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The making of indigenous vascular prosthesis

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***Background & objectives*: Vascular illnesses are on the rise in India, due to increase in lifestyle diseases and demographic transition, requiring intervention to save life, organ or limbs using vascular prosthesis. The aim of this study was to develop indigenous large diameter vascular graft for treatment of patients with vascular pathologies.**

***Methods*: The South India Textile Research Association, at Coimbatore, Tamil Nadu, India, developed seamless woven polyester (Polyethylene terephthalate) graft at its research wing. Further characterization and testing followed by clinical trials were conducted at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India. Fifteen *in vivo* experiments were carried out in 1992-1994 in pigs as animal model. Controlled (phase I) clinical trial in ten patients was performed along with control graft. Thereafter, phase II trial involved 22 patients who underwent multi-centre clinical trial in four centres across India.**

***Results*: Laboratory testing showed that polyester graft was non-toxic, non-leeching and non-haemolytic with preserved long-term quality, further confirming in pigs by implanting in thoracic aorta, comparable to control Dacron grafts. Perigraft incorporation and smooth neointima formation which are prime features of excellent healing characteristics, were noted at explantation at planned intervals. Subsequently in the phase I and II clinical trials, all patients had excellent recovery without mortality or device-related adverse events. Patients receiving the test graft were followed up for 10 and 5 years, respectively. Serial clinical, duplex scans and CT angiograms performed periodically confirmed excellent graft performance.**

***Interpretation & conclusions*: Indigenously developed Chitra vascular graft was comparable to commercially available Dacron graft, ready for clinical use at affordable cost to patients as against costly imported grafts.**

Key words Aortic aneurysm - coarctation of aorta - surgical repair - vascular diseases - vascular prosthesis

Ever since the historic use of Vinyon-N for arterial replacement by Voorhees *et al*[1] in 1952, tubular grafts made from textile fabric have been firmly established in modern vascular surgery. However, technological data regarding their physical, chemical and toxicological properties which lead to biological healing after implantation of these grafts, were not available for manufacturing these in India. Hence, it was considered worthwhile to initiate planned development of arterial prosthetic grafts indigenously so that these could be made available readily at an acceptable cost to patients. This prosthesis was intended to serve as a permanently implanted device for treatment of patients with vascular pathologies like aneurysm or arterial occlusive disease, to replace or bypass the diseased blood vessel, so as to preserve life and/or restore vital blood supply to the affected organ(s).

As per the Joint council of the Society of Vascular Surgery and International Society of Cardio-vascular Surgery (SVS/ISCVS)[2], the ideal characteristics of vascular prosthesis are indicated in Table I. The objective of this programme was the development of straight woven vascular prosthetic grafts of polyester in the size range of 10 to 25 mm internal diameter initially which should be comparable in safety and efficacy to the widely used commercially available vascular prostheses.

Table I. Ideal characteristics of vascular prosthesis (as per Joint council of SVS/ISCVS)[2]

1.	Cosmetic attributes - satisfactory feel and appearance
2.	Cleanliness and sterility - free from debris, grease, *etc*. Available in a sterile pack
3.	Consistency - all grafts to conform to common acceptable standards
4.	Cost - competent and affordable
5.	Porosity - adequate to improve healing characteristics, without producing blood leak
6.	Handling characteristics - pliable, easy to suture and good suture retention, minimal fraying
7.	Strength - no dilation or aneurysm formation
8.	Flow surface - thrombo-resistant
9.	Bio-mechanical properties - comparable visco-elastic properties to that of natural arteries
10.	Infection resistance - 100% free from risk of graft infection
11.	Durability - 100% long-term patency
12.	Easy and readily available

Material & Methods

Development & in vitro testing: Designed and fabricated at South India Textile Research Association (SITRA), Coimbatore, the main testing and clinical work of woven grafts was carried out at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, Kerala, India. The prosthesis was essentially made of polyester polyethylene terephthalate (PET). The design and developmental process included yarn preparation, weaving of the tubular fabric, crimping and sterilization. The fabric was a seamless woven tube of 75 denier texturised polyester yarn which was suitably crimped (Fig. 1) to provide large diameter vascular graft with a porosity of 200±50 ml/min/cm^2 of normal saline at 120 mmHg. The physical characteristics of the yarn, in terms of the yarn count, yarn twist and tensile strength to weave the grafts were determined by SITRA. The chemical composition of the material was analysed by infrared spectroscopy using spectrophotometer (Shimadzu, Japan), and differential thermal analysis at the laboratory for technical evaluation of biomaterials at the biomedical Division of SCTIMST. Toxicity/ biocompatibility studies such as acute systemic toxicity, intracutaneous irritation, *in vitro* haemolysis and implantation in muscle were studied as per international standards[3] for the vascular graft material. Acute systemic toxicity was studied in mice using physiological saline and cotton seed oil extracts of the material. Intracutaneous irritation was done by injecting the extracts of material and control intradermally into rabbits under ketamine anaesthesia. Grading of erythema and oedema of material in experimental and

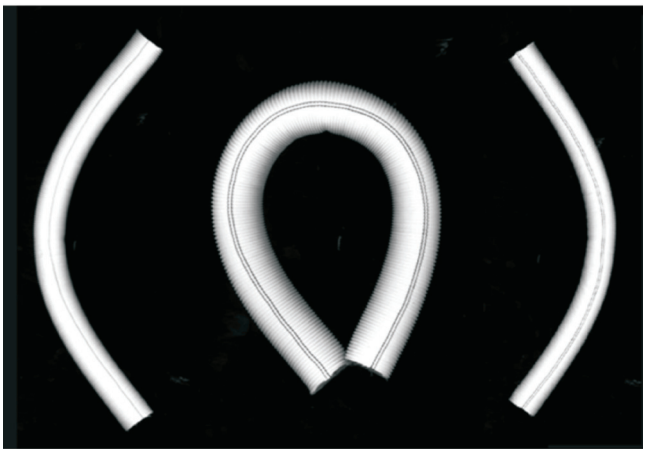

Fig. 1. The test vascular polyester polyethylene terephthalate (PET) graft with internal diameter ranging from 8-30 mm.

