UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL AND BIOPHYSICAL PROPERTIES OF BOVINE PARIETAL PERICARDIUM AND TUNICA VAGINALIS XENOGRAFTS IN A RAT MODEL

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FPV 2005 10
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By

ABDEL HAFEEZ YAGOUB MOHAMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2005
DEDICATION

To my sisters and brothers,

to my wife Aziza Yousif Hama

to my sons Albara, Awab and Awfa

for their moral support and encouragement
The study was conducted with the main objectives to evaluate the macroscopic, microscopic and biomechanical properties of lyophilized and glycerolized bovine parietal pericardium and tunica vaginalis used for repair of full thickness abdominal wall defect in the rat. Expanded polytetraflouroethylene (ePTFE) Mycro Mesh® was used as positive control. In addition, the effects of preservation methods used in this study on the biomechanical properties of the pre-implanted grafts were also studied.

Fresh bovine parietal pericardium and tunica vaginalis sacs collected from abattoir were processed and preserved by lyophilization and glycerolization. A total of 180 adult male Sprague Dawley rats (300-400g) divided into six groups of 30 rats each were used in the study. Full thickness mid ventral abdominal wall defects of 3×2.5 cm in size were created in each rat. The defects in the first four groups of rats
were repaired with the same size (3×2.5 cm) of lyophilized pericardium (IFDBP), lyophilized tunica vaginalis (IFDTV), glycerolized pericardium (GBP) and glycerolized tunica vaginalis (GTV) respectively. The remaining two groups were used as positive control and repaired with polytetrafluoroethylene (ePTFE) Mycro Mesh®. The negative control group underwent a U shape sham-operation. Six rats from each group were sacrificed at post-implantation intervals of 1, 3, 6, 9 and 18 weeks for macroscopic, microscopic and biomechanical evaluations.

Biomechanical evaluation of the pre-implanted grafts revealed that freeze-drying has no significant effect (P>0.05) on biomechanical properties of the fresh bovine parietal pericardium and tunica vaginalis. While gamma sterilization caused significant decrease (P<0.05) in biomechanical properties of the freeze-dried bovine pericardium and tunica vaginalis. Glycerol preservation caused significant (P<0.05) decrease in the biomechanical properties of fresh bovine parietal pericardium, while it has no significant effect on the biomechanical properties of fresh bovine parietal tunica vaginalis.

Macroscopically, 97.66% of the rats survived until their predetermined sacrifice date. Adhesions, infections and seroma were encountered in 7.22%, 2.77% and 1.67% respectively of the rats operated. No serious post-surgical complications such as hernia, fistula and intestinal obstruction were encountered in the study. Glycerolized and lyophilized grafts were gradually resorbed and replaced by recipient tissue, while the ePTFE implants apparently remained without marked structural
changes. Glycerol preservation seemed to delay the grafts resorption while lyophilization seemed to enhance grafts resorption.

Microscopically, the pre-implanted bovine parietal pericardium and tunica vaginalis were mainly fibro-collagenous in nature with few cellular and vascular elements. Freeze-drying and gamma sterilization has severe damaging effects on ultrastructural features of the grafts. In contrast, glycerol preservation seems to preserve the ultrastructural features of the grafts.

Microscopically, the lyophilized and glycerolized grafts were replaced by collagenous tissue. Foreign body giant cells were detected in fibrous capsules around ePTFE Mycro Mesh implant starting from week three post-implantation onward. Calcium deposition was demonstrated in matrix of the ePTFE Mycro Mesh implant at 18 weeks post-implantation. No foreign body giant cells or calcium deposition were demonstrated in rats implanted with grafts of bovine origin or in sham-operated rats.

The immuno-gold labeling showed that bovine type I collagen remained detectable in the implanted areas throughout the study period. The immunoperoxidase staining demonstrated that the intensity of the rat’s type I collagen was increased with the advance of post-implantation intervals, while the intensity of rat’s type III collagen showed slight changes with advance of post-implantation intervals.

