



UNIVERSITI PUTRA MALAYSIA

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

ABDEL HAFEEZ YAGOUB MOHAMED

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**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*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Md Zuki Abu Bakar, PhD

Faculty: Veterinary Medicine

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 polytetrafluoroethylene (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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Kajian ini dijalankan dengan objektif utama menilai perikardium dan tunika vaginalis lembu yang digunakan sebagai gantian dinding abdomen tikus yang disisih secara eksperimen. Perubahan keatas dinding gantian dikaji dari segi makroskopi dan mikroskopi. Ciri-ciri biomekaniknya dibandingkan dengan ePTFE Mikro Mesh yang digunakan sebagai kawalan positif. Disamping itu kesan keatas cara-cara pengawetan graf yang digunakan dalam kajian ini dan ciri-ciri biomekanik sebelum implan juga dinilai.

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.

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