control rabbits were recorded at 24, 48 and 72 h. *In vitro* haemolysis was carried out with material and extract of the material using centrifugation (REMI R-8C DX, India) with rabbit blood. The muscle implantation was carried out in nine albino rabbits. The implanted animals were sacrificed at the end of one, four and 12 wk, the tissue with the implanted materials were collected, sectioned using RM2255 microtome (Leica, biosystems, India) and subjected to histopathological analysis (Axio Imager Z1 microscope, Carl Zeiss).

Pre-clinical animal testing: During 1982-1983, as part of the pilot study[4], 30 pigs had received the test graft. Further, *in vivo* experiments in 1992-1994, reported herein, involved 15 pigs as per Ethics Committee requirements in accordance with draft document of Association for the Advancement of Medical Instrumentation[5]. Protocol was drawn to evaluate 11 mm test graft and 12 mm USCI DeBakey graft (USA) as concurrent controls (Fig. 2).

Four to six months old large white Yorkshire pigs (*Source*: Government pig farm, Parassala, Thiruvananthapuram) weighing 40-45 kg, were used for each experiment after due conditioning as per animal testing guidelines[6]. Following overnight fasting and premedication, general anaesthesia was employed using thiopentone sodium (10 mg/kg) and muscle relaxants and maintained with endotracheal intubation and inhalation anaesthetic agents. Open cut down was employed to obtain central venous access through saphenous vein and arterial access through femoral artery. Continuous monitoring of heart rate, blood pressure, electrocardiogram (ECG) and arterial blood gases (ABG) at regular intervals formed the standard protocol. Left postero-lateral thoracotomy was performed and pleural cavity entered by excising 4th or 5th rib. Lung was gently retracted and mediastinal pleura over descending thoracic aorta incised and aorta dissected and looped. Dissection was continued as far as feasible towards suprahiatal aorta whenever long grafts had to be implanted. Heparin (1 mg/kg) was administered three minutes prior to clamping in some cases except where blood retrieval strategy was employed in which case 3 mg/kg was required[7]. Cardiotomy reservoir with suction device was utilized to retrieve shed blood during most procedures. After completion of arterial grafting (Fig. 3A), protamine was administered and haemostasis achieved. Thoracotomy was closed without indwelling chest drainage tube.

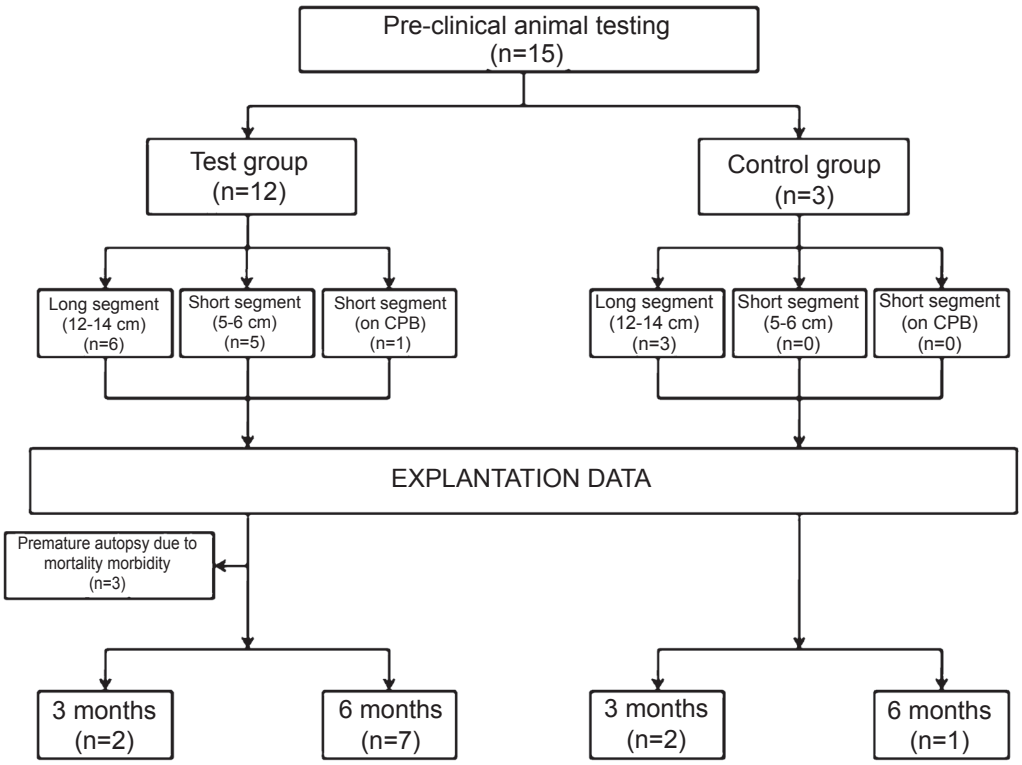

Fig. 2. Protocol of pre-clinical testing in Yorkshire pigs using test and control grafts and the explantation data. CPB, cardio pulmonary bypass.

