VitaCyte®
Unravelling Cell Isolation

Webinar

Collagenase Selection & Process Optimization for High Yield Hepatocyte Isolation

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Dimension Inx

Agenda

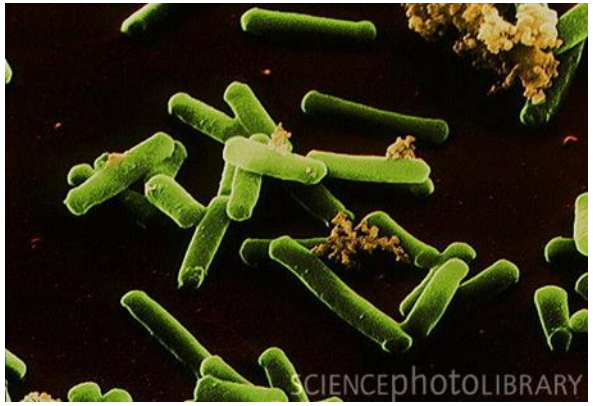
- **Introduction and strategy for choosing Collagenase**
- **Hepatocyte Isolation and process optimization**
- **How to get most cells out of the liver - examples**

*Disclosure: All data in this presentation has been publicly presented and/or do not include any proprietary or confidential information

Collagenase - Introduction

Clostridium histolyticum

- An anaerobic, motile, gram-positive bacterium that thrives in feces and soil
- The ammonia and proteases it produces, including several collagenases, digest proteins outside its body into amino acids, which it eats.



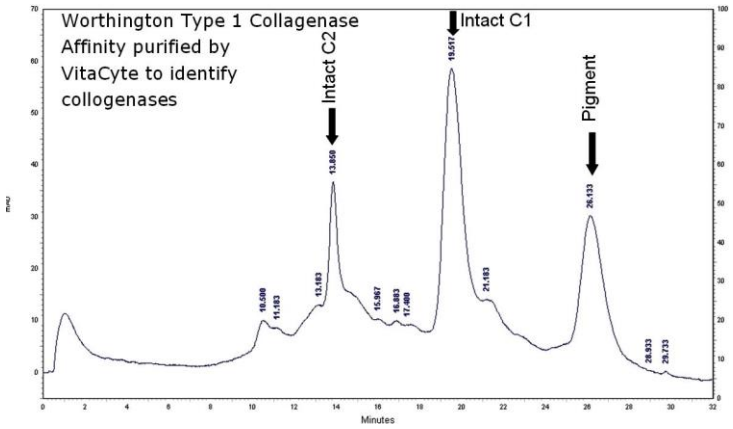
Crude collagenase is minimally purified *Clostridium histolyticum* culture supernatant that is later lyophilized: **high lot to lot variability**

Contains essential enzyme for tissue dissociation; collagenase and neutral protease but also other components: other enzymes, endotoxin, pigment

Enriched collagenase products are further purified to reduce pigment and increase enzyme activity but still reflects **lot to lot variability** of the culture supernatant

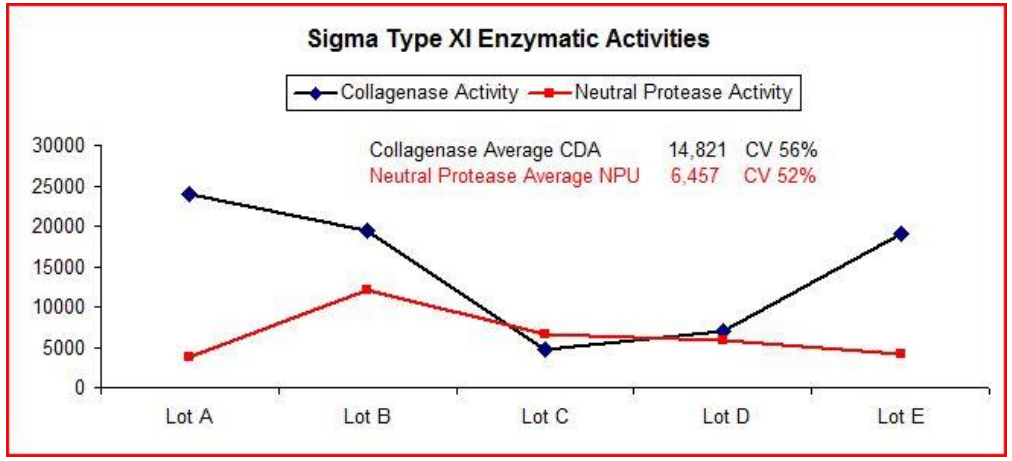
Key biochemical concepts that define Collagenase

