Isolation of rat hepatocytes

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Organisation

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Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Ratus norvegicus
Type of cells/tissues/organs	Liver

DESCRIPTION

Method keywords

Hepatocytes

cell culture

isolation

Scientific area keywords

Toxicology
basic research
fundamental research

Method description

This method describes the steps from a living rat to a single cell solution of primary hepatocytes. This requires surgery on the lab animal, a perfusion with buffer solution, a digestion with collagenase and a filtration step to obtain primary hepatocytes.

Lab equipment

Peristaltic pump, perfusion material, carbogen (5% CO2, 95% O2 gas).

PROS, CONS & FUTURE POTENTIAL

Advantages

Quick (120 min);
Reproducible;
High cell viability (90+ %);
High number of cells (200-400 million cells per isolation).

Challenges

Interspecies differences (Rat-human);
Terminal experiment for the lab animal.

Modifications

This is an established method and no major modifications are proposed.

Future & Other applications

An adaptation of this method can be used on different animals or humans. However as human liver(parts) are very hard to obtain, the application of this method on human liver is rare.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Papeleu P. et al. (2006) Isolation of rat hepatocytes. Methods Mol Biol. 2006;320:229-37. - Seglen P.O. (1976) Preparation of isolated rat liver cells. Methods in Cell Biology 13: 29-83

De Smet K., Beken S., Vanhaecke T., Pauwels M., Vercruysse A. and Rogiers V. (1998) Isolation of rat hepatocytes. In: Methods in Molecular Biology (vol. 107): Cytochrome P450 protocols, Phillips I.R. and Shepard E.A. (Eds.), Humana Press, USA: 295-301

Other remarks

- Sterilize the perfusion equipment with 70% (v/v) ethanol solution.
- Rinse with bidest water.
- Sedate the rat (e.g. 87.5 mg/kg ketamine and 12.5 mg/kg xylazine)
- Shave the abdomen.
- Disinfect the abdomen with 70% alcohol solution.
- Make a U-shape incision and put the intestines outside the abdomen.
- Put 2 surgical sutures on the bile duct, close the lower suture. - Make an incision in the bile duct and cannulate.
- Close the higher suture, fixing the cannula.
- Put 2 surgical sutures on the vena porta without closing them.
- Put 1 surgical suture on the vena cava inferior without closing it.
- Inject 1 ml of diluted Heparin solution (200IU/ml) in the vena saphena medialis.
- Close the lower suture on the vena porta.
- Make an incision in the vena porta and cannulate with the glass cannula.
- Close the higher suture on the vena porta.
- Close the suture on the vena cava.
- Excise the liver.
- Perfuse the liver with the perfusion equipment (15min, 30 ml/min).
- The animal dies of exsanguination.
- Digest the liver with collagenase solution (18400 Units
- Filter with a perlon filter to obtain a single cell solution

Material:

- Bile cannula
- Glass cannula
- Sterile surgical material (forceps, scissors, ...)
- Sterile glass petri dish
- Sterile injection needles (3-26G3/8)
- Sterile drape
- Shaving equipment (electronic and/or razor)
- Surgical suture (mersilk, 2-0)
- Sterile glass recipients
- Laminar Air Flow (LAF)
- Peristaltic pump
- Perfusion equipment
- Perlonfilter
- Sterivex® filter
- Carbogeen (5 % CO_2 and 95 % air)
- Bidest water
- Heparin (5000 IU/ml)
- Sedation (e.g. 87.5 mg/kg ketamine and 12.5 mg/kg xylazine
- Krebs-Henseleit-buffer (KHB) pH = 7.4
- Krebs-Henseleit-buffer with calcium pH = 7.4 - 70% (v/v) ethanol solution
- Collagenase solution: 18400U in 10 ml KHB+Ca^{2+}