Development and pre-clinical evaluation of an indigenous large diameter polyethylene terephthalate (PET) vascular graft coated with a fluoropolymer and sealed with a biodegradable hydrogel

Project Report

From 1st January 2011 to 31st December 2013

Under the guidance of

Prof. M. Unnikrishnan, MCh

Head, Division of Vascular Surgery

Department of CTVS

By

Lt Col (Dr) Vivek Agrawal, MS

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

Thiruvananthapuram – 695011, India

Travancore, an erstwhile province of pre-independent India, was ruled by Sree Chitra Tirunal Maharajah until the country became independent in 1947. The Government of India took over the province after the independence.

Known for their munificence, the Maharajah and members of the royal family of Travancore considered themselves 'dasas' (servants) of Lord Padmanabha, the reigning deity of Travancore. Interestingly, they wore a turban instead of a crown as a mark of respect to Lord Padmanabha. Their philanthropy finds expression in their countless contributions to the country, then and now.

On a visit to a super-specialty hospital in Europe, Sree Chitra Tirunal Maharajah was seized with a deep desire to establish a similar institution in Kerala. Those were the times when tertiary cares in cardiovascular and neurological diseases were not available in the State.

In the summer of 1974, the Maharajah's dream was fulfilled when the royal family made a gift that carried in its womb the beginnings of what later turned out to be the Sree Chitra Tirunal Institute for Medical Sciences & Technology. About this time, Dr. M.S. Valiathan, trained abroad in surgery and biomedical science, returned to India to guide the destiny of the Institute. Supported magnificently by Shri. C. Achutha Menon, the then chief minister of Kerala, the Government of Kerala took the unusual step of placing the center under the Department of Science and Technology in the State.

The Sree Chitra Tirunal Institute for Medical Sciences & Technology (SCTIMST), Thiruvananthapuram is an Institute of National Importance established by an Act of the Indian Parliament. It is an autonomous Institute under the administrative control of the Department of Science and Technology, Government of India.

The Institute signifies the convergence of medical sciences and technology and its mission is to enable the indigenous growth of biomedical technology, besides demonstrating high standards of patient care in medical specialties and evolving postgraduate training programs in advanced medical specialties, biomedical engineering and technology, as well as in public health.

ACKNOWLEDGEMENT

I have great pleasure to place on record my debt of gratitude to **Prof M Unnikrishnan**, MCh, Professor and Head of the Division of Vascular Surgery, Dept of CTVS, SCTIMST, my esteemed teacher, who introduced me to the field of vascular surgery, provided updated information, suggested improvisations and guided me to imbibe vascular surgical skills during the course.

I am very much grateful to **Prof Jayakumar K**, Professor and Head, Department of CTVS, SCTIMST for his wholehearted support during my course.

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Oct/2013 **Dr Vivek Agrawal**
Thiruvananthapuram

DECLARATION

I, **Lt Col (Dr) Vivek Agrawal**, hereby declare that the project in this book was undertaken by me under the supervision of Prof M Unnikrishnan, MCh, Professor and Head of the Division of Vascular Surgery, Department of CVTS, Sree Chitra Tirunal Institute for Medical Sciences and Technology. Thiruvananthapuram

Lt Col (Dr) Vivek Agrawal

Date: Senior resident, Vascular Surgery

Forwarded

The candidate, **Lt Col (Dr) Vivek Agrawal**, has carried out the required work in this project .

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Department of CVTS
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CERTIFICATE

Certified that this is bonafide record of **Lt Col (Dr) Vivek Agrawal,** the work done at Vascular Surgery division, Department of CVTS, as part of MCh Course of Vascular Surgery at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, for a period of three years from 1st January 2011 to 31st December 2013

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TITLE OF PROJECT

Development and pre-clinical evaluation of an indigenous large diameter polyethylene terephthalate (PET) vascular graft coated with fluoropolymer and sealed with biodegradable hydrogel.

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INTRODUCTION

Vascular grafts are devices intended to replace compromised arteries in the body and grafts made of polyethylene terephthalate (PET) fabric have been used mainly for reconstructing procedures involving medium to large diameter blood vessels. Being a fabric PET grafts are porous. Though porosity of the graft permits tissue in-growth, it would lead to bleeding through the graft walls immediately after implantation. So it is essential to seal the pores either by preclotting with patient's own blood or by other sealing materials prior to implantation in order to prevent blood leakage through the graft wall. In an effort to seal the pores at implantation and improve tissue response to PET fabric, Roy Joseph et al coated a fluoropolymer, polyvinylidine fluoride (PVDF), on PET fabric by dip coating technique. The coating was found to be uniform and no significant changes occurred on physical properties such as water permeability and burst strength. Cell culture cytotoxicity studies showed that coated PET was non-cytotoxic to L929 fibroblast cell lines. In vitro studies revealed that coating improved haemocompatibility of PET fabric material. Coating reduced platelet consumption of PET fabric by 50%. Upon surface modification leukocyte consumption of PET was reduced by 24%. About 60% reduction in partial thromboplastin time (PTT) observed when PET was coated with PVDF. Results of endothelial cell proliferation studies showed that surface coating did not have any substantial impact on cell proliferation. Overall results indicate that coating has potential to improve haemocompatibility of PET fabric without affecting its mechanical performance.

Saraswathy Manju et al. had shown in their study that the alginate dialdehyde cross-linked gelatin hydrogel was nontoxic, hemocompatible, and was efficient in sealing the pores of the graft. Blood perfusion study showed that when hydrogel-coated grafts were exposed to blood for 30 min, they showed little affinity toward platelets or leukocytes. Hemolytic potential of PET was significantly reduced when it was coated with hydrogel. Improved adhesion and proliferation of endothelial cells were observed when PET grafts were coated with hydrogel. Results also showed that coating with hydrogel did not affect the burst strength of the PET graft. Investigations conducted on

water permeability in the static and pulsatile conditions, in vitro cell culture cytotoxicity, hemocompatibility, and endothelial cell adhesion and proliferation of the coated grafts showed that hydrogel coated grafts offer great promise as implants.

AIMS AND OBJECTIVES OF STUDY

Aim of this work was to perform the pre-clinical evaluation of large diameter polyethylene terephthalate (PET) vascular graft coated with a fluoropolymer and sealed with a biodegradable hydrogel.

The hydrogel was a reaction product of oxidized alginate and gelatin. The product was developed at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. The animal model selected was pig.

This study was intended to collect and analyze data from pig descending aorta implantations to evaluate the capacity of the prosthesis to maintain physiological function when used in the circulatory system and to determine the response of the host and the response of the prosthesis.

The objectives of the study were to evaluate safty and efficacy associated with the test device following orthotopic implantation in a porcine model, namely -

- Thrombosis, embolic events, occlusions and stenosis
- Leakage (haematoma, hemorrhage, blood leakage)
- Adverse tissue response
- Graft disruption (anastamotic, suture line, dehiscence)
- Seroma
- False aneurysm/ Pseudoaneurysm
- True aneurysm/ Dialatation
- Infection/ Sterility
- Graft performance as assessed by the surgeon (such as suturability and handling characteristics)

REVIEW OF LITERATURE

As the incidence and frequency of vascular procedures increase over the years, researchers are actively looking for modified natural materials as a compromise between autografts and purely synthetic grafts made of materials such as expanded polytetrafluoroethylene and polyethylene terephthalate (PET) [1]. Vascular grafts are used for the treatment of blood vessel aneurysms, occlusions and fistulas, by replacing and reconstructing diseased arteries in other locations in the body. Whenever possible, the best choice for vessel replacement is an autograft where sections of the patient's healthy blood vessels (usually veins) are harvested, nevertheless unsuitable and inadequate in aortic / large arterial domain. Many patients, however, especially those with pre-existing vascular disease or patients that have already had autograft procedures do not have blood vessels that are healthy enough to adequately serve as replacements. In these cases, the most common form of treatment has been the use of synthetic polymeric materials to form either permanent or resorbable replacements for the damaged vessels.[1] In cases where the graft can be of a large diameter (greater than 6 mm), the synthetic materials have been effective. However, in situations where a smaller vessel diameter is required, the synthetic materials cannot be used due to high rates of stenosis and thrombus formation. [2] One possible solution is to use natural materials like collagen, either modified or combined with a synthetic material, to form a graft that more closely mimics the body's natural function and has low thrombogenicity and low incidence of restenosis. [3] It has been recognized that the porosity of vascular graft plays an important role in their long-term potency and biological performance. [4–6] One main disadvantage of highly porous vascular graft is their high permeability to blood during implantation, which may result in severe blood leakage through the graft wall. Therefore, the pores of the graft must be sealed before implantation to obtain zero or near zero permeability. Sealing of the pores are often achieved either by preclotting with patient's own blood or by other sealing materials. Preclotting with patient's own blood is not favored as there is often residual clot formation. The resulting graft hemorrhage is troublesome, increasing operative time and need for blood transfusion. Many studies have been done to develop vascular

grafts that are blood tight during implantation, thus eliminating the need for preclotting of the graft and sufficiently porous to facilitate the tissue in-growth and biological healing. [7]