Two classes of collagenase – class I (C1) and **class II (C2)** – initially defined by different substrate specificities, degrade collagen synergistically



Crude collagenase
(HPLC)

Collagen degradation activity (CDA) is the critical enzymatic activity required for tissue dissociation



Types of Collagenase

- **Type 1** crude collagenase has the **original balance** of collagenase, caseinase, clostripain and tryptic activities.
- **Type 2** contains **higher** relative levels of protease activity particularly clostripain.
- **Type 3** contains **lowest levels of secondary proteases (that means clostripain and caseinase)**.
- **Type 4** is designed to be especially **low in tryptic** activity to limit damage to membrane proteins and receptors.

- Hepatocytes in a normal non-fibrotic liver are sensitive to Trypsin and can be easily damaged if Collagenase has strong tryptic activity
- Fibrotic livers will require stronger Tryptic activity to release hepatocytes from underlying ECM

Collagenase – Strategy for Selection

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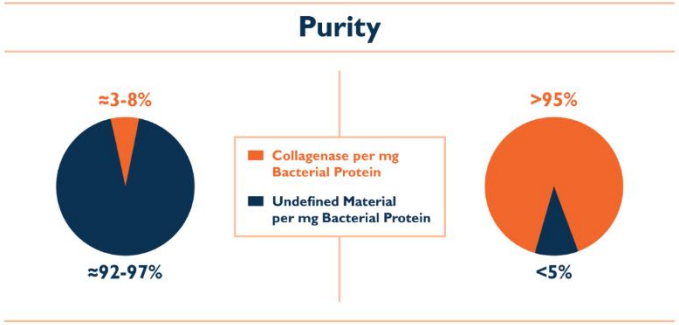
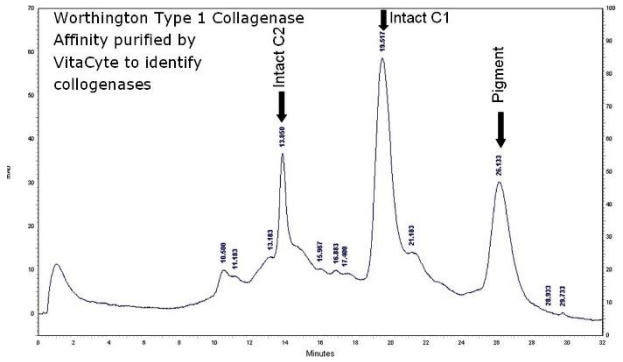
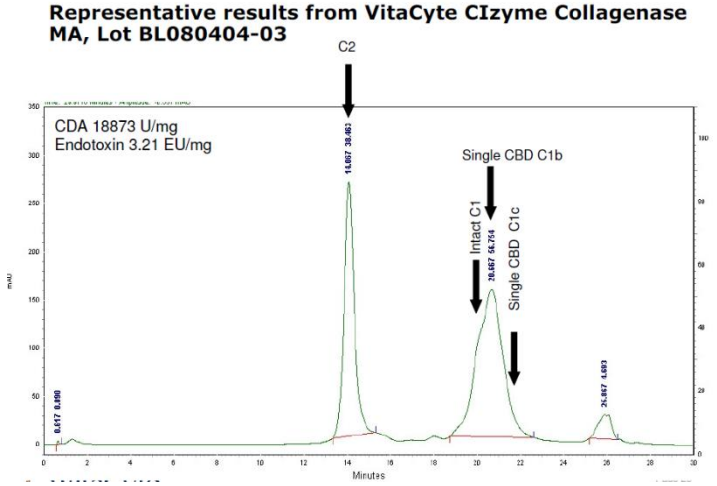
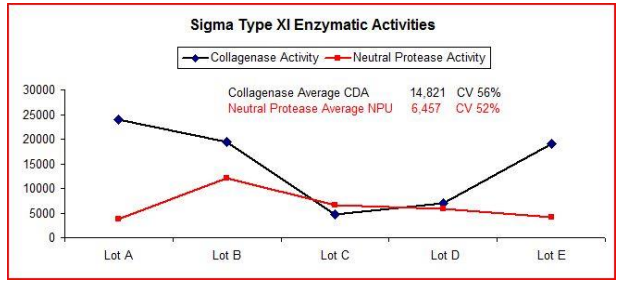
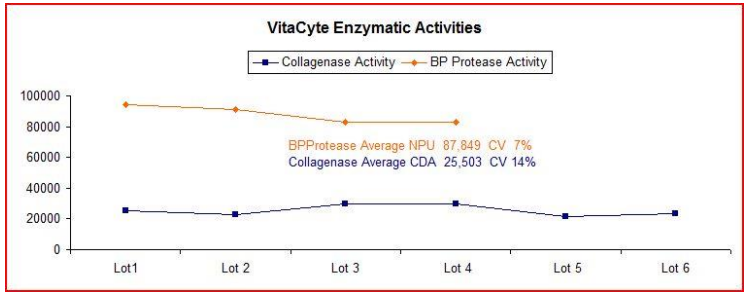
Traditional Collagenase vs **VitaCyte Collagenase**











