Rat Hepatic Parenchymal Cell Isolation and Culture Kit

Cat.No. : P-CA-615

Size：3Tests/10Tests

Background

This kit is based on an improved classical perfusion digestion method for isolating and extracting rat hepatic parenchymal cells. After each experiment (per T), at least 2 × 10^7 rat hepatic parenchymal cells can be obtained, following the perfusion digestion of liver tissue from one rat, as well as the isolation and purification. Immunofluorescence identification shows that the cell purity (CK-18 positive rate) is > 90%.

Scope of Application

This product is suitable for Sprague-Dawley、Wistar-Imamichi and other different species of rats aged 14-56 days. After perfusing and digesting the liver tissue, performing low-speed centrifugation, using trypan blue staining and cell counting, a yield of >2 × 10^7 cells can be obtained with the cell purity (CK-18 positive rate) exceeding 90%. The obtained hepatocytes continue to differentiate after 24 hours of cultivation, gradually losing their morphological characteristics, and the entire cultivation cycle can last for 1-2 weeks.

Components of Kit

The components of this kit is shown in the table below

Table 1. Composition and corresponding information of the Kit

Name	Size	Appearance	Storage and Expiration Date
Pre-perfusion Solution For Rat Hepatic Parenchymal Cells	3Tests (300 mL) 10Tests (500 mL×2)	Colorless Clear Liquid	2-8℃, 1 year
Diluent Of Perfusion Digestive Enzymes For Rat Hepatic Parenchymal Cells	3Tests (300 mL) 10Tests (500 mL×2)	Red Clear Liquid	2-8℃, 1 year
Perfusion Digestive Enzyme For Rat Hepatic Parenchymal Cells	3Tests (1.5 mL) 10Tests (5 mL)	Brown Clear Liquid	-5~-20℃, 1 year
Specialized Washing Solution For Rat Hepatic Parenchymal Cells	3Tests (250 mL) 10Tests (500 mL)	Red Clear Liquid	2-8℃, 1 year
Coating Buffer For Rat Hepatic Parenchymal Cells	3Tests (20 mL) 10Tests (50mL)	Colorless Clear Liquid	2-8℃, 1 year
Basic Culture Medium A For Rat Hepatic Parenchymal Cells	3Tests (75 mL) 10Tests (250 mL)	Red Clear Liquid	2-8℃, 1 year

Continued

Name	Size	Appearance	Storage and Expiration Date
Supplement A For Rat Hepatic Parenchymal Cells	3Tests (7.5 mL) 10Tests (25 mL)	Yellow Transparent Liquid	-5~-20℃, 1 year
Basic Culture Medium B For Rat Hepatic Parenchymal Cells	3Tests (150 mL) 10Tests (500 mL)	Red Transparent Liquid	2-8℃, 1 year
Supplement B For Rat Hepatic Parenchymal Cells	3Tests (15 mL) 10Tests (50 mL)	Red Transparent Liquid	-5~-20℃, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	Room temperature, 3 years
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Green	Room temperature, 3 years

Note: Please store each component at the temperature indicated on the label of the reagent tube. After thawing, the digestive fluid should be stored at 4℃ for 30 days. Following the first use of the kit, it is recommended to aliquot the digestive fluid into single-use portions according to the instruction and store at a -20℃ refrigerator. Thaw again before use to avoid repeated freezing and thawing.

Notes

1. In this experiment, you need to prepare PBS buffer, trypan blue staining solution, syringes, scissors, forceps, culture vessels, venous indwelling needle, and other reagents and consumables. If the cultivation needs to be expanded, please prepare complete medium independently.
2. If the isolation process of primary hepatocytes is not handled properly, contamination can occur easily. Ensure that all equipment used in the experiment is sterile.
3. Primary hepatocytes cannot be passaged. Do not use various digestive enzymes to digest and passage the fully confluent hepatocytes after extraction.
4. Hepatocytes are sensitive to fluid shear forces. During the isolation process, avoid vortexing or excessive pipetting when resuspend the cell pallet, in order not to affect cell viability.
5. Liver tissue perfusion can be performed by peristaltic pumps, infusion machines and manual operation. During the process, pay attention to controlling the perfusion flow rate to avoid incomplete or excessive tissue digestion. The following steps take peristaltic pumps and manual perfusion as examples, and the perfusion method can be adjusted according to specific laboratory conditions.
6. The operation of rat liver perfusion digestion is complicated. Prior to formal experiments, it is recommended to practice pre-perfusion to familiarize yourself with operational procedures and avoid unnecessary waste.