Most commonly used method includes coating or impregnation of porous graft with a biodegradable component. Coated or impregnated graft is blood tight during implantation. Owing to the gradual dissolution and degradation within the body, the resorbable material creates increasingly large pores in the initially porous graft, allowing in-growth of endothelial cells (EC).[8,9] Various proteins have been used as biodegradable components for coating or impregnation of the grafts. They include fibrin, albumin, gelatin, dextran, and collagen.[9–15] The grafts pretreated with these proteins now represent a high percentage of vascular grafts. However, proteins have some drawbacks, they are generally unstable, hard to obtain in the pure form, not easy to cross-link and control resorption rate, expensive, and difficult to render compatible with standard storage and sterilization procedures. Polysaccharide- based soft materials are a potential solution to avoid this risk of contamination and can be used as a coating material for porous vascular graft prosthesis.[16] Hydrogel derived from natural proteins and polysaccharides are biocompatible in nature and are widely used for medical applications such as plasma expanders, blood substitutes, bone healing promoters, wound dressings, and drug delivery.[17] They resemble extracellular matrices of tissue comprised of various amino acids and sugars based macromolecules thus have the potential to direct the migration, growth, and organization of cells during the tissue regeneration.[17,18] A hydrogel derived from alginate dialdehyde (ADA) and gelatin is an in situ forming biodegradable polymer of well-known biocompatibility and bioresorbability without employing any extraneous cross-linking agent.[19] However, rapid cross-linking and gelation is possible between oxidized alginate and gelatin in the presence of borax. [20] In a study by Joseph et al in 2008, hydrogel derived from ADA and gelatin was used for coating porous woven PET grafts with a view to seal the pores and to obtain implantable grafts with no blood leakage and low-thrombogenicity. Coated grafts were studied in vitro to evaluate its potential to remain impervious to blood while retaining porosity for tissue in-growth and biological healing. The study concluded that Hydrogel coated on PET graft samples effectively

sealed the pores of the graft leading to reduced water permeability. The coating did not adversely affect mechanical properties of the graft. Over 90% reduction in water permeability was achieved by hydrogel coating under static conditions. The soft rubbery nature of the hydrogel would minimize mechanical and frictional irritation to the surrounding tissues. A perfusion experiment revealed that hydrogel-coated PET graft is highly hemocompatible. Cytocompatibility tests showed that the coating would enhance the performance of the PET graft with better adhesion of EC. [21]

It has been widely recognized that PET is minimally thrombogenic. [22,23] which in turn adversely affect healing behaviour when used as implant .[24,25] Many authors attempted to reduce thrombogenicity by modifying material surface of PET implants. In most of these studies surface characteristics were altered by coating PET implants with antithrombogenic materials. Yoneyama et al.[26,27] attempted coating of 2-methacryloyloxyethyl phosphorylcholine on polyester fabric luminal surfaces and produced thrombus free small diameter vascular grafts. In another study surface modification of vascular and cardiac valve models using arginine–glycine–aspartic acid (RGD) peptide resulted in the formation of an endothelial like layer on PET, and promoted formation of a significantly thinner neointima (pannus) on PET patches in dog. Greater amount of thin pannus and less thrombus were seen on coated PET sewing cuffs. [28] Dewanjee et al. [29] grafted polyethylene oxide (PEO) onto PET fibres in the sewing ring of mechanical heart valve using gamma irradiation. Effect of grafting PEO on graft thromobogenicity was evaluated by in vivo animal experiments. No significant improvements in platelet thrombosis or wound healing were found in their study. Another research group pre-treated vascular graft with tissue factor pathway inhibitor and implanted in mongrel dogs.

Analysis of the grafts retrieved after 3 months implantation showed that the coating reduced thrombogenicity of the graft.[30] Recently some authors reported the effect of a novel absorbable coating on improving the biocompatibility of PET grafts.[31,32] When the PET material surface was passivated with certain fluoropolymer surface treatment substantial improvements in thrombogenicity and platelet accumulation were observed. [33-35] Fluoropolymer coating seem to mask the polar ester groups of PET and expose inert non-polar fluorine atoms on the surface of the graft material. In one study by

Joseph et al, a commercially available PET graft material was coated with a fluoropolymer, namely, polyvinylidene fluoride (PVDF), characterized for mechanical and in vitro biological properties Following conclusions could be drawn from the studies described above: [36]

1. Scanning electron microscopic observations revealed that PVDF form uniform coating on the PET surface.
2. Cell culture cytotoxicity study showed that PVDF coated PET is non-cytotoxic.
3. PVDF coating and the coating technique employed have little effect on the burst strength of the vascular graft fabric.
4. PVDF coating causes marginal reduction in water permeability of the graft. However, the values are within the acceptable range.
5. PVDF coating appears to improve the hemocompatibility of PET fabric.
6. Short term in vitro endothelial cell adhesion and proliferation studies did not show any appreciable difference between coated and uncoated PET.

In this work the above concepts were merged together and made a new vascular graft. A fluoropolymer coating was applied on the graft by dipping the graft in a fluoropolymer solution and the pores of the woven fabric graft were sealed by spraying hydrogel forming material on the external surface of the graft. This modified graft was used for pre-clinical studies.

MATERIALS AND METHODS

Unmodified woven PET vascular grafts were obtained from M/s. TTK Healthcare, Thiruvananthapuram, India. This was dip coated with fluoropolymer. The pores of the fabric grafts were sealed by spray coating with biodegradable hydrogel. The coating processes were developed and carried out by scientists of BMT Wing, SCTIMST, Trivandrum and grafts were supplied by them for implantation

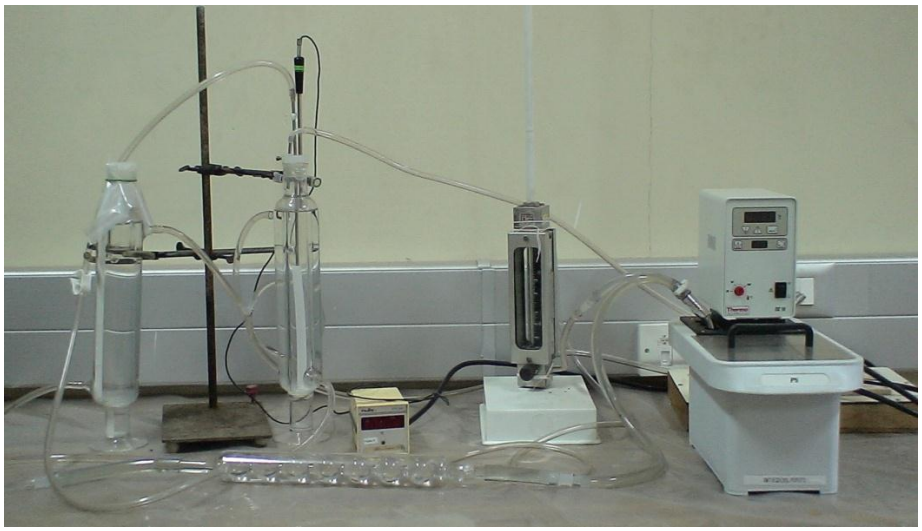


Fig No 1 - Fluoropolymer dip coating unit

The set up used for dip coating of vascular graft is shown in figure no 1. The coated grafts were dried in an air oven and later it was subjected to spray coating process. The steps involved in the hydrogel coating process are shown in figure no 2 and the unit used for spray coating of grafts is shown in figure no 3.