All animals except one could be extubated at the end of procedure and walked back to cage 4-6 h after completion of the procedure. They were given water and feeds from next day morning. Streptopenicillin injection was given for five days for infection prophylaxis. The surviving animals were followed up for three and six months as per protocol.

Grafts were explanted to study the healing characteristics, blood/tissue interactions and patency rates. Explantation was performed at three months or six months following graft insertion under general anaesthesia; 1 mg/kg heparin was administered to avoid misinterpretation of pre- or postmortem thrombus.

Phase I clinical trial:

(i) Design - Prospective randomized study was designed to prove non-inferiority of the test prosthesis with concurrent controls using commercially available prosthetic grafts. Objective patency assessment was made by non-invasive haemodynamic indices, duplex ultrasound and catheter angiography/CT angiography.

(ii) Parameters evaluated were (a) ease of pre-clotting; (b) pliability/conformability; (c) ease of suturing/suture retention; and (d) short-term patency at one year, long-term patency at five years and beyond.

(iii) Institutional Ethics committee (IEC) gave permission to use large diameter prosthesis of length less than 10 cm for a total of 10 patients during phase I trial as a matter of abundant caution. This restricted its use only for repair of abdominal aortic aneurysms (AAA) and coarctation of thoracic aorta (CoA). Special and detailed informed consent was obtained in every patient included in the study.

(iv) Methods - Between September 1998 and November 1999, 10 patients were included in phase I clinical trial using the test graft at SCTIMST, six of whom underwent repair for AAA and four for CoA (Table II). AAA involved the infra-renal segment of aorta which was repaired through xipho-pubic midline laparotomy and transperitoneal approach to the aorta. Standard inclusion graft technique was performed using appropriate (14-20 mm internal diameter) test graft using 3/0 monofilament polypropylene suture for proximal anastomosis and 4/0 suture for distal. Inferior mesenteric artery was re-implantated into the prosthesis using Carrel's patch technique[8] in selected cases upon indication. CoA repair was performed through left postero-lateral thoracotomy. Excision of coarct segment and interposition graft placement using 14-16 mm ID test graft was done in one patient while

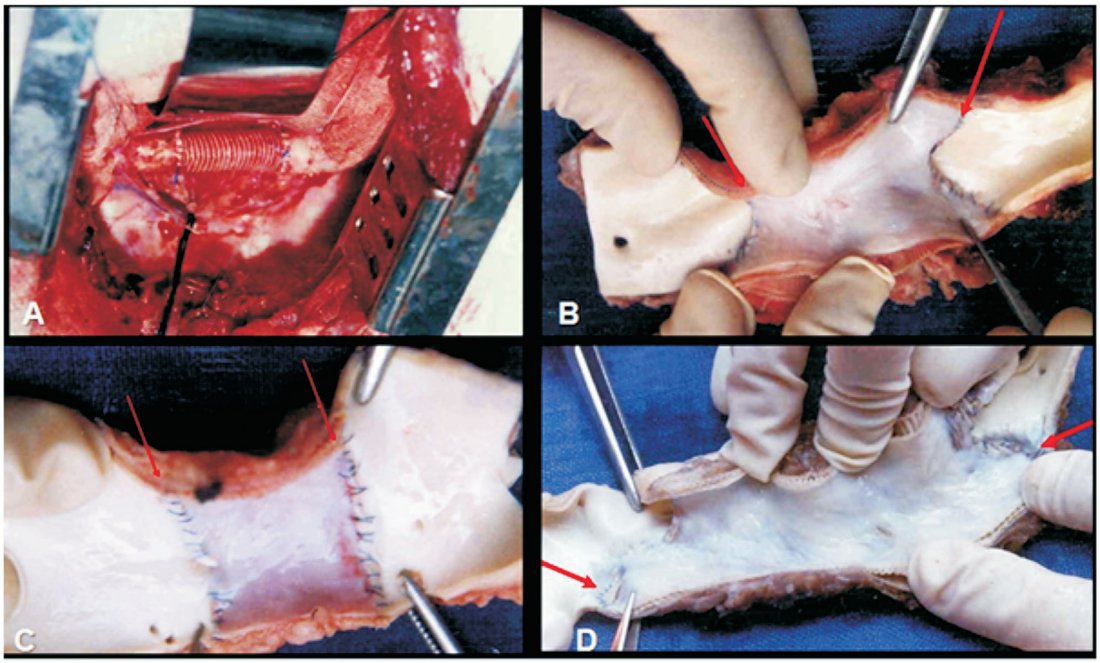

Fig. 3. Replacement of segment of descending thoracic aorta in pig with the test graft using standard protocol via left postero-lateral thoracotomy **(A)**. Gross healing characterstics of test vascular graft at explant post-6 month *in vivo* in pig in 8, 4 and 12 cm lengths respectively **(B, C, D)**. Neointima was found to be smooth in short replacements while it was less regular and even, nonetheless thrombus-free, in long grafts.

