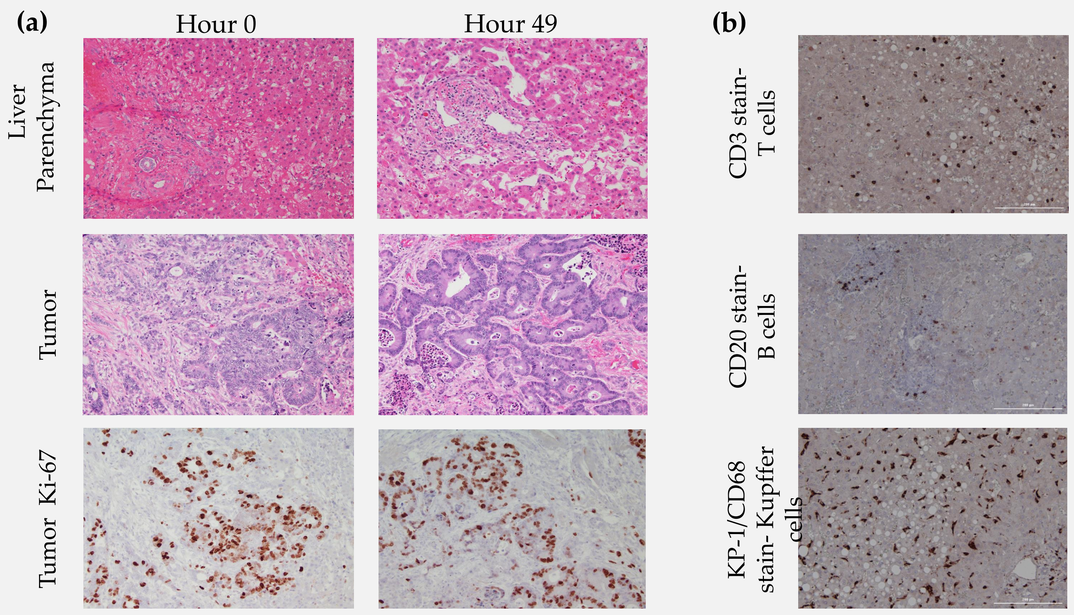


**Figure 5: (a)** Hematoxylin and eosin stains of liver parenchyma and tumor demonstrate an intact architecture between final biopsies (at ex vivo end time point) and baseline (at ex vivo starting timepoint). Tumor Ki-67 stains show continuing cell proliferation at final ex vivo timepoint. **(b)** CD3, CD20 and KP-1/CD68 stain of final biopsy shows presence of T cells, B cells and Kupffer cells, respectively. Stains demonstrate presence of immune cells on final biopsy in expected amounts.

## Discussion

We demonstrate that the perfusion of resected tumor-bearing human livers can be successfully established, and that the ex-vivo perfusion of liver specimens can maintain liver viability and functionality. Proof-of-concept of a liver compression device to reduce liver swelling due to lymph accumulation and maintain liver viability is demonstrated. Liver specimens displays evidence of physiologic function; tumor architecture remains intact and histologically unchanged; and immune and stromal components similarly remain intact and functional.

## Acknowledgements

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