- VitaCyte Collagenase is consistent from batch to batch and has high Collagen degradation activity (CDA)
- **VitaCyte offers ability to optimize isolation process and maximize hepatocyte yield per gm/tissue**

VitaCyte®
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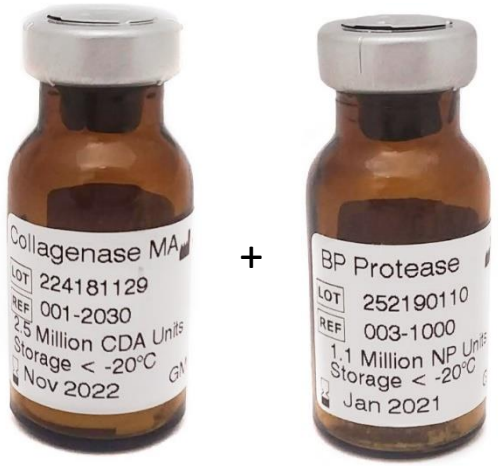
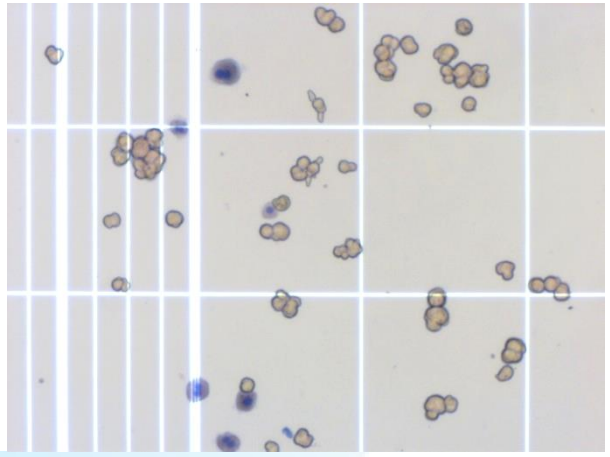
VitaCyte Working Collagenase = Collagenase MA + BP Protease

Collagenase – separation of cells from ECM
Protease, EGTA – separation of cells from each other

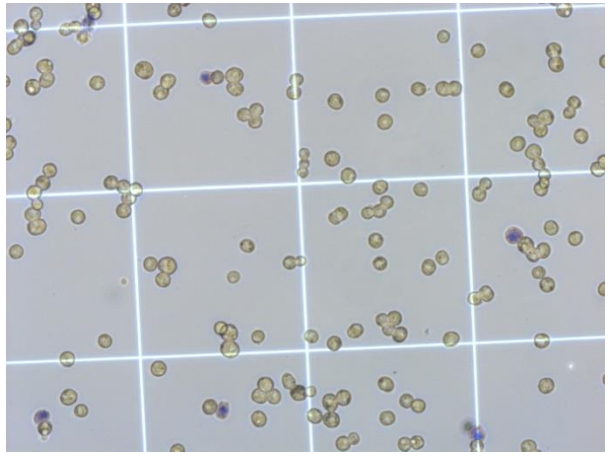


By adjusting perfusion time for Buffer 1 (EGTA) and adjusting concentrations of collagenase and protease, a “perfect” combination of digestive enzymes can be established for all specie hepatocyte isolations that would allow maximum cell recovery (yield) and minimal damage to cellular membranes (viability and cryo).