Table II. Clinical details and results of phase I (controlled) clinical trial

S.No	Age/Sex	Pathology	Procedure	Prosthesis size (I.D x length)	Mortality	Complications	Post-op ABI	Patient on follow up	Long follow up status (as in 2014)
1	69/F	AAA	Repair	16mm x 6cm	No	Nil	1.1	Yes	Doing well
2	70/M	AAA	Repair	20mm x 6cm	No	Paralytic ileus	1.0	Yes	Died after 12 yr (old age)
3	38/M	CoA	Jump graft	14mm x 10cm	No	Nil	0.85	Yes	HTN controlled on 2 drugs
4	24/M	CoA+ DTAA	Inclusion repair	16mm x 9cm	No	Paraplegia	0.90	Yes	Paraplegia persists otherwise active
5	36/M	CoA	Jump graft	12mm x 9cm	No	Nil	1.0	Yes	Doing well
6	69/M	AAA	Repair	18mm x 8cm	No	Paralytic ileus	0.92	Yes	Died after 15 yr (old age)
7	53/M	AAA	Repair	16mm x 10cm	No	Nil	1.0	Yes	Doing well
8	39/F	CoA	Jump graft	12mm x 10cm	No	Nil	1.0	Yes	Doing well
9	65/M	AAA+ CIAA	Repair	14mm x 10cm	No	Respiratory infection	1.0	Yes	Died after 10 yr (MI)
10	55/F	AAA	Repair	16mm x 4cm	No	Nil	1.1	Yes	Doing well

AAA, abdominal aortic aneurysm; CoA, coarctation of aorta; DTAA, descending thoracic aortic aneurysm; CIAA, common iliac artery aneurysm; ABI, Ankle-brachial pressure index; HTN, hypertension; ID, internal diameter

left subclavian artery (LSA) to descending thoracic aorta bypass (jump graft) using 12-14 mm ID graft was done in the other three. The latter involved end-to-side anastomosis to the dilated LSA proximally and post-coarct aorta distally using 4/0 polypropylene suture. In the control group, eight patients with AAA and two with CoA underwent elective implantation of the control Dacron graft.

Phase II clinical trial: Twenty two patients who underwent indexed vascular reconstructions at four designated hospitals *viz*. Medwin Hospital, Hyderabad; G. Kuppuswamy Naidu Memorial Hospital, Coimbatore; Medical College Hospital, and SCTIMST, Thiruvananthapuram; formed the basis of this part of the study conducted from August 2005 to September 2008. Patients chosen were all symptomatic and investigated, operated upon and followed up using an already established and common protocol. Age ranged from 19 to 74 yr with mean age of 55 yr; four patients were female (>4:1 male: female ratio). Clinical indications for graft implantations included CoA in three, middle aortic syndrome in one, thoraco-abdominal aortic aneurysm (TAAA) in two, AAA in 10, iliac artery occlusion in five and iliac artery aneurysm in one patient. Pre-operative ankle brachial index (ABI) was noted in all patients. Internal diameter of prosthesis used ranged from 10-18 mm.

Parameters were carefully noted regarding handling qualities of prosthesis and procedure related complications or mortality. Postoperative status regarding distal pulses and ABI were documented (Table III). Patients were followed up for a maximum period of five years using clinical and imaging documentation of graft patency and integrity.

Long-term follow up till date: Patients recruited in the clinical trials that involved implantation of the test graft at the institute as well as the control group were followed up by clinical examination and duplex ultrasound on yearly basis. CT scan/magnetic resonance angiography (MRA) were performed yearly for first two years and thereafter once in 2-3 years of follow up.

Parameters evaluated were *(i)* Dilatation and elongation - No progressive dilatation > 15 per cent/ that of control grafts (as measured in systole). *(ii)* Structural stability - should be non-biodegradable.

Table III. Clinical details and results of phase II (multicentric) clinical trial

S.No	Age/Sex	Pathology	Procedure	Month & year	Graft size (mm)	Mortality	Complications	Post-op ABI	Patient on follow up	Current long follow up status
1	73/M	AAA	Inclusion repair	August 2005	16	No	Nil	1.1	Yes	Doing well Last reviewed in December 2006
2	32/M	TAAA	Inclusion repair	August 2005	16	No	Respiratory infection	1.0	Yes	Underwent TEVAR for anastomotic psuedoaneurysm in 2012 Last reviewed in February 2013
3	52/F	CoA	Jump graft	August 2005	12	No	Wound infection	0.8	Yes	Doing well Last reviewed in October 2014
4	54/M	TAAA	Inclusion repair	August 2005	16	No	Transient renal dysfunction	0.9	Yes	Doing well Last reviewed in April 2012
5	65/M	AAA	Inclusion repair	December 2005	16	No	Nil	1.0	Yes	Expired in 2008 due to head injury following RTA. No issues till then
6	23/F	CoA	Interposition grafting	February 2006	12	No	Nil	0.9	Yes	Doing well Last reviewed in October 2012
7	65/M	AAA	Inclusion repair	February 2006	16	No	Nil	1.0	Yes	Developed stroke in 2010. Now has mild disability Last reviewed in May 2012
8	20/F	MAS	Bypass grafting	April 2006	10	No	Nil	0.8	Yes	Doing well Last reviewed in June 2010
9	65/F	AAA	Inclusion repair	May 2006	14	No	Nil	1.0	Yes	Doing well Last reviewed in July 2009
10	19/M	CoA	Jump graft	May 2006	14	No	Nil	0.9	Yes	Berry aneurysm clipping in 2006. Otherwise doing well Last reviewed in September 2013
11	60/M	AAA	Inclusion repair	November 2006	16	No	Paralytic ileus	1.0	Yes	Doing well Last reviewed in August 2014
12	55/M	Rt CIA occlusion	Bypass grafting	August 2007	10	No	Nil	0.7	Yes	Doing well Last reviewed in June 2014
13	60/M	AAA	Inclusion repair	October 2007	18	No	Nil	1.0	Yes	Doing well Last reviewed in July 2013
14	72/M	AAA	Inclusion repair	November 2007	18	No	Nil	1.0	Yes	Doing well Last reviewed in November 2008

Contd...