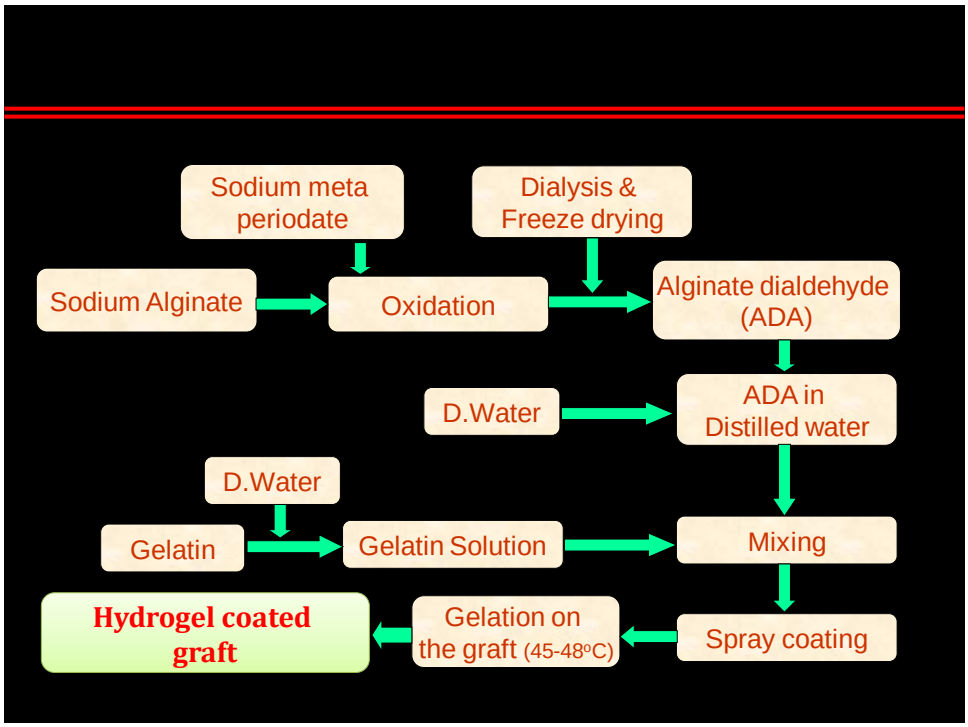


Fig no 2 -Hydrogel Preparation and coating process

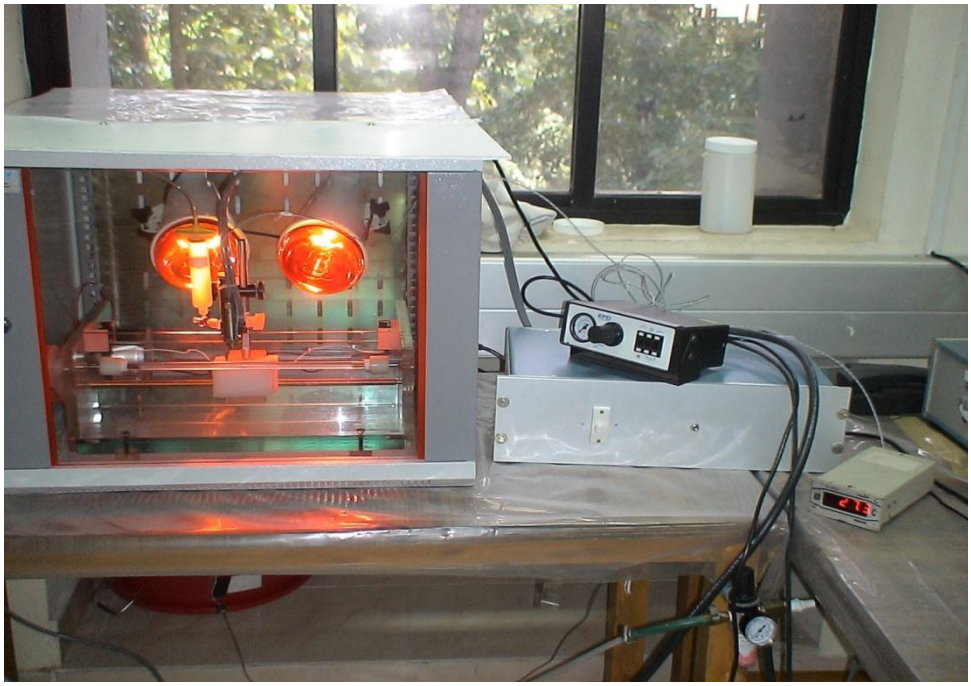


Fig no 3 - Hydrogel Spray coating unit

Coated grafts were examined under scanning electron microscope (SEM) for evaluating the morphology of the coated grafts. Coating was found to be uniform and it had sealed the graft pores, which is shown in Fig No 4

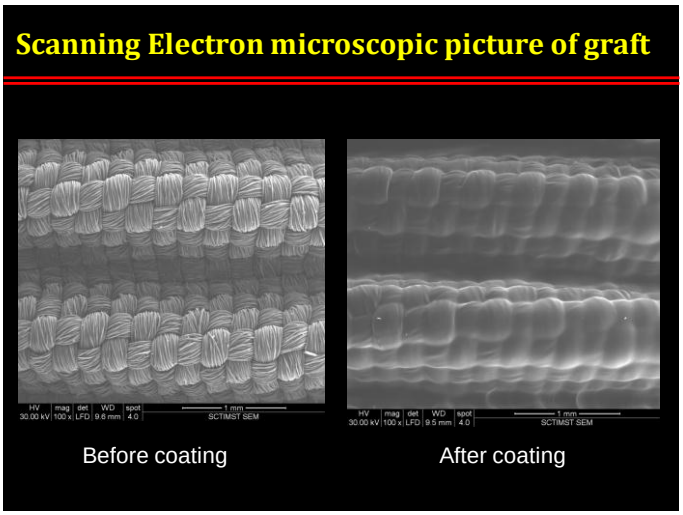


Fig No 4 - Grafts examination under scanning electron microscope (SEM) before and after coating with hydrogel

As control graft, collagen impregnated polyester vascular graft with brand name 'Wovex®' manufactured by M/s. Bard was used. Fig No 5

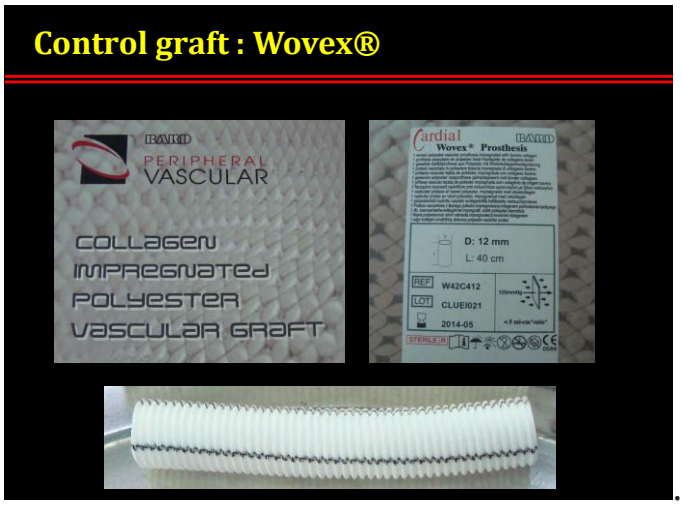


Fig No 5 -control graft Wovex® manufactured by M/s. Bard

Study Sponsor & Test Facility

Name and address of the sponsor:

Dr. Roy Joseph, Scientist F, Polymer Processing Laboratory, BMT Wing, SCTIMST.

Er. Muraleedharan C.V, Scientist G, DAIO, BMT Wing, SCTIMST.

Name and address of the test facility and test sites involved:

Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Biomedical Technology Wing, Poojappura, Thiruvananthapuram- 695012, INDIA

Division of In-vivo Models and Testing

Histopathology Laboratory

Name and address of the Clinical investigator:

Dr. M. Unnikrishnan, Professor, Department of CVTS , SCTIMST.

Name and address of other investigators :

Dr. Vivek Agrawal, Senior resident, Vascular surgery, SCTIMST

Study dates

Study started in Jan 2012

All implantation finished in Dec 2012

Protocol used for pre-clinical study of vascular graft

Test Methods: The tested graft was implanted in pig descending aorta as interposing graft after requisite length of native artery is resected. After implantation the following prospectively defined questions were answered.