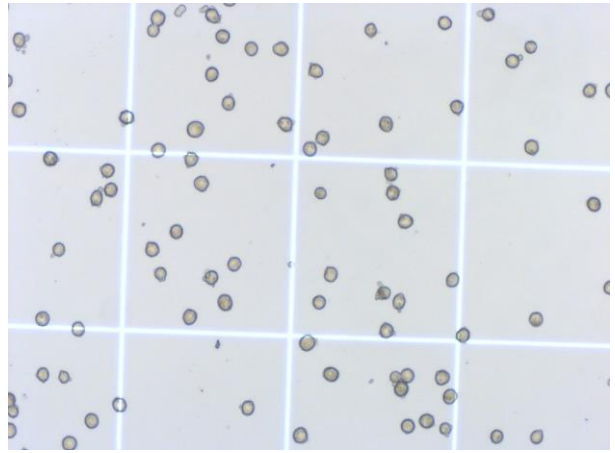
Mouse



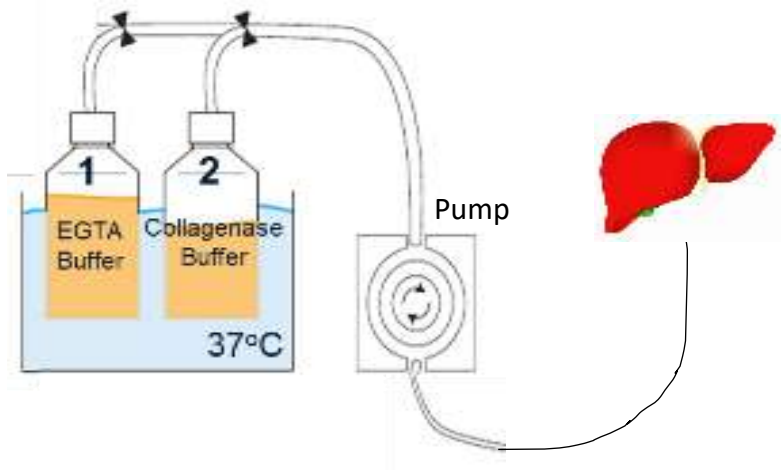
Rat



Human



Hepatocyte Isolation - Introduction



Standard 2 Step Collagenase Isolation of Hepatocytes

(Step 1) – Perfusion with 37°C Calcium free buffer + EGTA

- blood removal
- re-inflation of capillaries – perfusion flow speed is based on liver inflation
- relaxation of E-Cadherin junctions by EGTA
- time dependent process

(Step 2) – Perfusion with 37°C buffer containing Calcium and Collagenase

- start with Vitacyte recommended enzyme concentrations, then optimize
- time and Collagenase concentration dependent process
- digestions stopped when tissue disaggregation visible
- too short digestion (under-digested) = low yield, low viability (bellow 70%)
- too long digestion (over-digested) = high yield, low viability (bellow 70%)

Approximate Number of Hepatocytes:

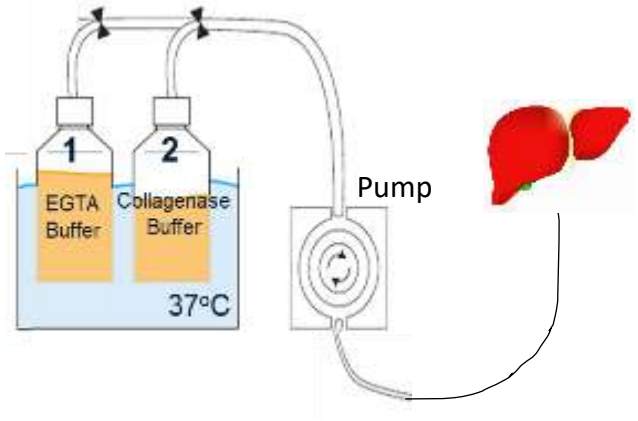
- **Human Liver**: 100-160 Billion Hepatocytes
- **Rat Liver**: 1.5-2 Billion Hepatocytes
- **Mouse Liver**: 100 – 150 Million Hepatocytes