7. Due to the bloody process of rat perfusion, this manual does not provide actual photos for reference, but only schematic diagram. If necessary, please contact the technical support to obtain actual photos of the experimental process.

Operational Procedures

1. Pre-experiment Preparation

1) Preparation of Culture Medium: Prepare Adhesion Culture Medium For rat Hepatic Parenchymal Cells and Maintenance Medium For Rat Hepatic Parenchymal Cells according to the experimental dosage.

① Adhesion Culture Medium For Rat Hepatic Parenchymal Cells

Basic Culture Medium A For Rat Hepatic Parenchymal Cells: Supplement A For Rat Hepatic Parenchymal Cells=10: 1.

② Maintenance Medium For Rat Hepatic Parenchymal Cells

Basic Culture Medium B For Rat Hepatic Parenchymal Cells: Supplement B For Rat Hepatic Parenchymal Cells=10: 1.

Note:Adhesion and maintenance culture medium: 2–8℃, valid for 3 months. When preparing above-mentioned culture medium, it can be prepared according to the usage amount. Remaining supplements should be aliquoted proportionally and stored at -20℃ to avoid repeated freeze-thaw cycles.

2) Self-supplied Reagents and Consumables: dissection board (foam board can substitute), ice plate or ice box, surgical instruments (at least 2 pairs of ophthalmic scissors, 3 pairs of straight forceps, 2 pairs of curved forceps, 2 pairs of microscopic forceps, hemostatic clips), 6 cm culture dishes, and several 15 mL/50 mL centrifuge tubes.

3) Preparation of Perfusion Digestive Working Solution For Rat Hepatic Parenchymal Cells:

Thaw Perfusion Digestive Enzyme For Rat Hepatic Parenchymal Cells at 4℃. Open the Diluent Of Perfusion Digestive Enzymes For Rat Hepatic Parenchymal Cells in a clean bench. Add 500 µL of Perfusion Digestive Enzyme For Rat Hepatic Parenchymal Cells into 100 mL of Diluent Of Perfusion Digestive Enzymes For Rat Hepatic Parenchymal Cells, mix thoroughly.

4) Before the experiment, equilibrate the Pre-perfusion Solution For Rat Hepatic Parenchymal Cells and Perfusion Digestive Working Solution For Rat Hepatic Parenchymal Cells in a 37℃ water bath for about 30 min.

5) Coat the culture dish with Coating Buffer For Rat Hepatic Parenchymal Cells. Incubate at 37℃ for more than 2 hours, or coat overnight at 4℃.

2. Rat Perfusion Digestion

1) Anesthesia and Fixation of Rats: After anesthetizing rat with anesthetics, spray the rat all over with 75% alcohol. Transfer them to a super clean bench, and secure the rat in a supine position on the dissection board with needles.

2) Rat Anatomy:

① Preparation: Place two clean culture plates in the ultra clean bench. Arrange sterilized scissors and forceps in pairs from left to right on two clean culture plates (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Straight Forceps 2, Curved Forceps 1, Curved Forceps 2, and Curved Forceps 3).

Note: The distal third of the instruments should extend beyond the plates to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contamination.

② Dissection: First, incise the rat skin along the midline of the abdomen with Ophthalmic Scissors 1. Lift the skin along the incision site with Straight Forceps 1, and use Ophthalmic Scissors 1 to cut open the skin horizontally towards the limbs. Bluntly dissect the skin and subcutaneous tissue toward both sides to expose the superficial muscle layer of the abdominal wall. Continue to use Straight Forceps 1 to pull the skin to both sides and peel it down below the abdomen. Then use Ophthalmic Scissors 2 to incise the diaphragm of the rat abdomen, and use Straight Forceps 2 to gently retract the diaphragm down below the abdomen.

Note: (Optional Operation) After incising the abdominal diaphragm, proceed to incise the thoracic diaphragm along the upper edge of the liver. Find the inferior vena cava above the liver and clamp the vein at this site with a hemostatic clip (Figure 1). This step allows the subsequent digestive solution to fully flow through the liver tissue and exit through the hepatic portal vein, improving digestion efficiency. If it is not possible to accurately locate the inferior vena cava above the liver and promptly complete the subsequent steps, please ignore this step.

3) Rat perfusion: The rat perfusion method depends on the specific laboratory conditions. If peristaltic pump perfusion is used, please follow steps a - c. If manual perfusion is used, please follow steps d - g.