Perioperative period

1. Incidence and extent of risk associated with the tested device as mentioned in objective.
2. Tested device performance characteristics.

At termination

i. Graft patency as evaluated by Angiography.
ii. Incidence and extent of risk associated with the tested device as mentioned in objective.
iii. Systemic adverse effects as evaluated by serum biochemistry and hematology.

Test System

Source of supply: Large Animal House, DIMT, BMT Wing, SCTIMST, Poojappura, Thiruvananthapuram.

Characterization of the Test System:

Species, strain	:	Swine (Ankamali cross-breed pig)
Origin	:	Large Animal House, SCTIMST
Age	:	Adult.
Weight at beginning	:	40 kg -60 kg.
Number and sex	:	33, either sex (random selection)

Fig No 6 - Swine (Ankamali cross-breed pig)

Method of unique identification of Test System: Ear tagging/RFID

Justification of the Test System:

The porcine aortic implantation model has been widely used for large diameter vascular evaluation as indicated in the reference cited. This model has been characterized for vascular graft evaluation in this laboratory.

Quarantine procedures: 6 weeks quarantine for newly procured animals. Animals are dewormed and vaccinated for HS during this period. Tetanus toxoid is also administered during this period.

Acclimatization interval: 1 week.

Admission and exclusion criteria:

The respect of the above mentioned criteria was checked before the beginning of the study. Any abnormality revealed by the initial clinical examination had lead to the exclusion of the affected animal(s).

Any pregnant candidate was excluded from the study.

Sex and body weight was controlled prior to the beginning of the study

Animal Husbandry

Facility conditions: The experimental animals were housed away from the pig house in roofed experimental holding areas with provision for separation of groups. Animals were fed once daily as per their DM requirement and potable water is available ad libitum.

Housing conditions:

In conformance with the CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines 1998 and investigations follow the Guide for the Care and Use of Laboratory Animals, Indian National Science Academy, India.

Environmental conditions:

Temperature : Ambient Temperature.

Lighting : Natural lighting, Artificial lighting

12 hours out of 24 hours for acclimatization and postoperative period.

Feed and water description:

Feed: Broiler finisher feed of minimum DE of 3.3 Mcal/Kg, ME of 3.1 Mcal/Kg and crude protein of 14%. Information on contaminants is not available.

Water: Potable drinking water suitable for drinking purpose as per IS 10500: 1991.

Feed and water source:

Feed: SKM Animal Feeds and Foods (India) Ltd., Erode, Tamil Nadu.

Water: BMT Wing, SCTIMST.

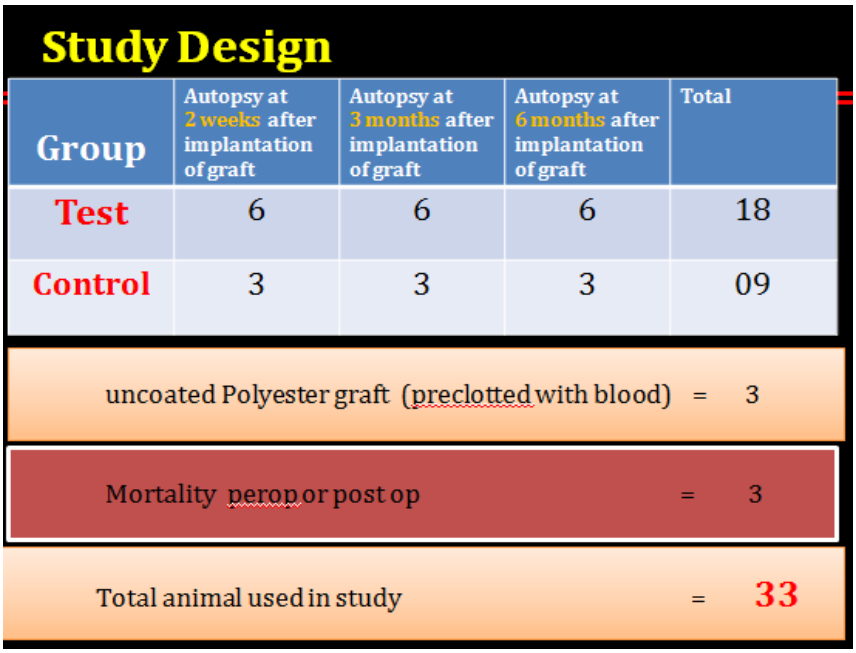
Test/Reference Item Administration

Implantation sequence and number of animals

The test/ control device were implanted in the descending aorta of pig as interposing graft following resection of required length of native aorta. Thus, each animal was provided one test/ control device. The time-periods were of two weeks, 3 months and 6 months. Each time period had 6 test and 3 control devices. Thus the number of test devices implanted were 18 and control devices were 9.

Experimental design

Random block design was used in this study. Animals were randomly assigned to test and control groups.(Table no-1)



Study Design

Group	Autopsy at 2 weeks after implantation of graft	Autopsy at 3 months after implantation of graft	Autopsy at 6 months after implantation of graft	Total
Test	6	6	6	18
Control	3	3	3	09

uncoated Polyester graft (preclotted with blood) = 3

Mortality perop or post op = 3

Total animal used in study = 33

Table No 1: showing the durations and number of test and control device/ animals in each group.

Total number of test graft	18
Total no. of control graft	9
Uncoated polyester grafts	3
Mortality *	3
Total number of animals used in study	33

* We had Three mortality

- One animal died during surgery due to bleeding & fibrillation
- Two animals died in immediate post op due to anticoagulant related bleeding

Therefore we added three more experiments in our study. Therefore total number of animals used in study were 33.

Explantations were done on completion of the scheduled duration. Clinical status of the animal, angiography of the graft, serum biochemistry and hematology and blood culture of the animal was performed and observations recorded.

A complete autopsy of the animal was done by pathologist and observations were recorded.

Data were analyzed and presented appropriately.

Anaesthesia and peri-operative control:

Animals were premedicated with atropine sulphate, Xylaxine, and Ketamine. A venous line were established on the marginal ear vein. Anesthesia was induced with Thiopentone sodium, intubated and maintained by 1% Isoflurane inhalation. The animal was secured on right lateral recumbence. Left lateral thorax was scrubbed with povidone soap and prepared with povidone iodine solution. Surgery was performed under standard aseptic techniques. Carotid artery cut down was made to insert arterial canula for monitoring blood pressure. Heart rate, SpO2, 5 lead ECG, arterial blood pressure, arterial blood gas, activated clotting time, rectal temperature and EtCO2 was monitored using cardiac monitor. Animal was infused Ringer lactate at 3ml/Kg/ hour peri-operatively.

Surgical implantation

Under general anaesthesia and using standard aseptic procedures, left lateral thoracotomy was made through 5 to 6^{th} intercostal space. Under heparinisation (3 mg/Kg body weight) descending aorta was isolated, looped and clamped. Test/control graft of 10-12 mm size and adequate length was implanted as an interposing graft with end to end anastamosis using 4/0 monofilament Prolene sutures. Hemostasis was ensured after implantation. The chest was closed in layers. The surgical wound was closed as per standard technique. The wound was dressed as routine. Analgesics and antibiotic coverage was given for the next five postoperative days. Ceftriaxone 25mg/Kg TID, and Gentamycin 1mg/kg BID was given as antibiotic coverage.

Meloxicam 1mL and Paracetamol 2mL IM was given TID as anti-inflammatory/ antipyretic. Tramadol 50mg BID as analgesic. Anti-platelet therapy was in the order. Aspirin 75mg daily, starting two days before implantation and continuing up to the end of the study. Heparin was partially reversed post surgically. Two doses of Fraxiparine (0.4ml each) were given subcutaneously at 12 hours interval post implantation.

Follow up of the test system

Animals were observed daily for any clinical abnormality for one week postoperatively and then periodically. In case of clinical abnormality detected, a complete veterinary clinical examination and recording was realized.

On completion of the study period, the animal was examined for clinical abnormality followed by angiographic evaluation of the implanted graft. For this, under general anesthesia carotid artery cut down was made and a 7F introducer sheath was placed. Aortogram was acquired by injecting a non-ionic contrast through a 7F pig tailed catheter place in the aortic arch. Animal was euthanized and a complete autopsy was made by Pathologist.