What to expect in number of isolated hepatocytes if process is optimized:

- **Human Liver**: **flow at 1/3 of tissue mass, 20-25 min**, **20-40 Million /gm** tissue (Adult liver, sectioned), → 20-40 Billion total, 60-80% viability
  flow at 1/3 of tissue mass, 20-25 min, **50-75 Million/gm** tissue (pediatric, sectioned or whole) → 15-25 Billion total, 80-95%
- **Rat Liver**: **15-20ml/min, 15-20 min**, **0.8-1 Billion** Hepatocytes per rat, 80-95% viability
- **Mouse Liver**: **6-10ml/min, 10-12 min**, **50-100 Million** Hepatocytes per mouse, 80-90% viability

Hepatocyte Isolation – High Level Summary

Ex vivo Isolation Method



- Enzymes selection
- Type of isolation buffers
- Whole organ vs resection perfusion
- Single pass vs recirculation

Liver Tissue

- Tissue acceptance criteria
- Donor age
- Tissue "quality"
- WIT/CIT

Digestion

Hepatocyte preparation

- Process variations
- Purification methods
- Immediate processing vs recovery
- Temperature of processing

Cryopreservation

- Crygenic solution
- Handling and Storage (Vapor Phase LN2)
- Vial to vail variability
- Shipping conditions

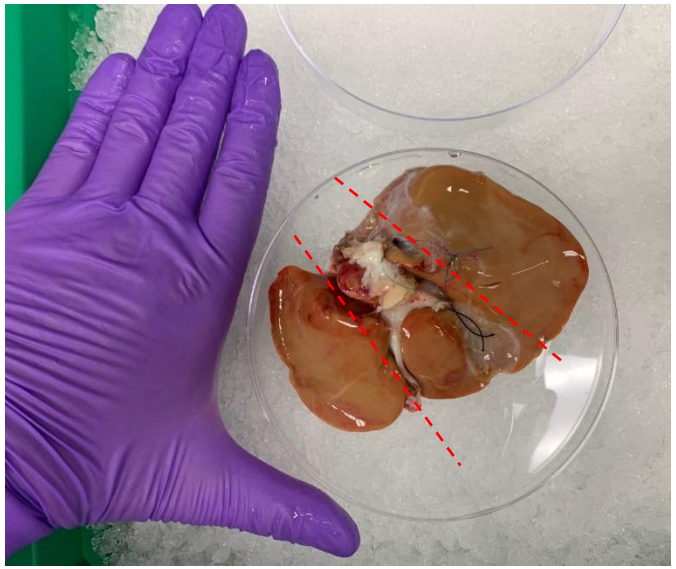
- Isolation of primary human hepatocytes is a **highly variable process** that can substantially differ from group to group
- **Each isolation process is "unique"**
- Industry developed analytical methods to help standardize selection of good quality lots for their customers – CofA

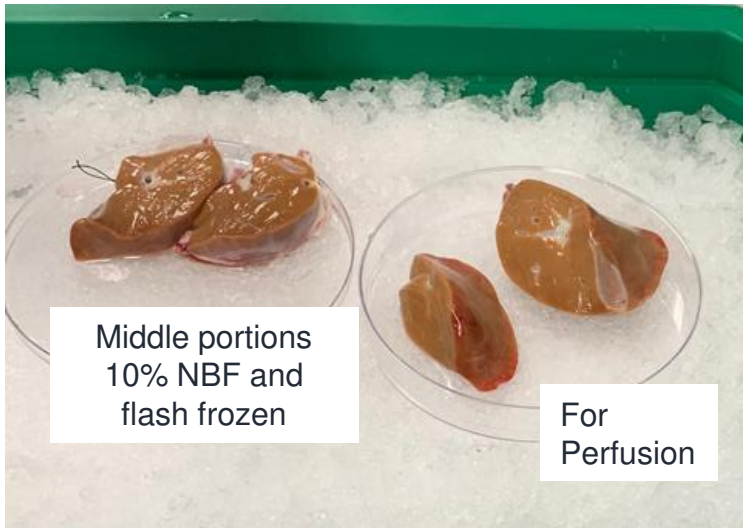
Liver preparation – Pediatric, cut, Vitacyte Collagenase MA

