Primary mouse hepatocyte isolation

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Method Article

Keywords: hepatocyte isolation, mouse hepatocyte, primary tissue culture

Posted Date: September 4th, 2019

DOI: https://doi.org/10.21203/rs.2.12018/v1

Abstract

This protocol is intended to isolate primary hepatocytes from *mus musculus* (mouse) for the purposes of culturing primary hepatocytes. Hepatocytes can be used for approximately 5 days from the time of isolation, and cannot be frozen for future use. A single isolation from a single mouse, including time to prepare reagents, takes approximately 2 hours.

Introduction

Reagents

Note: all solutions and buffers should be made the day of procedure, not in advance

In order to make the buffers needed, first prepare the following reagents.

1. HEPES solution: 7.2 g HEPES in ~25 mL of 1xHBSS to final volume of 30 mL

2. CaCl2 solution: 74 mg of CaCl2 and 10 mL of 1xHBSS

3. EGTA solution: 95 mg of EGTA and 5 mL of 1xHBSS. *In order for EGTA to dissolve, the solution must be made basic with the addition of NaOH. Add incrementally until dissolved.*

Perfusion buffer:

400 mL 1xHBSS

25 mL HEPES solution (see step 1 above)

5 mL EGTA solution (see step 3 above)

pH solution to 7.5 with NaOH and complete volume to 500 mL with HBSS; Place solution in 37°C bath until use

Collagenase buffer

80 mL 1xHBSS

10 mL CaCl2 (see step 2 above)

5 mL HEPES (see step 1 above)

24 mg collagenase (*added right before use*)

pH solution to 7.5 with NaOH; Place solution in 37°C bath

Mass collagenase in a microcentrifuge tube and place on ice until use.

For *hepatocyte cell culture*, prepare the following medias:

Washing media

500 mL M199 media

5 mL 100X glutamax

**May be adjusted depending on number of mice (~50 mL of washing media per mouse); Filter sterilize and place in 37°C bath*

Plating media

174 mL M199 media

2 mL 100x glutamax

2 mL 100x pen/strep

2 mL 20% BSA

20 mL FBS

**Filter sterilize and place in 37°C bath*

Equipment

Collagenase from Clostridium histolyticum release of physiologically active rat hepatocytes tested, Type IV, 0.5-5.0 FALGPA units/mg solid, ≥125 CDU/mg solid(Sigma Aldrich Cat #C5138-500MG)

Masterflex® L/S® Precision Variable-Speed Perfusion Machine (Cole Parmer #HV-07528-10)

60mm TPP Tissue Culture Dishes (LightLabs #TC-1015)

Mesh strainer, 70 micron (Bd Biosciences 352350)

Glutamax (Invitrogen 35050061)

Avertin (Sigma Aldrich T48402-25G)

Masterflex platinum-cured silicone tubing, L/S 15, 25 ft (Cole Parmer HV-96410-15)

Terumo™ Surflo™ Winged Infusion Sets (Fisher scientific 22-289912)

Qtips (Fisher scientific 22-029-488)

Collagen coated coverslips (GC-12, Neuvitro)

Procedure

1. Setup perfusion station: Place metal platform within the glass chamber. Run the tubing through perfusion machine and clamp the end of the tubing with a large binder clip. Attach a butterfly needle to each port. [**See Supplementary Picture 1**].

2. Place the unclamped end of tubing in the perfusion buffer and turn on perfusion machine. Let the buffer flow until it is seen coming out of each needle, then clamp each butterfly needle tubing. Pause the perfusion machine until ready to use.

3. Take anesthetized animal (via IP injection of Avertin) and place supine on metal platform and tape all limbs down.

4. Remove the abdominal skin, preserving the muscle and peritoneum. Open the peritoneum and open the abdominal cavity for full visualization of the liver and abdominal organs.

5. Push the stomach and intestines to the right side of the animal and identify the inferior vena cava (IVC) and portal vein (PV).

6. Cannulate the IVC with the butterfly needle ensuring that the bevel of the needle is facing up. Use a hemostat to secure the needle within the IVC and tape the tubing down to the metal platform. Unclamp the butterfly needle tubing. Turn on the perfusion machine. If the IVC was successfully cannulated, the liver should begin to engorge and lighten in color.

7. Immediately identify and cut the PV. At this point the animal should rapidly bleed out and perfusion buffer should begin to flow out of the PV.

8. Pass at least 100 mL of perfusion buffer through a single mouse.

9. While the perfusion is running, add the collagenase (transported on ice) to the collagenase buffer and mix well.

10. Pause the perfusion machine and switch the tubing to the collagenase buffer. Resume perfusion and run through entire volume of collagenase buffer.

11. When the perfusion is finished, remove the liver by cutting along the vasculature and connective tissue; avoid cutting the liver parenchyma. Place the liver in a petri dish with 10 mL of perfusion buffer.