Post Mortem

Methods of euthanasia

Animals was euthanized by an excess dose of intravenous anesthetic Thiopentone sodium followed by KCl and Pancuronium bromide given intravenously.

Handling of animals found dead

Autopsy was performed to identify the cause of death. For animals dying on the table, probable cause was recorded.

Necropsy:

A complete necropsy of the animal was performed with an emphasis to record incidences of thromboembolism in the distal organs. Other observation pertaining to the device associated risks such as graft leakage (hematoma, hemorrhage and blood leakage), graft disruption, seroma, aneurysm and graft infection (abscess formation) was made.

The observation done is as follows

Vascular graft: Observation of:

- Graft Type
- Location/position of graft
- Dimensions
- Appearance-intactness
- Endo-leaks
- Structural integrity
- Any other findings in other organs
- Gross observation of vascular graft:
- Lumen patency
- Presence/absence of thrombosis – internal and external
- Presence of tissue layer on flow surface
- Presence of purulent material on flow surface
- Anastomosis at both ends
- Microscopy: H&E and special stained paraffin sections
- Healing at anastamosis
- Endothelialization

- Tissue ingrowth and cellular infiltrate
- Material debri (degraded graft or coating material)
- Inflammation
- Neo-intima – thickness, cells, matrix
- External capsule, haematoma formation

Blood parameters monitored before implantation, during study and at autopsy

- Blood culture
- Total count.(RBC and WBC)
- Differential leukocyte count.
- Platelet count.
- Hemoglobin
- Haematocrit.
- Clotting time
- Bleeding time
- Prothrombin time
- Random blood glucose level.
- ALT
- AST
- Creatinine
- Blood urea nitrogen.
- Total protein

IMPLANTATION PROCEDURE USED FOR PRE-CLINICAL STUDIES IN PIG

1. Anaesthesia - Procedure was done under general anaesthesia with endotracheal tube intubation.
2. Carotid – Femoral shunt was placed. For this left carotid artery and left femoral artery was exposed. 5 Fr sheath was placed in both exposed arteries. These were connected to each other with circuit. This shunt was opened during cross clamping of aorta. Carotid – Femoral shunt helps in maintaining blood pressure and circulation to distal tarso during cross clamping of aorta.

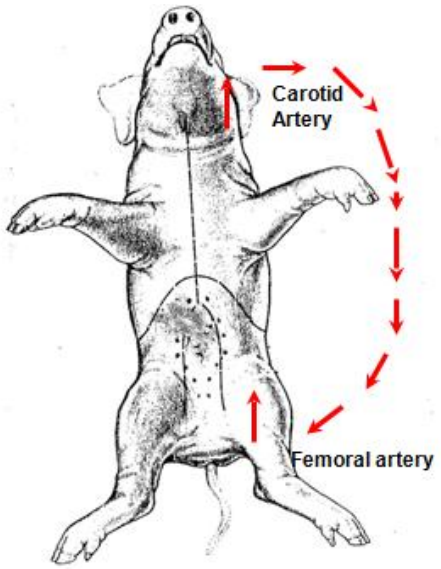


Fig 5.1 scheamatic diagram of Carotid – Femoral shunt

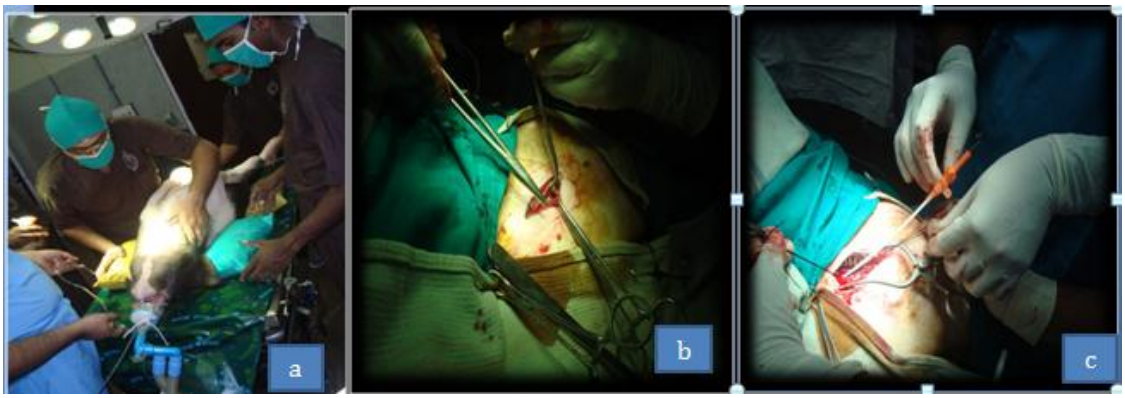


Fig 5.2 For Carotid – Femoral shunt placement (a) Positioning of animal, (b) Exposure of carotid artery (c) Placement of sheath in carotid artery

3. Position - Animal was placed in right lateral decuibitus position.

4. Incision - Single space or double space entry thoracotomy was performed.

a) When we were planning to use short graft we do thoracotomy via 5th intercostal space and 6th rib was excised for better exposure.

b) If we were planning for long graft implantation we do double space entry via 4th and 8th intercostal space.

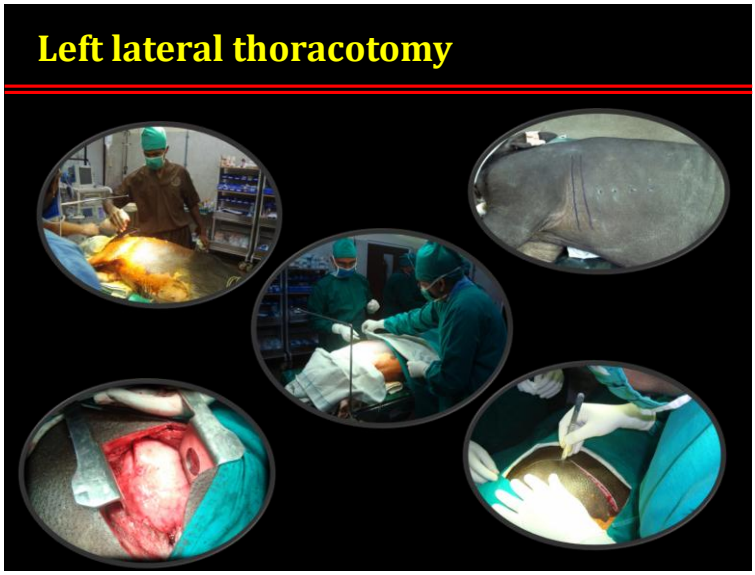


Fig No 5.3- Position of pig in right lateral decuibitus position and left lateral thoracotomy

5. Descending thoracic aorta was exposed. Azygos vein was ligated and divided.

6. 3 mg/kg heparin was given and when ACT was gone up to 400/ sec., Descending thoracic aorta was cross clamped & carotid femoral shunt was opened.

7. A segment of Descending thoracic aorta in between clamps was resected. (Fig No 5.4)

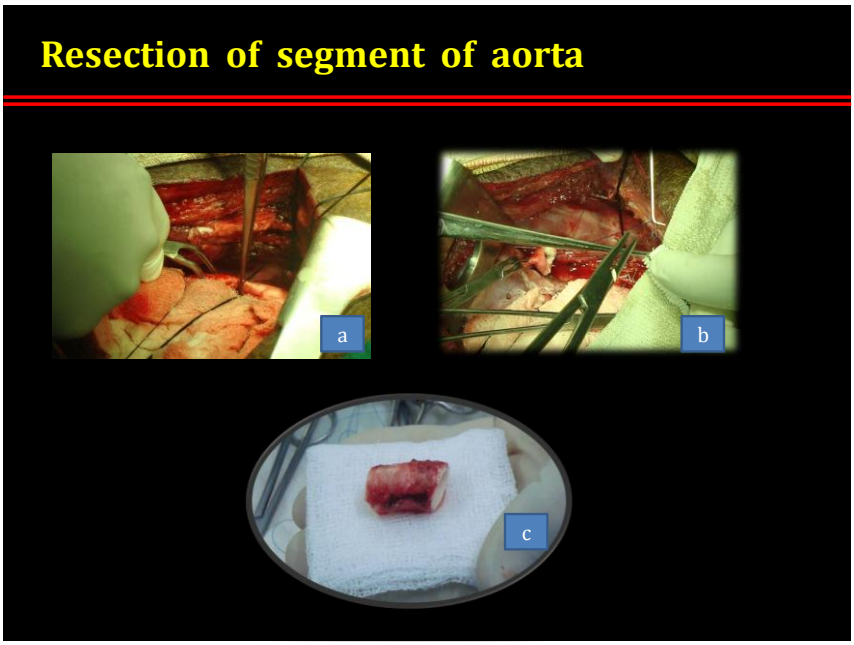


Fig No 5.4- Resection of segment of aorta after Cross clamping (a) Clamping of Descending thoracic aorta (b) Resection of segment of aorta (c) Resected segment