S.No	Age/Sex	Pathology	Procedure	Month & Year	Graft size (mm)	Mortality	Complications	Post-op ABI	Patient on follow up	Current long follow up status
15	53/M	Lt CIA occlusion	Bypass grafting	November 2007	10	No	Wound infection	0.7	Yes	Expired in 2009 from cerebral haemorrhage No issues till then
16	74/M	Rt CIAA	Bypass grafting	December 2007	10	No	Nil	0.8	Yes	Doing well CAD on medical management Last reviewed in June 2014
17	67/M	AAA	Inclusion repair	January 2008	18	No	Nil	1.1	Yes	Doing well Last reviewed in November 2009
18	61/M	AAA	Inclusion repair	January 2008	18	No	Paralytic ileus	1.0	Yes	Doing well Last reviewed in February 2013
19	65/M	Rt CIA occlusion	Bypass grafting	April 2008	10	No	Nil	0.9	Yes	Doing well Last reviewed in October 2014
20	56/M	AAA	Inclusion repair	September 2008	18	No	Transient renal dysfunction	0.9	Yes	Doing well Last reviewed in February 2014
21	50/M	Rt CIA occlusion	Bypass grafting	December 2005	10	No	Nil	0.7	Yes	Reviewed at 3 months Lost to follow up
22	55/M	Lt CIA occlusion	Bypass grafting	January 2006	10	No	Nil	0.8	Yes	Reviwed at 3 months Lost to follow up

AAA, abdominal aortic aneurysm; CoA, coarctation of aorta; MAS, middle aortic syndrome; TAAA, thoraco-abdominal aortic aneurysm; CIA, common iliac artery; CIA, common iliac artery aneurysm; ABI, ankle-brachial pressure index, RTA, road traffic accident; TEVAR, thoracic endovascular aneurysm repair; CAD, coronary artery disease

Table IV. Reporting standards generated by an Ad-hoc Committee of Joint Councils of Society for Vascular Surgery and International Society for Cardiovascular Surgery, North American Chapter[2]

Performance standards in nutshell for vascular prosthesis		
(a)	*At implantation*	
	(i)	Ease and security of pre-clothing
	(ii)	Pliability/comformability
	(iii)	Ease of suturing and suture retention
	(iv)	Bleeding through the graft
	(v)	Fraying at edges
(b)	*Long-term*	
	(i)	Absence of dilationg and elongation
	(ii)	Structural stability
	(iii)	Thrombus free lumen
	(iv)	Absence of pseudoaneurysm formation
	(v)	Resistance from infection
	(vi)	Healing characteristics - perigraft capsule, neointima formation
	(vii)	Status of anastomotic suture line

Source: Ref. 2

(iii) Anastomotic characteristics - Freedom from pseudoaneurysm formation. *(iv)* Infection - <1 per cent in intracavity grafts, <2 per cent for grafts crossing inguinal ligament and <3 per cent for extremity grafts.

Results

In vitro testing: The results of the study did not show any significant irritation or systemic toxicity with physiological saline and cotton seed oil extracts of the material. The percentage of haemolysis induced by the material and extract was under acceptable range. Results of the histopathological evaluation suggested that the material did not produce any histopathological changes. Hence the toxicity study concluded that the material was non toxic, non irritant, non haemolytic and biocompatible.

Pre-clinical animal testing

(i) Immediate - All animals, except one (15th), were extubated at the end of procedure and walked back to their cage 6-8 h thereafter. The 15th animal which underwent interposition graft using cardio-pulmonary bypass to support circulation succumbed to air embolism at the end of an otherwise satisfactory procedure. Another animal died on 2nd post-operative day following hyperpyrexia of 104°F and at autopsy graft was noted to be patent. The third animal developed delayed paraplegia 24 h after operation that involved 45 min of aortic cross-clamping, had to be sacrificed and at autopsy the prosthetic graft was found to be patent and void of any thrombus. Hence the remaining 12 animals were available for explantation to study the healing characteristics.

(ii) Explantation data - Explantation was performed at three and six months after graft implantation under general anaesthesia. Animals weighed 55 and 65 kg, respectively; 1 mg/kg heparin was administered to avoid misinterpretation of pre- and post-mortem thrombus.

Gross examination showed that the grafts were well incorporated in a 1-4 mm thick perigraft capsule. No pseudoaneurysm was noted in either of the groups. Cut surface showed that suture lines were tidy and clean with no evidence of thrombus formation. The body of the prosthesis was glistening white with well organized neo-intima. However, neoimtima inside long grafts appeared irregular and uneven but well organized in contradistinction to smooth glistening thin lining in short replacements (Fig. 3B-D)

Histologically, the inner lining was thin and measured 0.6-1.0 mm, and showed uniform regular neointima of fibro-collagenous tissue, a few islands of calcification along with cellular infiltration of lymphocytes and a few foreign body giant cells (Fig. 4).

Phase I clinical trial: First patient following repair of AAA was discharged from hospital on 10th day. At discharge, her blood pressure was controlled with 50 mg Atenolol (beta-aderenergic blocker) and her ankle brachial index was 1.1. She was maintained on aspirin and iron supplementations as well. CT scan prior to discharge showed patent graft, intact suture lines, smooth luminal outline and patent inferior mesenteric artery. She was evaluated in person five years after surgery. In 2013, a telephonic interview confirmed her wellbeing with no complaints regarding abdomen.

All patients survived major surgical procedure and made satisfactory early recovery except one patient (4th) who underwent surgical repair for complex post-subclavian coarctation of aorta with large heavily calcified post-coarct aneurysm, who developed

paraplegia. Prolonged aortic cross-clamp time of >60 min along with significant bleeding requiring blood transfusions led to spinal cord dysfunction. Minor sequelae in others included prolonged paralytic ileus in two and respiratory infection in one (Table II). All patients were discharged from hospital on or before 10th postoperative day except the patient who developed paraplegia (20 days). All patients underwent Duplex ultrasound and CT scan before discharge from hospital which showed satisfactory repair, patent graft and smooth regular luminal surface of prosthesis.