Post-implantation biomechanical evaluations revealed that the healing biomechanical properties between the implanted materials and the recipient abdominal tissues increased.
with advance of post-implantation intervals. However, there were no significant
differences (P>0.05) among the overall mean values (n=15) of healing tensile strength,
maximum load at break and Young’s modulus of elasticity of all groups of implanted
materials.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN MORFOLOGI DAN BIOFISIK KE ATAS XENOGRAF DARIPADA PERIKARDIUM DAN TUNIKA VAGINALIS LEMBU DALAM TIKUS

Oleh

ABDEL HAFEEZ YAGOUB MOHAMED

July 2005

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Perikardium dan tunika vaginalis lembu yang diambil dari rumah sembelih telah diproses secara kering-beku dan diawet dalam gliserol. Sebanyak 180 ekor tikus dewasa jenis Spraque Dawley (300-400g) yang dibahagikan kepada enam kumpulan dengan setiap kumpulan mengandungi 30 ekor tikus (n=30) telah digunakan dalam kajian ini. Dinding abdomen tikus berukuran 3 x 2.5 cm telah dipotong dan
disisih bagi semua tikus kecuali bagi kumpulan kawalan negatif. Dinding abdomen yang telah dibuangkan daripada tikus dalam kumpulan 1–4 telah digantikan masing-masing dengan perikardium kering-beku (IFDBP), tunika vaginalis kering-beku (IFDTV), perikardium yang diawet dengan gliserol (GBP) dan tunika vaginalis yang diawet dengan gliserol (GTV). Tikus dalam kumpulan 5 bertindak sebagai kawalan positif dan dinding abdomen digantikan dengan graf ePTFE. Tikus dalam kumpulan kawalan negatif menjalani pembedahan dinding abdomen berbentuk-U dan tidak digantikan dengan xenograf. Bagi semua kaedah ini enam ekor tikus dari setiap kumpulan telah di tamatkan pada minggu 1, 3, 6, 9 dan 18 pasca implan diperiksa dan dinilai graf yang di implan secara makroskopi, mikroskopi dan juga ciri-ciri biomekaniknya.

Dari segi makroskopi, 97.66% tikus didapati hidup sehingga tarikh tamat kajian. Pelekatan organ pada graf, jangkitan dan seroma yang berlaku masing-masing adalah 7.22%, 2.77% dan 1.67%. Tiada komplikasi teruk seperti hernia, fistula dan obstruksi usus didapati berlaku selepas implan dalam kajian ini. Graf yang diawet dalam gliserol dan yang di kering-beku telah diserap secara perlahan dan digantikan oleh tisu penerima, manakala graf ePTFE dilihat kekal tanpa perubahan struktur yang ketara. Pengawetan dalam gliserol didapati melambatkan proses penyerapan graf oleh badan manakala proses kering-beku meningkatkan kadar penyerapan.

Dari segi mikroskopi, perikardium dan tunika vaginalis lembu sebelum implan menunjukkan struktur berserat kolagen dengan sedikit elemen sel dan salur darah. Proses kering-beku dan pensterilan gamma keatas graft memberikan kesan kerosakan
yang teruk apabila periksa pada tahap ultrastruktur graf. Sebaliknya pengawetan dalam gliserol dilihat dapat mengekalkan struktur graf tersebut.

Secara mikroskop, graf-graf kering-beku dan yang diawet dalam gliserol telah digantikan oleh tisu kolagen. Sel gergasi badan asing telah dijumpai dalam kapsul berserat disekeliling graf ePTFE pada minggu ke-3 dan selanjutnya terdapat kalsium dalam matrik graf ePTFE pada minggu ke-18 pasca implan. Tiada sel gergasi badan asing atau kalsium ditemui dalam tikus yang di implan dengan graf berasal dari lembu atau dalam kumpulan kawalan negatif.

Penlabelan immunogold menunjukkan kolagen lembu jenis I masih ditemui dalam graf yang di implan sepanjang kajian ini. Pewarnaan imunoperoksidase menunjukkan kandungan kolagen tikus jenis I bertambah dengan pergerakan masa, manakala kandungan kolagen tikus jenis III menunjukkan sedikit perubahan pasca implan.

Penilaian biomekanik graf paraimplan menunjukkan proses kering-beku tiada kesan yang ketara (P>0.05) ke atas ciri-ciri biomekanik perikardium dan tunika vaginalis. Pensterilan gamma menyebabkan penurunan ketara (P<0.05) ciri-ciri biomekanik perikardium dan tunika vaginalis lembu yang telah di kering-beku. Pengawetan gliserol menyebabkan penurunan ketara (P<0.05) ciri-ciri biomekanik perikardium lembu, tapi tiada kesan ketara didapati ke atas ciri-ciri biomekanik tunika vaginalis lembu.

Penilaian biomekanik graf selepas implan menunjukkan ciri-ciri biomekanik diantara graf dan tisu penerima meningkat dengan gerakan masa. Walaubagaimanapun, tiada
perbezaan yang ketara didapati diantara purata keseluruhan nilai (n=15) bagi kekuatan tensil, beban maksima pada carikan dan ketegangan Young’s modulus bagi semua kumpulan yang di implan dengan graf.
ACKNOWLEDGMENTS

First, my praise to Almighty Allah for giving me the strength and resilience to complete this study and peace be upon His final Prophet and Messenger Mohamed.