8. Resected part of aorta was replaced with graft either test or control. Anastomosis was done with 4/0 prolene suture. (Fig No 5.5 and 5.6)

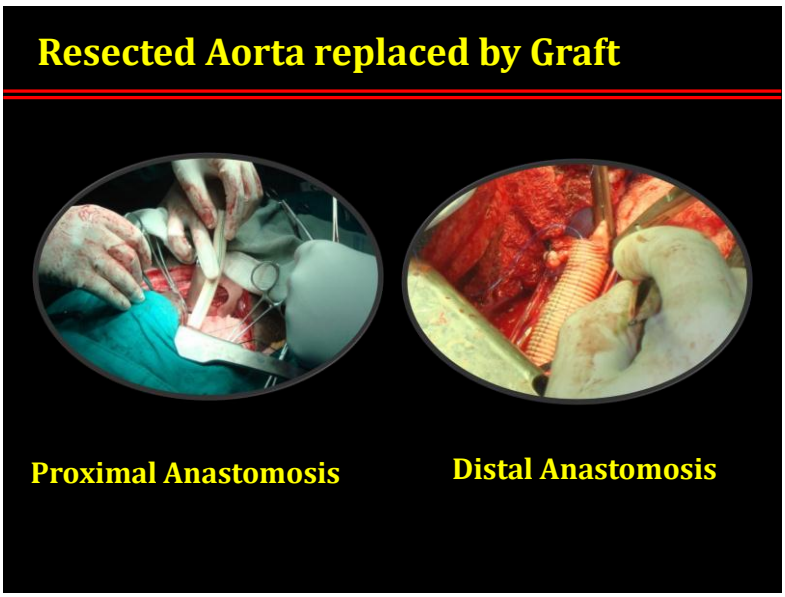


Fig No 5.5- Proximal and distal anastomosis for replacement of graft

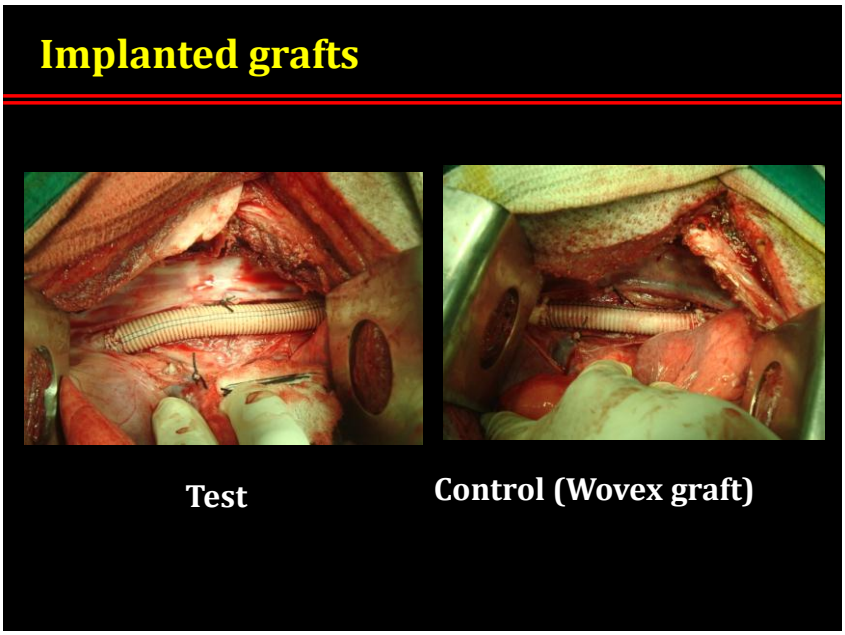


Fig No 5.6- Implanted Text and control grafts

9. Heparin was half reversed with protamine sulphate. Hemostasis was secured.

10. Thoracotomy wound was closed over ICD, which was removed after one day.

11. Check angiogram was done immediately after surgery to see graft patency and intact suture line. . (Fig No 5.7)

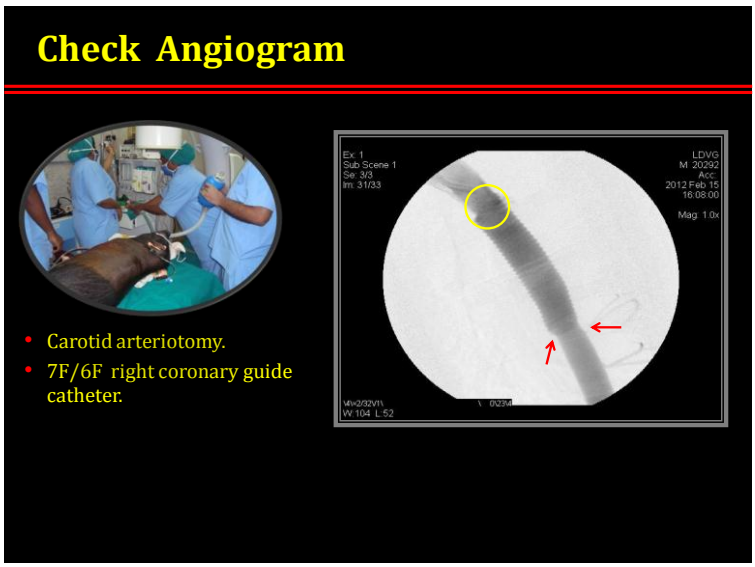


Fig No 5.7 – Post implantation Check angiogram. Yellow circle and red arrow indicate suture line

12. Animal was extubated on table and shifted to recovery room.

Fig No 5.8 Animal in recovery room

RESULTS

- Implantation of graft in animal was started in January 2012
- All implantations were completed by December 2012.
- We had planned graft implantation in 30 animals but we had three mortality one in periop and two in immediate postop. Therefore three more experiments were added in our study to complete our study and we did total 33 implantations.
- Out of 33 pigs.
- Sex of pig - Male pig - 15

  Female pig - 18

  Male female ratio - 1:1.2
- Weight of pig at time of implantation ranges from 33kg to 59 Kg (Mean weight 41kg).
- Weight gain after surgery

Study group	Weight Gain or Loss
2 weeks groups	Lost about 1.5 Kg
3 months groups	Gain weight about 4 to 5 kg
6 months groups	Gain weight about 9 to 10 kg

- We had three mortality (3/30- 10%)
  - One animal was died during surgery due to bleeding & fibrillation. (1/30- 3.33%)
  - Two animals died in immediate postop period due to anticoagulant related bleeding (2/30- 6.66%).

- **Observation made during surgery –**

  1- The grafts did not require preclotting or special preparation before being implanted.

  2- Graft was soft and good in handling.

  3- Suturing was easy.

  4- There was no bleeding from anastomotic line or graft surface.

- **Observations during postop period –**

  1- All animal did well post op expect two who died in immediate postop period due to bleeding.

  2- Animal had 100-150 ml ICD drain on immediate postop day. ICD was removed next day morning in all animals.

  3- Wound healing was normal in all animals. Sutures were removed on 10-12th postop day.

  4- Pigs were active after few days of surgery.

  5- They started their routine food after few days.

  6- All animal has normal growth and weight gain during post implantation period.

<u>Angiograms results before explantation of graft</u>

•Before explantation of graft check angiograms were performed.(Fig No 6.1 & 6.2)

•In all cases suture lines were intact

•Inner lining of graft became smoother which were suggestive of formation of neointima.