Pediatric Liver
BMI: 22.3
Tissue Weight: 150gm,
100gm perfused – 35ml/min

Post Isolation
Viability post isolation: 85%
Yield post isolation: 8.9 Billion (89 million/gm tissue)

Final Viability after cleanup: 93.2%
Final yield: 7.27 Billion (72 million/gm tissue)



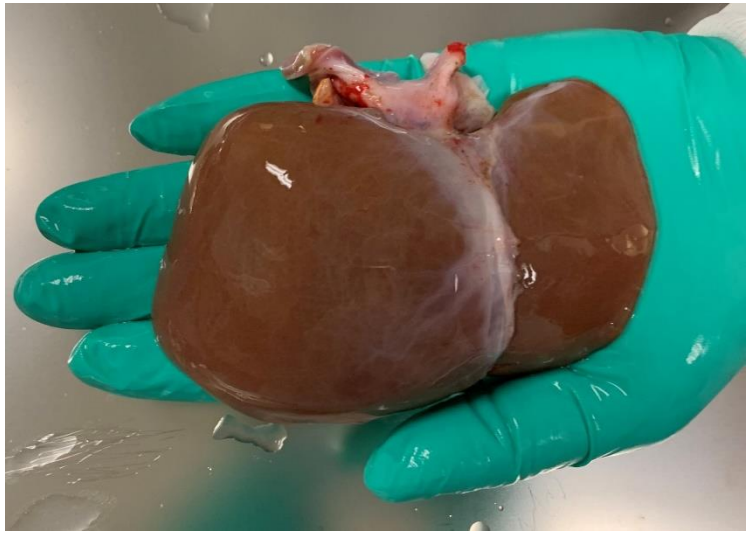


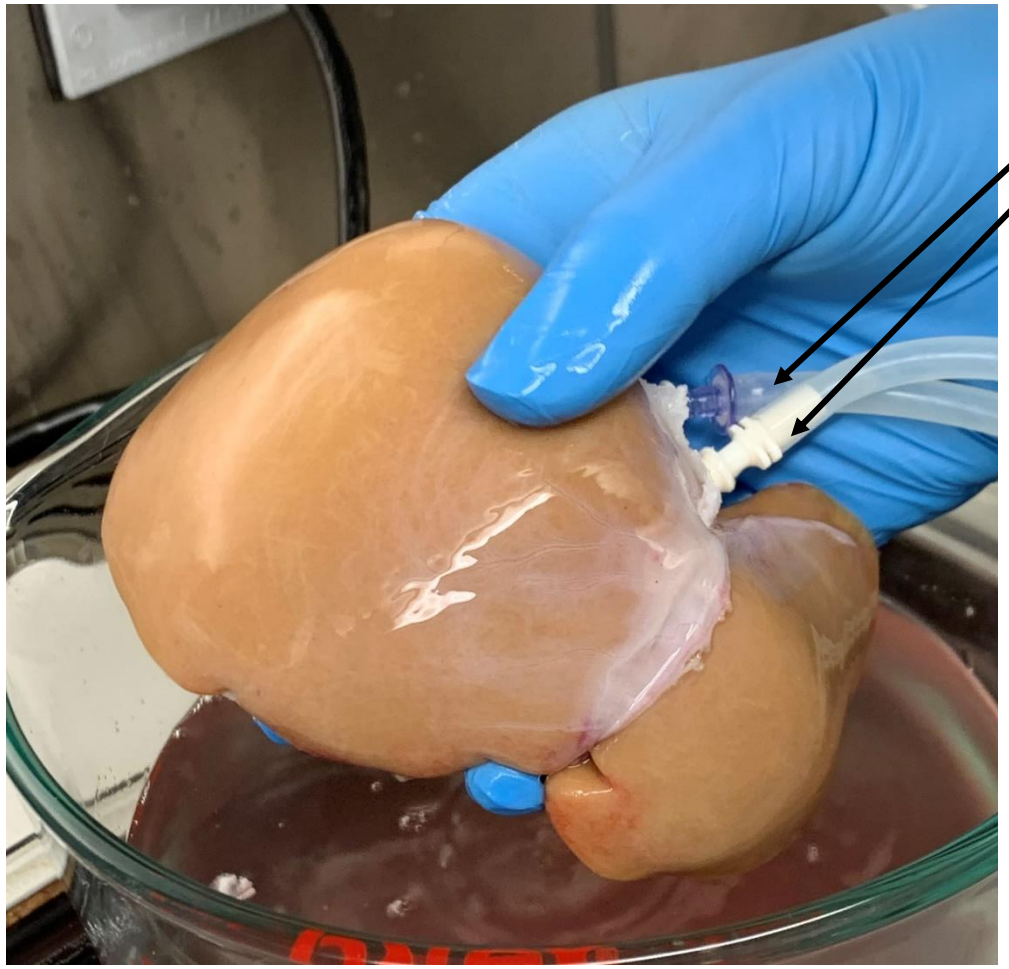
Liver preparation – Pediatric, whole, Vitacyte Collagenase MA