I would like to convey my sincere gratitude to Dr. Mohamed Zuki Abu Bakar Chairman of my Supervisory Committee for his invaluable advice, guidance, constant support and encouragement. I would like to extend my grateful thanks and appreciations to the members of my Supervisory Committee Associate Professor Dr. Noordin Mohamed Mustapha, Dr. Norimah Yusof and Dr Loqman Mohamed Yusof for their constructive criticism, advice and support throughout the course of this study.

I am grateful to Dr. Ainul Yuzairy and Dr. Ani Yardi for their valuable assistance during the surgical part of study. Thanks are also due to Mr. Zahid and Mrs. Asnah Hasan of MINT for their technical support. I am highly indebted to Mrs. Saphiah Jalal, Mr. Siva Soorian, and Mr. Rosely Sidik for their constant assistant and friendship. My thanks are also due to the staff of the Electron Microscopic Unit, Institute of Bioscience for their helps and co-operation.

I am grateful to the University of Khartoum and the (IRPA) Grant (No 54184), Ministry of Science and Technology (Malaysia) for the financial support. I wish to extend my thanks to the staff of the Faculty of Veterinary Medicine and Graduate School University Putra Malaysia (UPM) for their kindness co-operation and assistance during the period of this study.
It is worth to mention my colleagues and friends from the Sudanese community in UPM and Serdang area for their friendship and companion. Finally and importantly, I would like to extend my sincere appreciation to my wife Aziza Yousif and my sons Albara, Awab and Awfa for their patience, sacrifice and moral support during the course of my study.
I certify that an Examination Committee met on 25th July 2005 to conduct the final examination of Abdel Hafeez Yagoub Mohamed on his Doctor of Philosophy thesis entitled “Morphological and Biophysical Properties of Bovine Parietal Pericardium and Tunica Vaginalis Xenografts in a Rat Model” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 08 SEP 2005
DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ABDEL HAIFEEZ YAGOUB MOHAMED

Date: 16.08.2005
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A mid ventral abdominal skin incision of 4 cm long is being made.

A surgical defect of 3×2.5 cm is created in mid-ventral region of rat’s abdomen. The defect involves all abdominal wall layers except the skin.

A 3×2.5 cm rat’s abdominal wall musculature (A) against a bovine tunica vaginalis graft preserved in 99.5% glycerol (B).

A 3x2.5 cm mid ventral abdominal surgical defect is repaired with the same size of glycerolized bovine tunica vaginalis. A monofilament 4/0 prolene suture material is used with simple continuous pattern.

Post-operative supportive bandage materials applied around the abdomen after dressing the skin wound with tincture of iodine and with sterile gauze.

Clean rectangular sheet of bovine pericardium spread on carton paper.

Fresh bovine pericardium strips of 1x4 cm size.

Photographs of FDBP (A) and IFDBP (B) show the color changing of the radiation indicator from yellow to red after irradiation.

Rehydration of the freeze-dried strips in normal saline before measurement of thickness.

Mitutoyo non-rotating thickness gauge (Model EMD-57B-11M) for measurement of strips thicknesses. Note the graft strip (arrow) under the metal bar (arrowhead).

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Rats’ abdominal walls implanted with ePTFE Mycro Mesh (A and B) and GBP (C and D) at week one Pi. Note the peritoneal surface of the implants are partially (*) or completely covered (**) with the recipient tissue and neoperitoneum (np) with developing blood vessels (arrows).

Rats’ abdominal walls implanted with IFDTV A and B at week one Pi. Note the subcutaneous surface of the implants is partially A (*) or completely B (**) covered by the new developing tissue layer (nol).

Sham-operated rat’s abdominal wall at week one post-surgery shows the incisional site on the peritoneal surface (arrows) covered with neoperitoneum without much new connective tissue.

Peritoneal surface of rats’ abdominal walls implanted with A) ePTFE Mycro Mesh, B) IFDBP and C) sham-operated rat at week three Pi show the variability of the peritoneal lining, Note the blood vessels (arrows) on the fatty tissue lining (fl). No fat tissue lining in C (*).

Subcutaneous surface of rats’ abdominal walls implanted with A) IFDTV and B) GTV at week 6 Pi. Note that the IFDTV (*) was replaced with thin fibrous tissue while the GTV (**) implant remain without obvious change in size or shape.

Peritoneal surface of rats’ abdominal wall implanted with A) IFDTV and B) GTV at week 6 Pi. Note that the IFDTV implant has little fat tissue (*) as compared to GTV implant (**) which under lined with fatty tissue (fl).