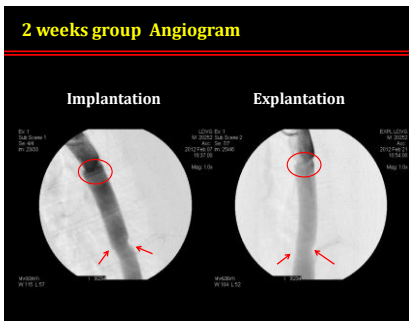


Fig 6.1 - check angiograms, Circle and arrow indicating suture line

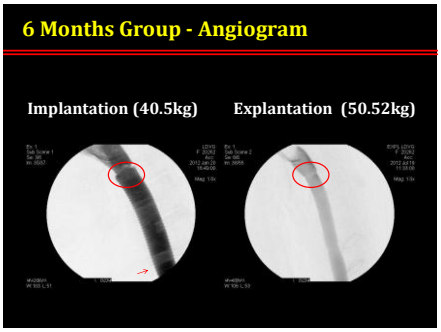


Fig 6.2 -check angiograms, Circle and arrow indicating suture line

Explanted graft during autopsy

1. Anastomotic Suture lines were found intact.

2. There was no pseudoaneurysm in any of the graft.

3. There were no obvious signs of infection seen.

4. Graft was surrounded with perigraft tissue.

5. On opening the graft longitudinally on naked eye examination we found that inner lining was smooth and uniform in both text and control grafts which was suggestive of neointima formation. Although inner lining of text graft was smoother and uniform and there were some petechial spots in control graft which may be due to platelet deposition. (Fig 6.3- 6.5)

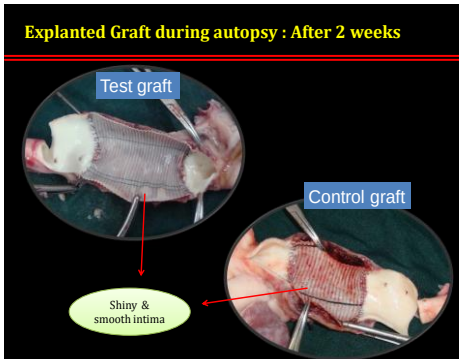


Fig 6.3 Explanted grafts after 2 weeks

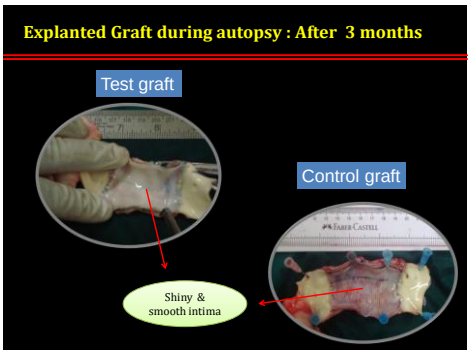


Fig 6.4 Explanted grafts after 3 months

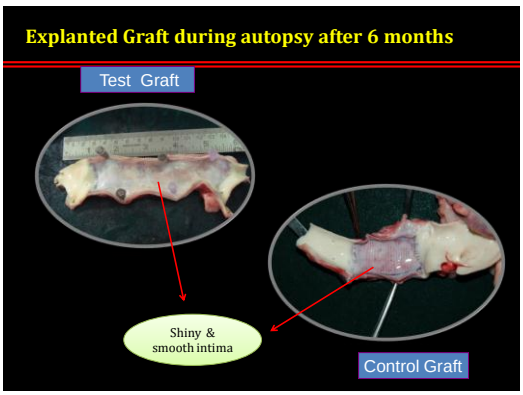


Fig 6.5 Explanted grafts after 6 months

Explanted graft under magnification

- Under magnification also all test and control grafts showed shiny, smooth and uniform neointima. (Fig No. 6.6 and 6.7)

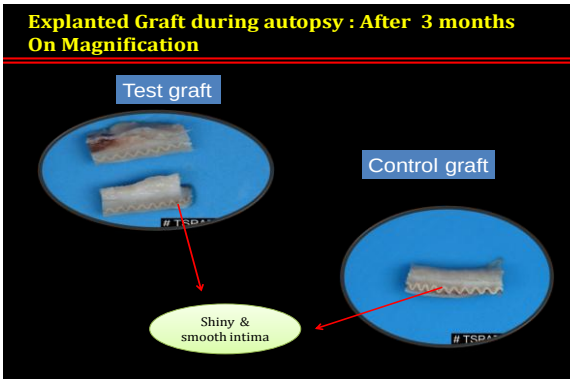


Fig 6.6 Explanted graft after 3 months under magnification

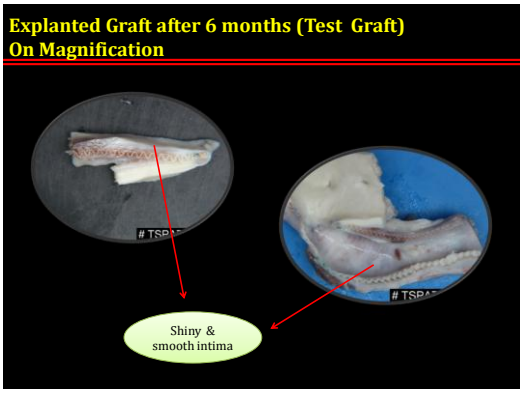


Fig 6.7 Explanted test graft after 6 months under magnification

Histopathology of Explanted graft (light microscopy, scanning electron microscopy, and transmission electron microscopy)

- There were endothelial like uniform cell lining over the inner surface of graft which was suggestive of neointima.
- Thrombosis was not seen at anastomosis site
- Tissue in-growth was seen in between fabric, which suggests that graft has been incorporated into body tissues. (Fig No. 6.8 – 6.10)

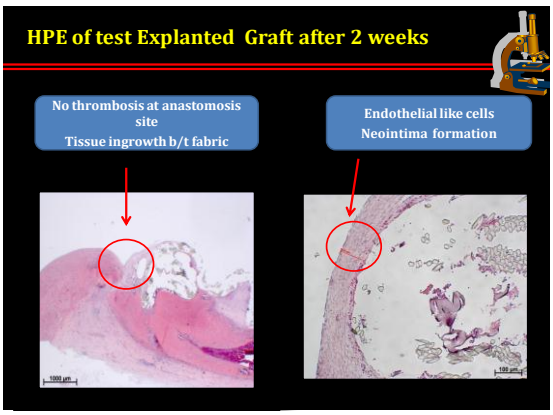


Fig 6.8 Histopathology of Explanted graft after 2 weeks

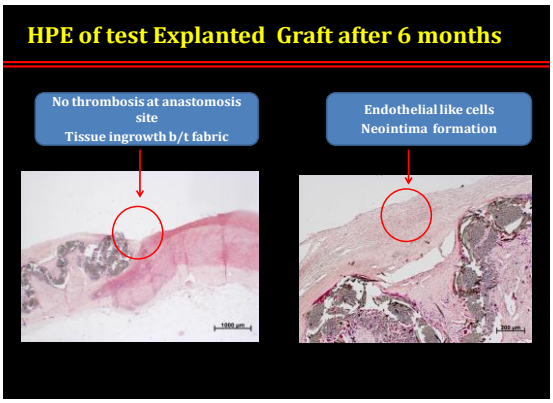


Fig 6.9 Histopathology of Explanted graft after 6 months

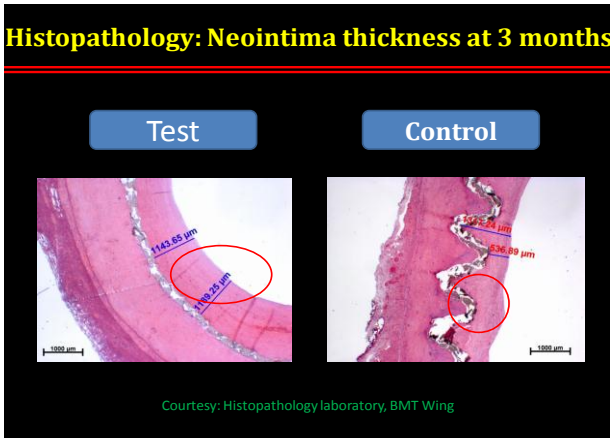


Fig 6.10 Histopathology of Explanted graft after 3 months

Histopathology of internal organs

All internal organs like heart, lung, liver, kidney and spleen did not show any evidence of embolic or thrombotic spots which suggest that graft was safe for all internal organs

DISCUSSION

In the field of vascular surgery the use of surgical bypass is fundamental to the treatment of a wide variety of arterial and venous disorders. In turn, the technical conduct as well as success of surgical bypass is directly dependent on the conduit used. The ideal conduit should be readily available, easy to handle, resistant to thrombosis and infection, durable, and inexpensive, as well as have characteristics similar to the vessel that it is replacing.

