Whole-liver decellularization of rat liver

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SCOPE OF THE METHOD

Alternative method relates to	Animal health, Human health
Alternative method is situated in	Basic Research
Type of alternative method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
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 colsed 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

Associated documents

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

PARTNERS AND COLLABORATIONS

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