① **Peristaltic Pump Perfusion**

a. Lift up the outer lobe of the rat liver using Curved Forceps 1 and push the intestinal tissue to the right, exposing the inferior vena cava and hepatic portal vein of the rat (a pipette tip can be placed under the rat to fully expose the inferior vena cava). Take a perfusion needle (exhaust all air) and connect the inlet end of it to a centrifuge tube or botttle containing Pre-perfusion Solution For Rat Hepatic Parenchymal Cells. The needle should be parallel to the inferior vena cava, with the oblique plane of the needle pointing upwards. Insert it into the inferior vena cava (the injection position is shown in Figure 1), and insert the needle tip above the bifurcation of the blood vessel. Fix the perfusion needle and use other auxiliary materials (such

as syringe needles) to keep the needle still and avoid puncturing the blood vessel. Gently pull out the needle core, then the blood in the blood vessel can be seen to flow out with the needle core. At this time, the outer tube is left in the blood vessel for perfusion.

b. Turn on the peristaltic pump and slowly infuse the Pre-perfusion Solution For Rat Hepatic Parenchymal Cells until the liver becomes fully distended. At this time, use Ophthalmic Scissors 2 to cut open the hepatic portal vein for drainage (marked in Figure 2), maintaining a perfusion rate at 20 mL/min until all blood in the liver are completely drained and the liver tissue turns waxy yellow(the perfusion process is shown in Figure 3).

Note: In this step, all liver lobes should turn waxy yellow. If there is any congestion, gently disperse it with a curved forceps. The amount of pre infusion depends on the age of the rat. It is recommended to infuse 30-50 mL for rats under 28 days old, 100 mL for rats between 42-56 days old, and try the infusion amount for older rats on their own. If only a portion of the liver lobes turn waxy yellow or there is a large area of red color that cannot be dispersed, it indicates perfusion failure. The specific reasons are detailed in Table 2 below.

c. Transfer the inlet end of the hose into a centrifuge tube or bottle containing 37℃ preheated Perfusion Digestive Working Solution For Rat Hepatic Parenchymal Cells, avoiding air entering the tube. Turn on the peristaltic pump and continue perfusion digestion, maintaining a flow rate at 10 mL/min. During the perfusion process, rhythmically squeeze the hepatic portal vein incision with Curved Forceps 1 (squeeze for about 3-5 seconds, with an interval of 0.5-1 min, as to fill the liver and improve digestion rate). The perfusion time varies from 10-20 minutes. When the liver tissue exhibits a honeycomb-like punctate pattern, demonstrates slow rebound after gentle pressure, and displays cracked striations on the surface, it indicates the completion of digestion.

② **Manual Perfusion**

d. Use two 50 mL syringes to separately aspirate the preheated Pre-perfusion Solution For Rat Hepatic Parenchymal Cells and Perfusion Digestive Working Solution For Rat Hepatic Parenchymal Cells. Remove the needle and connect the hub of venous indwelling needle to the tip of the syringe containing the Pre-perfusion Solution, ensure that it is stable with no leakage. Expel all air from the syringe and needle tube, then set aside for later use.

e. Lift up the outer lobe of the rat liver using Curved Forceps 1 and push the intestinal tissue to the right, exposing the inferior vena cava and hepatic portal vein of the rat (a pipette tip can be placed under the rat to fully expose the inferior vena cava). Take the perfusion needle (exhaust all air) which has been connected to the syringe containing Pre-perfusion Solution For Rat Hepatic Parenchymal Cells. The needle should be parallel to the inferior vena cava, with the oblique plane of the needle pointing upwards. Insert it into the

inferior vena cava (the injection position is shown in Figure 1), and insert the needle tip above the bifurcation of the blood vessel. Fix the perfusion needle and use other auxiliary materials (such as syringe needles) to keep the needle still and avoid puncturing the blood vessel. Gently pull out the needle core, then the blood in the blood vessel can be seen to flow out with the needle core. At this time, the outer tube is left in the blood vessel for perfusion.

f. Gently push the syringe to allow the Pre-perfusion Solution into the rat liver tissue. Use a timer to start the timing, control the infusion rate, and maintain the flow rate at around 20 mL/min. When the liver is full, use Ophthalmic Scissors 2 to cut open the hepatic portal vein for drainage (marked in Figure 2). Keep perfusion until the blood in the liver are completely drained and the liver tissue turns waxy yellow(the perfusion process is shown in Figure 3).