12. Agitate the liver to dissociate the hepatocytes.

13. Using a serological pipette, transfer the dissociated cell suspension to a 70 micron mesh filter over a 50 mL Falcon tube. Once filtered through, complete the volume with perfusion buffer to dilute the collagenase.

14. Immediately centrifuge at a minimum speed of 800 rpm for 5 minutes.

15. Discard the supernatant. Resuspend in washing media and centrifuge again at 800 rpm for 5 minutes.

16. Discard the supernatant. Resuspend in 10 mL (or 5 mL if the cell pellet is small) of plating media.

17. Suspended in plating media, the hepatocytes will remain viable for a few hours, until plated. Once plated, place in 37°C cell incubator. Cells should be plated on a collagen coated surface.

Troubleshooting

Time Taken

Anticipated Results

References

Acknowledgements

We have adapted this protocol from Dr. Dudley Lamming's lab, and we would like to thank Dr. Sebastian Arriola Apelo and Nicole Cummings for advice on hepatocyte isolation and culture.

Figures

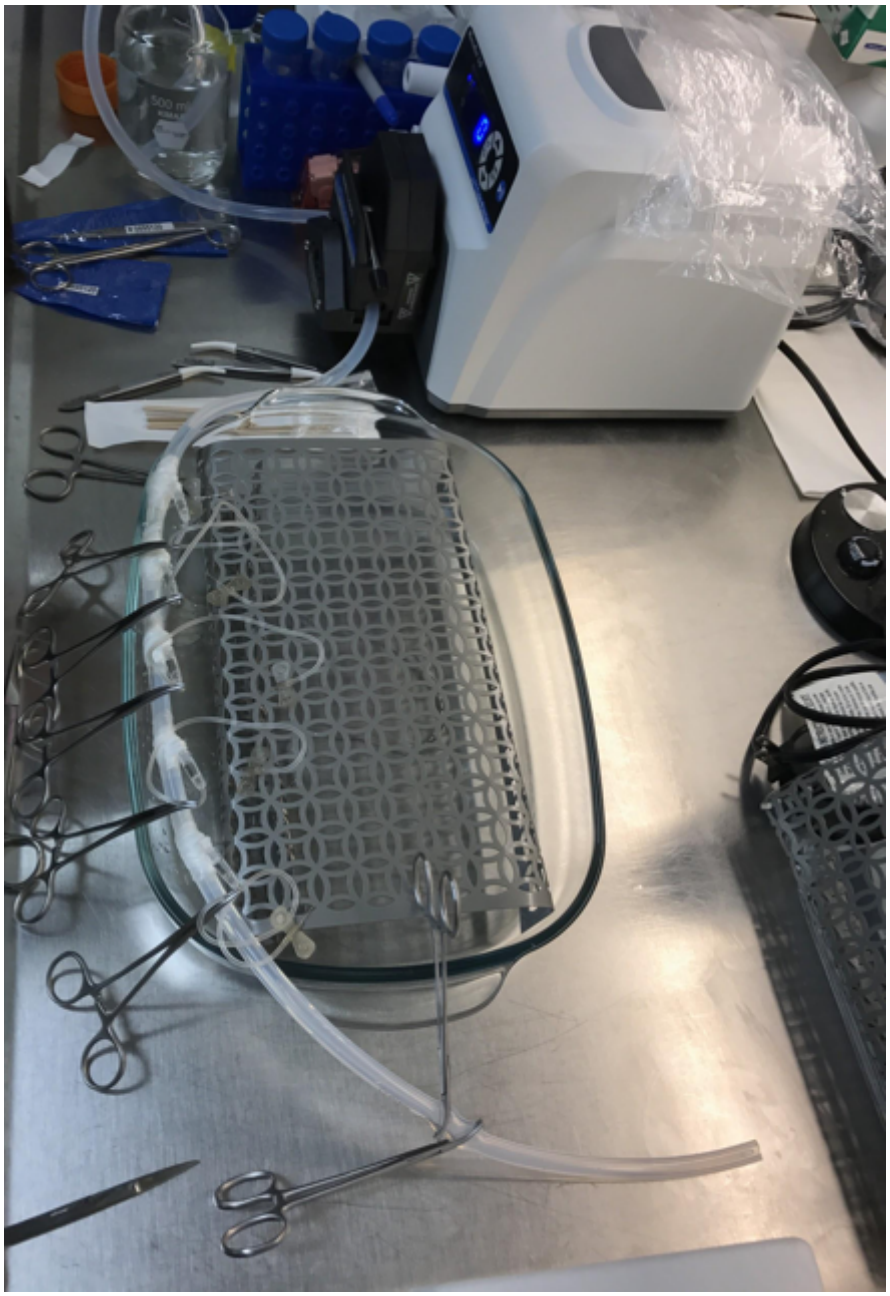

Figure 1

Perfusion station for primary hepatocyte isolation.