Complete follow up was available for all 10 patients at three, six months, one year and yearly thereafter. All patients were in good health and active except the 4th patient who did not recover from paraplegia inspite of aggressive physiotherapy. ABI at follow up ranged from 0.85 to 1.1. CT/conventional aortogram was done at discharge and on yearly follow up (Fig. 5). The inner diameter of the graft as measured in CT/aortogram was 0.5-0.7 mm less than the internal diameter of the appropriate graft size. This feature matched the control group of DuPont Dacron grafts used in similar locations.

All patients who received the test implant survived the procedures and recovered well except the patient who developed paraplegia; however, digital substraction angiography (DSA) at one year and CT aortogram at five years showed intact repair with patent graft. Host-graft interaction was reflected at three sites namely, aorta to graft interphase, outside the graft and its inner luminal surface. No untoward sequelae of excessive intimal hyperplasia or pseudoaneurysm formation were noted in and around the proximal and distal suture lines in these patients. The incorporation of the graft in the form of perigraft capsule appeared satisfactory. The luminal side of the grafts were clean and regular with no evidence of thrombus formation inside the test or control grafts.

Phase II clinical trial: Prosthesis was found to be surgeon friendly, with excellent suturing and suture retention qualities. All centres reported good graft handling qualities with pliability, lack of fraying, ease of pre-clotting and suturing and absence of excessive weeping following implantation similar to control Dacron grafts. Patients were electively ventilated for 4 to 24 h with mean of 8 h as dictated by their clinical condition. All 22 patients at 4 centres of study made satisfactory early recovery from appropriate procedures. Two patients (both TAAA) developed transient renal dysfunction (increase in serum creatinine >1 mg/dl higher than pre-operative level) eventually normalising at discharge. Follow up visits were planned at three and six months, one year and yearly thereafter. Apart from regular clinical assessment, duplex scan evaluation was performed in patients with graft implantation in abdomen. Check MR/CT angiogram was performed on follow up showed preserved patency with no evidence of graft-related complications (Fig. 6).

One patient succumbed to stroke 18 months after aorto-femoral graft procedure; and another to road traffic accident leading to irreversible brain damage three years after AAA repair. Post-operative ABI at 1-4 years were 0.9 or more in 15, 0.8 in 4, and 0.7 in 3 (Table III).

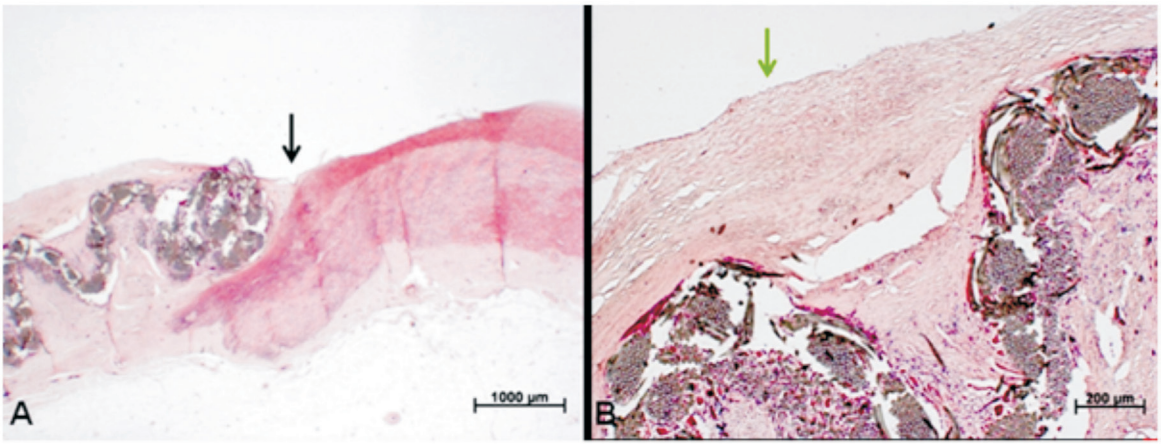

Fig. 4. Low power microphotographs using Hematoxylin & Eosin (H&E) staining showing lack of thrombosis at anastomotic site (black arrow) with tissue ingrowth between the graft **(A)**, and endothelial-lined neointima (green arrow) on the inner aspect of the graft **(B)** 6 months after implantation in porcine model.

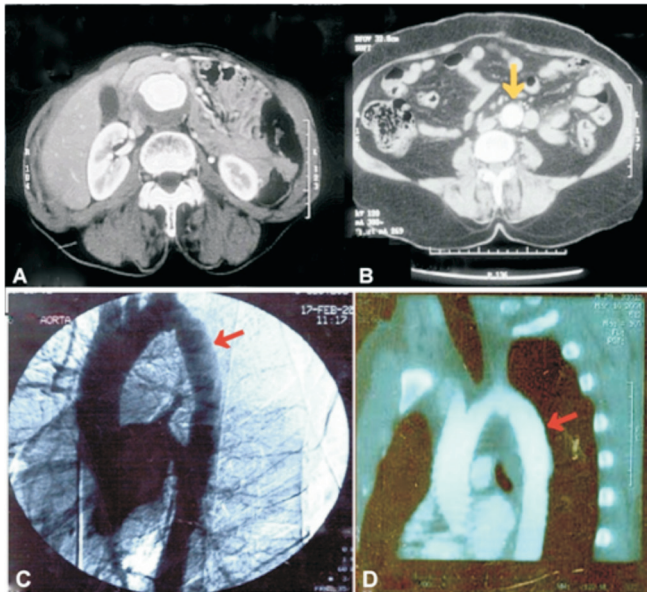

Fig. 5. Contrast-enhanced abdominal CT scan axial section of a symptomatic patient in phase I clinical trial showing an infra-renal abdominal aortic aneurysm **(A)**. Abdominal contrast enhanced CT scan of the same patient taken 8 years after repair showing intact and patent test vascular graft (yellow arrow) **(B)**. Follow-up imaging of an adult patient with coarctation of aorta treated by interposition grafting with test vascular graft as part of phase I trial. Digital subtraction angiogram (DSA) at 1 year **(C)** and CT angiogram at 6 years **(D)** showing preserved and patent graft (red arrows).