Note: In this step, all liver lobes should turn waxy yellow. If there is any congestion, gently disperse it with a curved forceps. The amount of pre infusion depends on the age of the rat. It is recommended to infuse 30-50 mL for rats under 28 days old, 100 mL for rats between 42-56 days old, and try the infusion amount for older rats on their own. If only a portion of the liver lobes turn waxy yellow or there is a large area of red color that cannot be dispersed, it indicates perfusion failure. The specific reasons are detailed in Table 2 below.

g. Grasp the needle holder of the indwelling needle, remove the syringe, and connect the hip of the indwelling needle to another syringe containing Perfusion Digestive Working Solution For Rat Hepatic Parenchymal Cells. Continue perfusion, start the timer, control the flow rate at 10 mL/min, and rhythmically squeeze the hepatic portal vein incision with Curved Forceps 1 during the perfusion process (squeeze for about 3-5 seconds, with an interval of 0.5-1 min to fill the liver and improve digestion rate). The infusion time is about 10-20 minutes. When the liver tissue exhibits a honeycomb-like punctate pattern, demonstrates slow rebound after gentle pressure, and displays cracked striations on the surface, it indicates the completion of digestion.

4) Cut the vascular and connective tissue connected to the liver along the diaphragm. Gently lift the liver tissue with Curved Forceps 2, cut off the remaining connecting tissue. Transfer the intact liver tissue to a pre-prepared culture dish containing Specialized Washing Solution For Rat Hepatic Parenchymal Cells. Secure the liver tissue at the junction of the liver lobes using Curved Forceps 2 in a super clean bench. After removing the gallbladder with Curved Forceps 3, gently peel off the hepatic capsule. Hepatic parenchymal cells can be seen flowing out like quicksand, when the tissue is gently shaked. The incomplete dispersed parts can be gently separated with forceps and dispersed into the cell washing solution (Figure 4).

3. Isolation and Purification of Hepatocytes

1) Place a **100 μm Cell Filter** and a **70 μm Cell Filter** over a brand new 50 mL centrifuge tube. Take 1-2 mL of

Specialized Washing Solution For Rat Hepatic Parenchymal Cells to rinse the filter. Use a 5 mL pipette to take the cell suspension from the culture dish in the above steps and filter it through the **100 μm Cell Filter** and the **70 μm Cell Filter** in sequence.Then transfer the filtrate into a 15 mL centrifuge tube.

2) Centrifuge 50 g for 5 minutes.

3) Discard the supernatant and add 20 mL of **Specialized Washing Solution For Rat Hepatic Parenchymal Cells** to gently disperse the cell precipitate. Gently shake the centrifuge tube to resuspend the cells in the cell washing solution.

4) Repeat steps 2 and 3 three times.

5) Discard the supernatant and add 10 mL of **Specialized Washing Solution For Rat Hepatic Parenchymal Cells** to gently disperse the cell precipitate. Gently shake the centrifuge tube to resuspend the cells in the cell washing solution.

6) After gently shaking, immediately aspirate a small amount of cell suspension and observe cell activity under a microscope using trypan blue staining

4. Hepatocytes Culture

1) Perform trypan blue staining on purified live hepatocytes, observe cell viability directly under a inverted microscope after staining, and then count with a cell counter.

2) According to the cell viability and the number of cells, inoculate at a viable cell density of 3×10^5 cells/cm^2.

3) Aspirate the Adhesion Culture Medium For Rat Hepatic Parenchymal Cells into the coated culture vessel. Pipette the corresponding volume of hepatocytes suspension according to the calculated inoculation density, evenly drop it into the culture medium. Gently shake the cell suspension and then pipette it up and down to evenly distribute the cells (if the seeding density is uneven, it can easily cause cell aggregation in the middle of the vessel, resulting in phenomena such as cell clustering and floating).

4) After 4-6 hours of cell growth, observe under a microscope, it can be seen that the cells are significantly large and round, with double nuclei. Replace the culture medium with Maintenance Medium For Rat Hepatic Parenchymal Cells.

5) After 24 hours of cell culture, cells begin to differentiate and gradually lose the characteristics of hepatocytes, with increasingly irregular morphology.

6) Change the medium every 2-3 days and the culture process can be sustained for 1-2 weeks.