Pediatric Liver
BMI: 21
Tissue Weight: 240gm – 80ml/min

Post Isolation
Viability post isolation: 89%
Yield post isolation: 16.8 Billion (70 million/gm tissue)

Final Viability after cleanup: 96%
Final yield: 15.2 Billion (63 million/gm tissue)





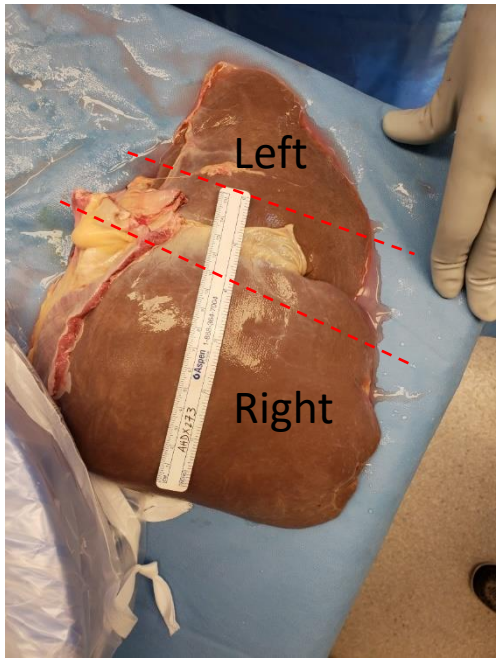
Liver preparation – Adult, cut, Vitacyte Collagenase MA

Adult Liver
BMI: 25.7
Tissue Weight: 1,450gm

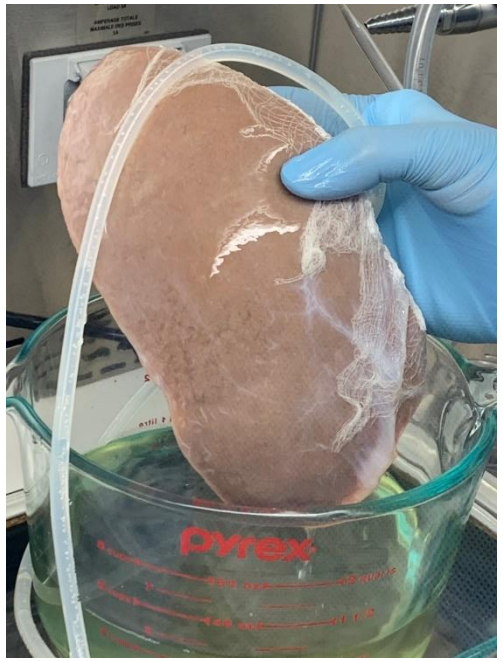
Left Lobe: 280gm – 100 ml/min
Right Lobe: 670gm – 140ml/min
Total perfused: 950gm

Post Isolation
Viability post isolation: 76%
Yield post isolation: 36 Billion (38 million/gm tissue)

Final Viability after cleanup: 92%
Final yield: 24.2 Billion (25 million/gm tissue)



