Whole-liver decellularization of rat liver

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Contact person

Paul Claes

Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)
Department Pharmaceutical and Pharmacological Sciences
Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology
Country Belgium

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

liver
decellularization
matrix

Scientific area keywords

basic research

Method description

This method describes the steps to go from a liver to a decellularized matrix. It uses mild and strong detergents to destroy cells and keep the extracellular matrix intact. This matrix can then be used for a variety of purposes, including (but not limited to) repopulation, basis for coating and basic research.

Lab equipment

Laminar Air Flow (LAF) ;
Peristaltic pump ;

Perfusion equipment.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Versatile tool: matirx can be used for an array of things.

Challenges

Very specific surgery and very dependent on the surgeon;
Small differences in cannule placement can have major implications;
Very hard to keep sterile;
Time consuming;
Remaining SDS is lethal for cells.

Modifications

This method can be modified: cannulation of vena cava and/of arteria hepatica to create an closed environment.

Future & Other applications

This method has a lot of potential, but faces a few big obstacles. If these could be removed, this method is would be a huge step forward.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

De Kock, Joery & Ceelen, Liesbeth & De Spiegelaere, Ward & Casteleyn, Christophe & Claes, Paul & Vanhaecke, Tamara & Rogiers, Vera. (2011). Simple and quick method for whole-liver decellularization: A novel in vitro three-dimensional bioengineering tool?. Archives of toxicology. 85. 607-12. 10.1007/s00204-011-0706-1

Other remarks

Material:
- Bile cannula
- Plastic 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
- 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
- 1% triton-x solution

- 1% SDS solution

Experimental procedure:

- 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 plastic 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 KHB, 30 ml/min).
- The animal dies of exsanguination.
- Perfuse the liver 1 hour with triton-X solution
- Perfuse the liver 1 hour with SDS solution
- Perfuse the liver 1 hour with Bidest water