Troubleshooting

Table 2. Common problems, causes, and solutions for isolation of Rat Hepatic Parenchymal Cells

Problem	Possible Cause	Solution
During the perfusion process, the kidneys, right iliac region, and abdomen were distended.	**The infusion needle punctured the blood vessel, causing the infusion fluid to flow to other areas.**	**Gently lift the venous vessel when inserting the needle, ensuring that the needle does not descend. Keep the needle stationary during the fixation process.**
During the perfusion process, fluid accumulated in the abdominal cavity, and the liver gradually shrinked from expansion.	**Excessive pressure can cause the rupture of blood vessels and tissues.**	**Ensure the hepatic portal vein is completely cut open, and do not clamp the hepatic portal vein for too long during the perfusion process.**
The needle fall off and caused the accumulation of fluid near blood vessels during the perfusion process.	**The length of the needle entering the blood vessel was too short.**	**It is advisable to insert the needle above the bifurcation of the inferior vena cava.**
During the perfusion process, no distention was observed in the liver, and large areas of blood-red residue still remained in the liver tissue after perfusion and lavage.	**The needle punctured blood vessels, the perfusion flow rate was too low.**	**Use scissors to clamp the hepatic portal vein, gently infuse the perfusion solution, and observe whether the liver is completely distended. If only partial or no distention occurs, it indicates that the needle has not been completely inserted into the blood vessel. Suggest re-experimenting.**
After perfusion, it was difficult to peel off the liver capsule, and no quicksand-like cells was observed after peeling. After gentle shaking, the tissue maintained its normal shape.	**Inactivation of digestive enzymes, prolonged preheating time, high flow rate during the perfusion digestion process led to insufficient digestion.**	**Check the storage conditions of digestive enzymes and complete the experiment within a short period of time after thawing. The preheating time should be controlled at around half an hour. During manual perfusion, use a timer to control the perfusion rate at around 5 mL/min.**
During staining observation, cell clusters and lumps were observed.	**Inadequate digestion.**	**Preheat the perfusion solution before perfusion. Ensure that an adequate amount of perfusion solution is infused.**
A large number of cells floated within 24 h after inoculation	**Excessive inoculation density can lead to cell death**	**Prior to inoculation, assess cell viability via staining, perform cell counting, and optimize the seeding density.**
Cells grow poorly after inoculation.	**Improper preparation of the culture medium and faliure to change the medium within the specified time.**	Operate **according to P**rocedure 1, Step (1), and Procedure 4, Step (4).
After inoculation, the cells tend to aggregate in the center of the culture dish while relatively fewer cells were observed inthe surrounding areas.	**Uneven distribute of cells**	When seeding, evenly drip the cell suspension dropwise and do not directly inject the entire volumn into the culture medium. After gently shaking the vessel, use a pipette tip or pipette to gently mix cells by pipetting up and down. Before placing the cell culture dish in the incubator, observe and confirm with a microscope.