Although the perfect conduit does not exist, autogenous blood vessels are closest to the ideal. Autogenous arterial conduits such as the internal mammary, radial, and gastroepiploic arteries have been used with great success in the coronary circulation[37-39]

Synthetic conduits were introduced to modern arterial reconstructive surgery by Voorhees and Blakemore in 1952 with the development of a vascular graft woven from Vinyon-N fibers, a copolymer of vinyl chloride and acrylonitrile.[37] Despite successful early clinical outcomes, first-generation vascular prostheses lacked long-term durability. Within 5 years, graft dilatation and aneurysmal degeneration were frequent, with an 80% loss in tensile strength.[38,39] A variety of candidate materials for synthetic arterial conduits were examined throughout the 1950s, including solid rigid metals, glass, and silk. Their failure led to the supposition that porosity and resistance to thrombosis were critical features for an arterial substitute. Innovations in polymer science and engineering in the period surrounding World War II led to the synthesis of polytetrafluoroethylene (PTFE; e.g., Teflon) in 1938 and polyethylene terephthalate (PET; e.g., Dacron) in 1941. Both polymers, when engineered as fibers or as a nonwoven fabric, proved to be more durable and biocompatible and have remained commercial standards. In the aortic position, the performance of prosthetic grafts is excellent, with susceptibility to graft infection the remaining major limitation. In the paediatric population, the inability of commercially available prostheses to match the growth of the child is an additional limitation of current synthetic large-calibre conduits.

Dacron is the DuPont trademark for PET, a highly durable polyester thermoplastic polymer that can be processed into synthetic fibers. DeBakey introduced the first knitted Dacron prosthesis in 1957; it was used extensively for arterial reconstruction of the thoracic and abdominal aorta, as well as proximal peripheral vessels.[40] This pioneering work, combined with developmental and clinical studies by Szilagyi, Wesolowski, Sauvage, and Cooley, contributed to the popularization of Dacron grafts.[41]

Historically, weaving and knitting have been the two common techniques for fabrication of Dacron fibers into a tubular conduit. The knit structure involves looping fibers in an interlocking chain, which yields a soft and stretchable fabric. In contrast, a woven structure assembles the yarn in an over-and-under pattern in the lengthwise and circumferential directions .[42-44] Woven grafts are stronger and less porous than knitted grafts but are less compliant and may fray when cut. Typically, highly porous knitted Dacron grafts required “preclotting,” a process that involved exposing the graft to an aliquot of the patient's blood before heparinization. Currently, most commercial knitted grafts are impregnated with gelatin,[45] collagen,[46] or albumin.[47] Despite some evidence that coated grafts may induce a greater inflammatory response than preclotted grafts,[48,49] both display similar patency rates.[50,51]

Dacron grafts are often crimped longitudinally to increase flexibility, elasticity, and kink resistance. However, these properties are lost soon after implantation as a consequence of tissue ingrowth.

Recently, several modifications of Dacron grafts have been approved for clinical use. To reduce surface thrombogenicity, grafts coated with bioactive heparin or passivated with fluoropolymers have been developed.[52] A silver-coated Dacron graft has also been introduced to decrease the occurrence of graft infection. Long-term follow-up studies will be required to determine whether these modifications improve outcomes.

Early versions of knitted grafts were prone to dilatation over time, with a 10% to 20% increase in graft size immediately after implantation,[53] followed by slow

expansion.[54] Nonetheless, correlation of graft dilatation and structural failure has not been established.[55] Most Dacron grafts have been extremely durable. In 1997, the Food and Drug Administration (FDA) disclosed a total of 68 cases of structural failure occurring at an average of 7.4 years after implantation. [56] Given that approximately 60,000 aortic reconstructions are performed annually, the overall rate of graft failure is small.[56] A recent single-center review noted a 0.2% structural failure rate at a mean follow-up of 12 years.[57]

In a study by Joseph et al in 2008, hydrogel derived from ADA and gelatin was used for coating porous woven PET grafts with a view to seal the pores and to obtain implantable grafts with no blood leakage and low-thrombogenicity. Coated grafts were studied in vitro to evaluate its potential to remain impervious to blood while retaining porosity for tissue in-growth and biological healing. The study concluded that Hydrogel coated on PET graft samples effectively sealed the pores of the graft leading to reduced water permeability. The coating did not adversely affect mechanical properties of the graft. Over 90% reduction in water permeability was achieved by hydrogel coating under static conditions. The soft rubbery nature of the hydrogel would minimize mechanical and frictional irritation to the surrounding tissues. A perfusion experiment revealed that hydrogel-coated PET graft is highly hemocompatible. Cytocompatibility tests showed that the coating would enhance the performance of the PET graft with better adhesion of EC.

In one study by Joseph et al , a commercially available PET graft material was coated with a fluoropolymer, namely, polyvinylidene fluoride (PVDF), characterized for mechanical and in vitro biological properties Following conclusions could be drawn from the studies described above: [36]

1. Scanning electron microscopic observations revealed that PVDF form uniform coating on the PET surface.
2. Cell culture cytotoxicity study showed that PVDF coated PET is non-cytotoxic.
3. PVDF coating and the coating technique employed have little effect on the burst strength of the vascular graft fabric.

4. PVDF coating causes marginal reduction in water permeability of the graft. However, the values are within the acceptable range.

5. PVDF coating appears to improve the hemocompatibility of PET fabric.

6. Short term in vitro endothelial cell adhesion and proliferation studies did not show any appreciable difference between coated and uncoated PET.

In this work the above concepts were merged together and made a new vascular graft. A fluoropolymer coating was applied on the graft by dipping the graft in a fluoropolymer solution and the pores of the woven fabric graft were sealed by spraying hydrogel forming material on the external surface of the graft. This modified graft was used for pre-clinical studies.

In our preclinical trial we observed following points

1- The grafts did not require preclotting or special preparation before being implanted.
2- Graft was soft and good in handling
3- Graft was strong , it was not fraying
4- Suturing was easy.
5- Suture retention was good.
6- There was no oozing from needle holes, anastomotic line or graft surface.
7- All animal did well post op except two who died in immediate postop period due to bleeding.
8- Wound healing was normal in all animals.
9- Pigs were active after few days of surgery.
10- All animal has normal growth and weight gain during post implantation period.
11- Check angiograms before explantation showed intact suture line and neointima formation.
12- When explanted all grafts were patent and covered with neointima.
13- There was no pseudoaneurysm or abnormal dilatation in any of the graft.
14- Graft was surrounded with perigraft tissue without signs of any infection.

15- On opening the graft longitudinally on naked eye examination we found that inner lining was smooth and uniform in both text and control grafts which was suggestive of neointima formation. Although inner lining of text graft was smoother and uniform and there were some petechial spots in control graft which may be due to platelet deposition.
16- Under magnification also all test and control grafts showed shiny, smooth and uniform neointima.
17- Histology of the explanted grafts (light microscopy, scanning electron microscopy, and transmission electron microscopy) showed
    a. Neointima formation.
    b. Anastomotic sites were free from thrombus .
    c. Tissue in growth was seen in between fabric, which suggests that graft has been incorporated into body.
    d. All internal organs like heart, lung, liver, kidney and spleen did not show any evidence of embolic or thrombolic spots which suggest that graft is safe for all internal organs.
18- Last but not the least this graft is indigenous. It will be cost effective and easily available for the use.

All these observation suggest that the hydrogel and fluropolymer is neither thrombogenic, antigenic, cytotoxic, or pyrogenic. They do not affect mechanical properties of graft. Therefore it can be used for preclotting large diameter polyester grafts.

CONCLUSION

Fluropolymer coated & Hydrogel sealed graft had excellent results in preclinical evaluation .We plan to go ahead for clinical trial. These grafts are good substitute to collagen coated imported graft in future from our animal studies performed so far.

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