Poster Presentation 16

USE OF CAM ASSAYS TO MEASURE REVASCULARISATION IN OVARIAN TISSUE TRANSPLANTATION

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Background: As cancer therapies improve and the long-term survival rate of cancer patients increases, there is growing demand for fertility preservation strategies. Ovarian tissue cryopreservation and transplantation has the potential to help 1200 young women within the UK per year. However, while 60 live births have been recorded worldwide, this procedure is still considered experimental and almost 50% of follicles are lost following transplantation due reperfusion injury and subsequent apoptosis. Therefore, the aim of this study was to assess the revascularization of bovine ovarian tissue treated in different ways using chorioallantoic membrane (CAM) assays as an in vivo method model to determine if improvement of angiogenesis in transplanted ovarian tissue can be made.
Methods: Bovine ovarian cortex (n=4) was dissected into approximately 20 (1-2mm x 5mm x 5mm) squares. The tissue was randomly assigned to one the six experimental groups. 1. frozen and thawed, 2. frozen, encapsulated in alginate and then thawed, 3. frozen and thawed followed by tissue culture without VEGF 4. frozen and thawed followed by tissue culture with VEGF, 5. frozen, encapsulated in alginate and then thawed and placed in tissue culture without VEGF and 6. frozen, encapsulated in alginate and then thawed and placed in tissue culture with VEGF. Tissue was then used to generate chorioallantoic membrane (CAM) assays. Following incubation, the assays were imaged, blood vessels quantified and tissue harvested for comparison between the treatment groups.
Results: A greater number of blood vessels were observed in the CAM assays that had been encapsulated in alginate and cultured prior to grafting ($P<0.05$). Overall alginate encapsulation lead to improved angiogenesis in the tissue post-thaw, compared with the other experimental groups. Interestingly, culturing the tissue with VEGF prior to transplantation did not yield a higher vascular count than tissue cultured without VEGF ($P<0.05$).
Conclusions: The preliminary results of this study showed that encapsulating ovarian tissue grafts in alginate hydrogels prior to cryopreservation, preserves the vascular scaffold allowing for improved angiogenesis and tissue oxygenation post thawing.

Poster Presentation 17

METAL FOAM BASED REWARMING OF VITRIFIED SYSTEMS

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Vascular tissues such as arteries and heart valves can be successfully vitrified by loading high molarity cryoprotective agents (CPAs) such as VS55 (8.4 M) and DP6 (6 M) and cooling at or beyond their critical cooling rates (CCR) of 2.5 and 40 °C/min respectively. However, successful rewarming from the vitrified state remains challenging in standard 1.8 mL cryovials, and larger systems, as rates of only 70 °C/min were achieved immersed in 37 °C water. This rate is below the critical warming rate for DP6 of 185 °C/min. This rate is also too slow for thicker tissues that are only partially loaded with VS55 or DP6 and will lead to devitrification or crystallization which reduces the viabilty of the tissue. Here, we present a new method that can dramatically increase the warming rate of vitrified systems by inductively warming commercially available metal foams, foils or mesh (Nitinol) embedded in vitrified samples with non-invasive radiofrequency (RF) fields (360 kHz, 20 kA/m). RF rewarming led to specific absorption rates (SAR) of 100 W/g Cu, 450 W/g Al and 1000W/g Nitinol within the systems. Tests with these systems routinely achieved warming rates between up to 1,000 °C/min and beyond. As a proof of principle, a partially CPA loaded 2 mm thick artery was successfully rewarmed with high viability while gold standard convection lead to substantial viability loss. Finally, computational modeling suggests that tissues up to 4 mm thick may benefit by being in contact with these heat sources and thereby distributing warming more effectively than gold standard convection in the tissues without cracking.

Poster Presentation 18

ORGAN ENGINEERING OF PERFUSION TECHNOLOGY FOR TRANSPLANTATION

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Machine perfusion is one of the most important technologies for organ preservation and organ resuscitation. For clinical applications, the utilization of grafts donated after cardiac death (DCD) would greatly contribute to the expansion of the donor organ pool. Machine perfusion technology can improve organ viability and can enable assessment of organ suitability for transplantation. Furthermore, machine perfusion technology could rescue marginal organs under controlled perfusion conditions. For future clinical use, machine perfusion technology will contribute to restoration from the cryopreserved condition and to cultivation of organs from stem cells. However, the implementation of such a strategy requires the development of novel preservation methods.
The aim of our study is the development of a novel technology to preserve liver function and to restore organ viability using optimized perfusion conditions for the temperature, oxygenation, pressure and flow rate. In this presentation, our organ engineering projects for perfusion technology are introduced to contribute for organ banking.
In our projects, porcine liver grafts kept at a warm ischemia time of <60 minutes were employed as a model of DCD grafts. The liver grafts were perfused under several temperature and oxygenation conditions. Here, one of the conditions of the experiments is introduced. Four hours of machine perfusion with modified UW-gluconate solution was conducted under several temperature conditions. The conditions were as follows: Group 1, liver grafts preserved with hypothermic machine perfusion preservation (HMP) at 8-10 °C; Group 2, liver grafts preserved at 4 °C and then gradually increased to 22 °C for 4 h (RMP); and Group 3, liver grafts preserved with subnormothermic machine perfusion preservation (SNMP) at 22 °C for 4 h. The relationship between oxygen consumption, the pressure of the portal vein and hepatic artery, and enzyme release rates were analyzed to determine the oxygenation effect during machine perfusion at different preservation temperatures.
As a result, the pressure of the portal vein and hepatic artery at subnormothermic temperatures were lower than those at hypothermic temperatures. This trend in portal vein pressure indicates that SNMP and RMP reduce the wall shear stress in the sinusoidal under high temperature conditions. In addition, the oxygen consumption was strongly related to liver graft temperature during machine perfusion. The effluent enzyme levels were lower in the higher oxygen consumption group than in the lower oxygen consumption group after 1-h machine perfusion.

Poster Presentation 19

PAPER-BASED CRYOPRESERVATION

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Contributions: This work uniquely demonstrates cryopreservation of mammalian cells within paper platforms. Cryopreserved cells using the novel method can be released using a thawing device that we developed. Alternatively, cells can be grown within the paper which permits 3D cell culture.