Anatomy Images for Reference

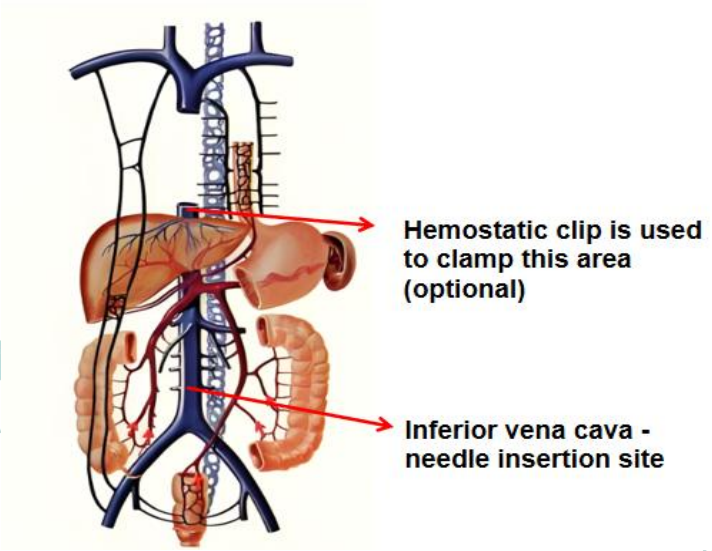


Figure 1. Portal vein system and perfusion needle site

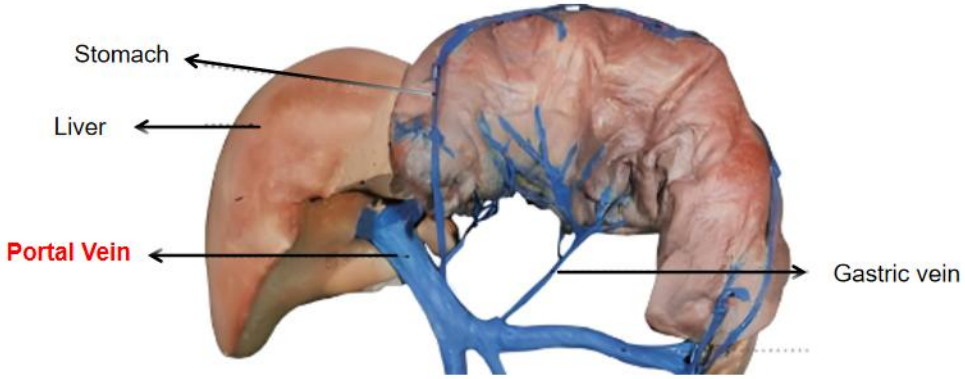


Figure 2. Schematic diagram of hepatic portal vein anatomy

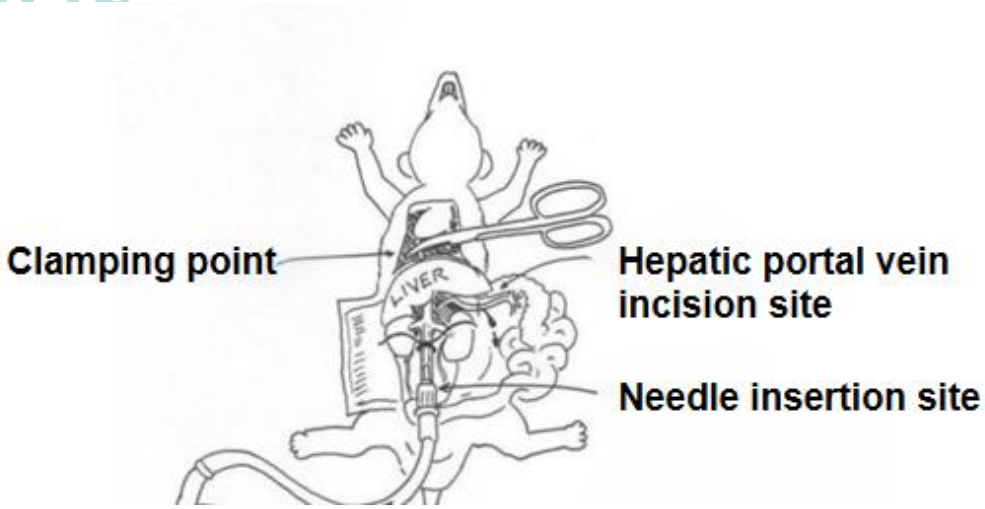


Figure 3. Overview diagram of perfusion process

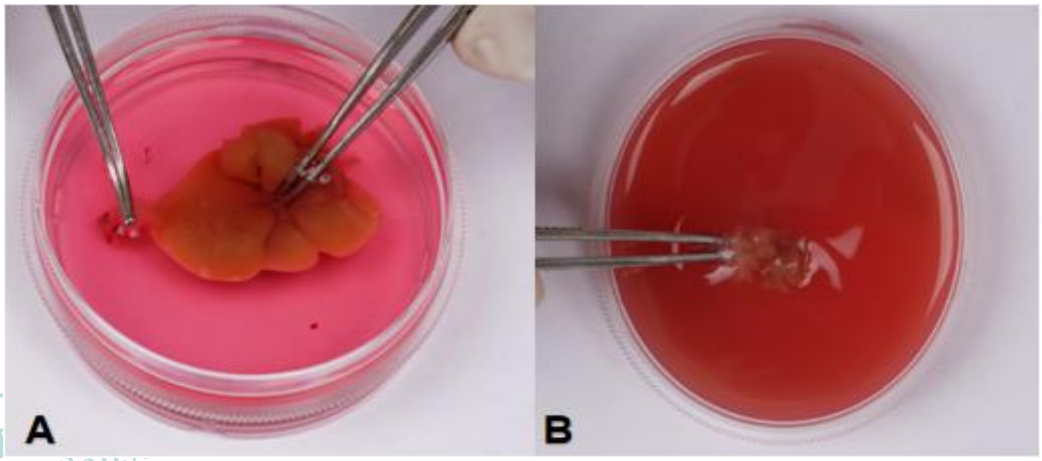


Figure 4. Treatment of the liver in vitro

A: Gently peel off the hepatic capsule. B: Gently shake the tissue to allow the cells to be released, leaving behind only a small portion of the residual tissue

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