Cut and sealed, ready for perfusion



Right lobe during perfusion

10B hepatocytes in 2L bottle

Liver digestion – how to tell when digestion is completed

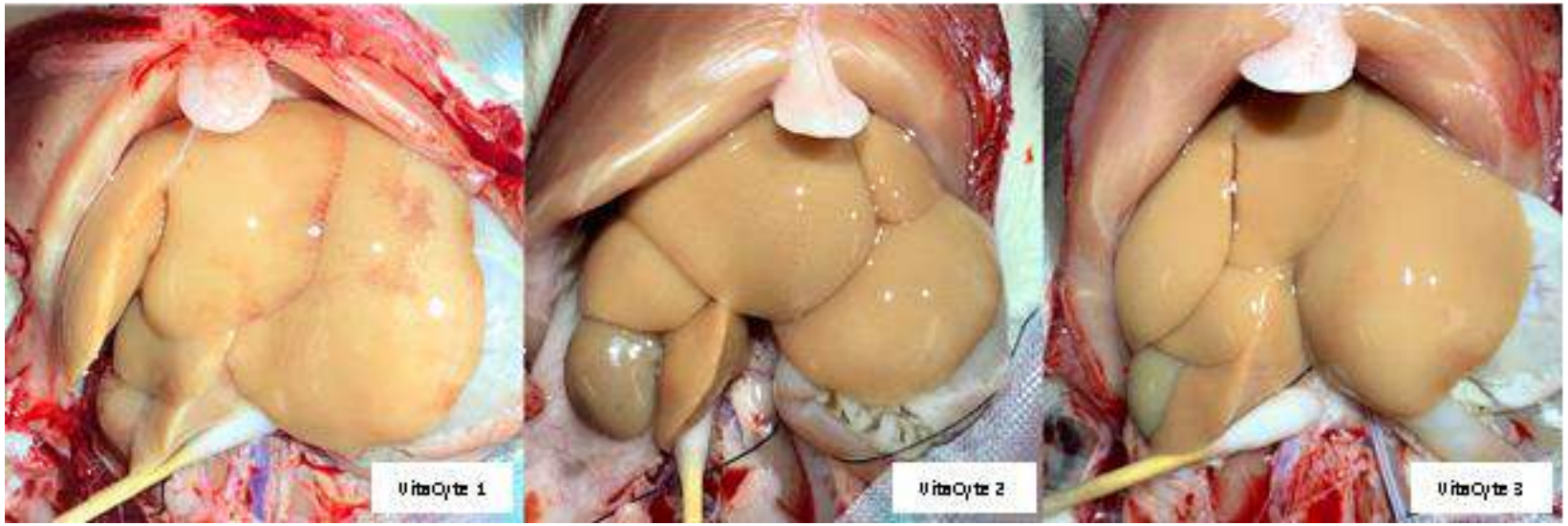
Tissue breakage indicates complete digestion

- Tissue disaggregation is uniform, cells fall apart easily,
- Need 3-5 min to shake the cells off the scaffold

- Visible ECM and nearly full tissue disaggregation

- 1,200 vials of hepatocytes at 15M hepatocytes/vial

Liver digestion – Rat isolation, Vitacyte MA Collagenase



			Pre-Percoll®				Post-Percoll®			
Liver	Liver mass (g)	Enzyme	Total yield	Viable yield	Viability	Viable yield/ g liver	Total yield	Viable yield	Viability	Viable yield/ g liver
1	15.4	VitaCyte® (MA/BP)	7.80E+08	6.30E+08	0.81	4.09E+07	3.23E+08	2.95E+08	0.91	1.92E+07
2	13.5		1.11E+09	9.90E+08	0.89	7.33E+07	9.00E+08	8.35E+08	0.93	6.19E+07
3	12.8		8.63E+08	6.90E+08	0.80	5.39E+07	6.30E+08	6.00E+08	0.95	4.69E+07

Conclusion

VitaCyte®
Unravelling Cell Isolation

- Hepatocyte isolation is a highly variable process
- The key to successful high yield isolation is to control variables
- Collagenase is a variable that can be controlled by using Vitacyte Collagenase
- Use of Vitacyte Collagenase allows for controlled process optimization that can result in high yield, high viability isolation process

Acknowledgments:

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