Long-term follow up till date: Follow up was complete in eight patients at >10 yr after implantation of test vascular prosthesis in phase I trial and >5 yr in 14 patients in phase II trial. Three patients who were around 70 yr of age in 1998-1999 died of old age and cardiac events beyond five years after the operation. Present evaluation has confirmed excellent clinical status of the survivors and satisfactory graft function with no incidence of pseudoaneurysm, aneurysm, infection, thrombosis, dilatation or any other untoward sequelae with reference to implanted vascular prosthesis (Fig. 7)[9], except one 32 yr old patient with thoraco-abdominal aortic aneurysm due to Takayasu's disease was noted to have pseudoaneurysm at distal aortic anastomosis five years following surgery requiring endovascular aortic stent grafting. Two patients were lost to follow up beyond one year.

Discussion

This study reports indigenously fabricated polyester graft developed and extensively tested *in vitro*, than tested *in vivo* using large animal, prior to clinical study by implantation in patients. Each batch of grafts was put through physical (yarn count, twist and tensile strength), chemical (infra-red spectroscopy and thermal analysis) and toxicity (intracutaneous injection, haemolysin and intramuscular implantation in mice) tests with satisfactory results in accordance with standards laid down by Association for the Advancement of Medical Instrumentation (AAMI) and American National Standards Institute (ANSI)[5].

Several animal models are described in literature for *in vivo* testing of prosthetic grafts. Classic animal experimental data reported by Adam Wesolowski delineated pigs as the most suitable model for *in vivo* experiments[10]. His original work on growing pigs weighing 20-39 kg formed the benchmark for

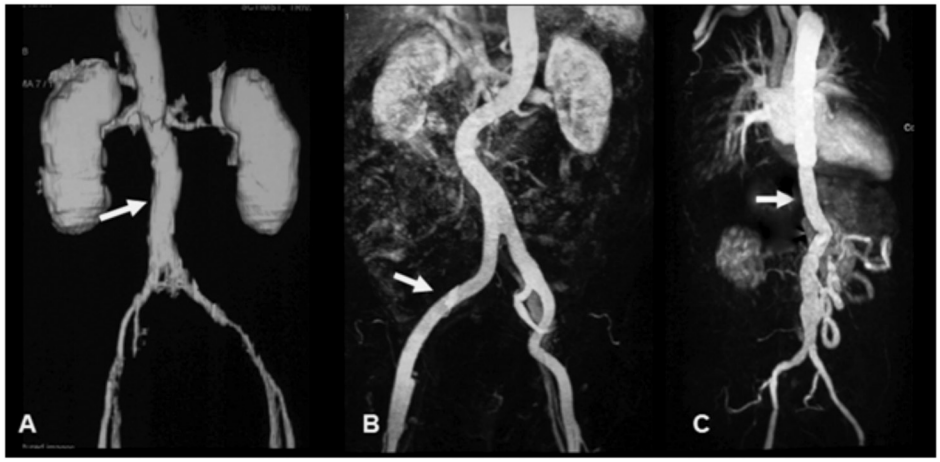

Fig. 6. Follow up CT/MR angiograms of patients in the multicentric trials who underwent vascular reconstruction using test graft (white arrows), showing good results following open repair of abdominal aortic aneurysm at 5 years **(A)**, aorto-femoral bypass for iliac occlusion at 4 years **(B)**, and open repair of thoraco-abdominal aortic aneurysm at 6 years **(C)**.

graft research as implantation data of three months in growing pigs in terms of healing patterns and characteristics were noted to be equivalent to three years in man.

Pore size of prosthesis is essential for ingrowth of fibroblasts and capillary buds from peri-graft tissue to form and stabilize neointima in the graft body. However, larger pore size results in bleeding through its interstices. Hence the optimal pore size is a trade off that minimizes blood loss through interstices while permitting ingrowth of fibroblasts and capillaries to augment neointimal healing[11]. The woven test graft porosity was 200 ± 50 ml/cm^2/min of normal saline at 120 mmHg. No bleeding problem was encountered through the prosthesis after blood pre-clotting when partial heparinisation (1 mg/kg) was employed. For the experiment performed under cardio-pulmonary bypass, plasma pre-clotted and autoclaved test graft appeared impervious to blood during implantation and thereafter.

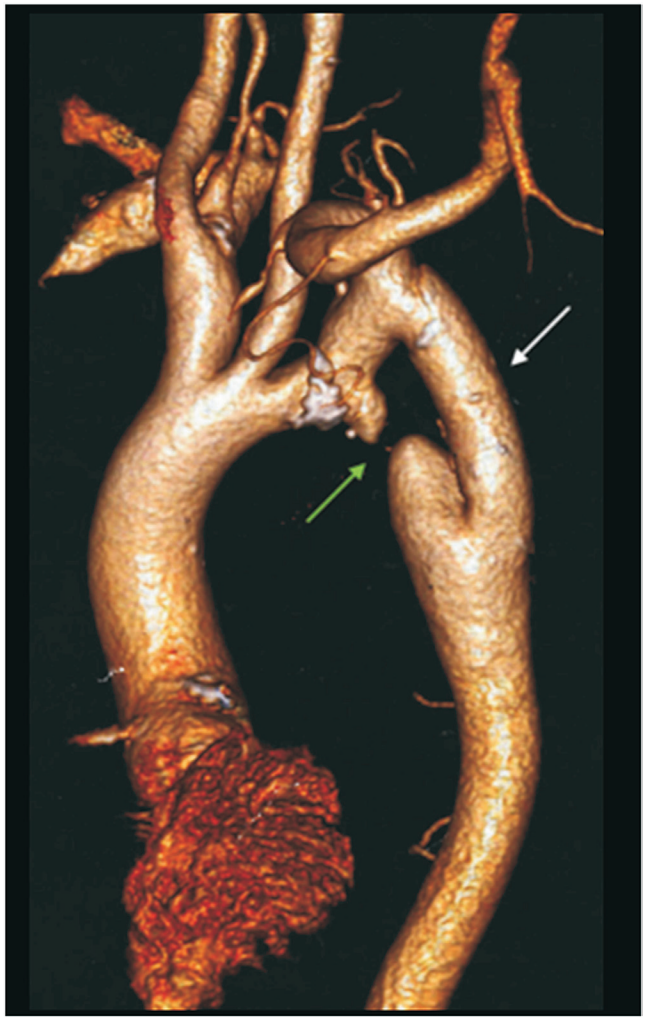

Fig. 7. CT angiogram, volume-rendered image at 14 years following jump graft bypass (white arrow) from left subclavian artery to descending thoracic aorta for coarctation of aorta (green arrow) using 14 mm test graft.
(*Source*: Ref. 9, Reproduced with permission).

Subsequent to general concerns of survival, toxicity, allergy and rejection, *in vivo* animal experiments primarily focused on study of explanted vascular grafts at varying intervals after implantation. Study of explanted graft, in turn, was further characterized by:

(i) Structural integrity of the prosthesis and freedom from dilatation/pseudoaneurysm formation.

(ii) Patency in general, indicating the functional ability of graft in conducting blood for distal perfusion.

(iii) Perigraft capsule formation signifying the incorporation of foreign device into the host tissue.

(iv) Neointima formation, which constitutes the most critical determinant of optimal functioning of prosthetic graft in living body, the quality of which is inversely proportional to thrombogenicity. Major sources of endothelialisation are as follows; across suture lines by direct growth from either ends, by deposition of fibroblasts and wandering endothelial precursor cells (EPCs) in the bloodstream onto the platelet-fibrin layer on the inner side, or by transmural ingrowth of fibroblasts and capillary beds from perigraft tissue entering through the pores of woven/knitted fabric[12].

Structural integrity was well preserved and the anastomotic suture lines appeared covered with a transparent fibrin layer separating the flowing blood in the prosthesis. All grafts were incorporated into the perigraft capsule and developed a compact layer of neointima, with a thickness from 0.1-1.0 mm depending upon the diameter of graft, size of parent vessel, distal run-off and, to a certain extent, the surgical technique. In all short segment grafts, neointima appeared very thin, pink, shiny and intimately adherent as expected as endothelialisation is easily possible from both ends of native aorta. However, in long grafts, neointima was thicker (1-1.2 mm), whitish, slightly irregular but still clean and free from thrombus.

In human studies, permanent implants like vascular prostheses must provide optimal safety for the recipient patient along with efficacy and performance for a long duration with uninterrupted functional integrity (Table IV)[2]. Reporting standards laid down by Ad-hoc

committee of the Joint Council of Society of Vascular Surgery and International Society of Cardiovascular Surgery, North American Chapter, mandates a preliminary assessment at two years and final report at five years before multicentric trials can be resorted to[2]. Safety, efficacy and performance standards of the graft were found to be excellent in this study, even beyond six years and in some patients continuing beyond 14 years. Similarly, patients in multicentric trial were followed up for duration of at least five years. No device-related complications occurred in any of the patients in clinical trials.

All patients had optimal graft performance status on follow up with continued patency and absence of thrombus formation, pseudoaneurysm formation, loss of tensile strength, progressive dilatation or infection. There was only one incidence of anastomotic pseudoaneurysm in the entire series which involved a 32 yr old patient who developed distal anastomotic pseudoaneurysm four years after an intact repair of thoraco-abdominal aortic aneurysm, who also had a previous history of carotid pseudoaneurysm following carotid bypass. The occurance of such anastomotic pseudoaneurysms is not uncommon in Takayasu's disease[13] especially in the setting of long-term steroid usage. This follow up study over a protracted period of 6-14 years has proven safety to patients. The results compared favourably with published data on long-term biostability of polyester vascular prosthesis which reported 95 per cent 10 yr patency rates and structural failure rate of 0.2-3.0 per cent[14-16].

In conclusion, indigenously designed, developed and thereafter comprehensively performed clinical trials have provided robust data regarding safety and efficacy of indigenously developed vascular prosthesis during an extended study period of over a decade. In a total of 32 patients, with diverse clinical conditions and ages thus studied in the clinical trials encompassing both single-centre and multicentric phases, no complications or adverse events occurred. The technique of coating the prosthesis to render it impervious to blood at implantation[17], thus obviating the need for preclotting prior to implantation, was introduced to the Chitra vascular graft as well. Laboratory testing and *in vivo* experiments in 30 pig models were completed and the coated graft will undergo clinical trial to prove its safety and efficacy before making it available on shelf.

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***Conflicts of Interest*:** None.

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