Indian J. Vet. Pathol., ***31***(1): 11-16, 2007

Implant pathology of polyvinylidene fluoride coated polyethylene terephthalate fabric in rabbits

T.V. Anilkumar[1†], R. Shelma[2], Roy Joseph[2], P.V. Mohanan[3] and C.V. Muraleedharan[4]

[1]Experimental Pathology, [2]Polymer Processing Laboratory, [3]Toxicology Division and [4]Division of Artificial Organs
Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology
Trivandrum-695 012, Kerala, India

ABSTRACT

Anilkumar TV, Shelma R, Joseph Roy, Mohanan PV and Muraleedharan CV (2007). Implant pathology of polyvinylidene fluoride coated polyethylene terephthalate fabric in rabbits. Indian J. Vet. Pathol. ***31***(1): 11-16.

Implant pathology of polyvinylidene fluoride (PVDF) coated polyethylene terephthalate fabric (PET) was studied in rabbits. The tissue response to the coated fabric was compared with that of an uncoated commercial biomaterial-grade fabric. The coating was aimed at enhancing the potential of PET for biomedical applications. The histopathologic response to PVDF-coated and uncoated fabric was similar in the rabbit model. Both these materials evoked chronic inflammation, fibrosis, granuloma and giant cell reaction. Quantification of the nature of tissue response was performed as per international norms. When assessed by international standards, the PVDF coated fabric was found biocompatible in view of specific applications of the biomaterial.

Key words: Implant pathology, biomaterials, surface modification, polyvinylidene fluoride, polyethylene terephthalate)

INTRODUCTION

Implant pathology is the study of pathologic effects of implanted materials in animals and man. Materials used for developing biomedical devices, the biomaterials, are often implanted in suitable animal models to study the physiological and therapeutic effects on the host. An assessment of pathogenic potential of the material, in view of its safety as an implant, is desirable before using it as a raw material for fabricating biomedical devices. In any case, knowledge of implant pathology is essential before clinical use of all biomaterials and biomedical devices[7]. In contrast to drugs, which may interact with the metabolic system of the host, biomaterials are relatively inert and do not interact with the native metabolic enzyme systems. However, the size, shape and composition of the implant material may induce lesions in the host. Some lesions are acceptable in view of specific application but a fair knowledge about the implant pathology is desirable for identifying appropriate biomaterials for designing and fabricating biomedical devices[7]. Robust standards are available to assess the biological features of biomaterials[3].

Fabric materials have been used for prosthetic surgery since very long time[1]. Polyethylene terephthalate (PET) is a commercially available fabric suitable for many biomedical applications[1,5,7]. It is biocompatible and satisfies essential hallmarks of a biomaterial. Its ability to retain mechanical strength and fatigue characteristics for a long period of time in the body encouraged biomaterial scientists to use it for many applications such as vascular graft, sewing cuffs of mechanical heart valves, sutures, *etc*. However, it has several drawbacks for specific applications, for example, potential blood clotting property when used as vascular grafts and mechanical heart valves. In an effort to reduce the blood clotting ability and enhance haemocompatibility several modification procedures have been developed recently[2,4,6,8]. We adopted an innovative coating with polyvinylidene fluoride (PVDF) on PET. The technique did not cause any deterioration of material characteristics but substantially improved haemocompatibility as evidenced by 47% reduction in platelet adhesion, 25% reduction in leucocyte adhesion and 40% reduction in partial thromboplastin time. *In vitro* cytotoxicity tests conducted at par with ISO10993-5 standard indicated that the material is non-toxic and biocompatible. This paper is about the implant pathology of the PVDF-coated PET and unmodified PET.

MATERIALS AND METHODS

Materials: PET vascular graft samples are prepared by weaving doubled 76 denier 34 filament texturised yarn. Weaving is carried out using modified conventional loom and the weaving parameters are adjusted to achieve a mean water porosity of 200 ml/min/cm^2. The weaving generates a tubular structure without seams. These tubular fabrics are further crimped to ensure that the grafts does not kink during use. The crimped grafts are cleaned in multiple stage cleaning/extraction process including ultrasonic cleaning, methanol extraction and water extraction to remove all chemicals and oils that might have accumulated on the fibres during the fabrication process. For implantation, strips of size 10mm x 6mm were cut using clean scissors, washed with distilled water thrice and finally with acetone and dried. This is taken as control. Surface

†Corresponding author.

modification of PET was done by coating PVDF solution. Samples were sterilized with ethylene oxide prior to implantation.

Scanning Electron Microscopy: The fabric samples were cut in discs of 4 mm diameter and washed with distilled water five times to remove loose fibres and powders formed during cutting. Samples were then dried at 40°C in a vacuum oven overnight. Dried samples were gold coated in vacuum and surface morphology was observed in Hitachi Scanning Electron Microscope.

Animal experiments: Implantation studies were conducted on albino rabbits weighing not less than 2.0 kg belonging to either sex. Sets of at least three animals were used for every implant (six to eight sites per animal) and time points (1 week, 4 week and 12 week) as per international guidelines prescribed for implantation studies for biocompatibility (ISO10993-6)[3]. Implantation was carried out aseptically in supra-vertebral muscles under general anaesthesia (Xylazin, 5 mg/kg, I/M); Ketamin, 80 mg/kg, IM). The skin of anaesthetised rabbits was lightly swabbed using 70% alcohol after close clipping of fur over the site. An incision was made at the site of implantation to expose the facia and muscle. Implants were inserted deeply into the muscle by blunt dissection about 25 mm apart, along one side of the spine. Multiple samples were implanted at contra-lateral sites. After implantation the incision was closed with sterile sutures.

At the end of the implantation period, animals were euthanized with an overdose of the anaesthetic agent and the muscle samples along with the implanted material were collected. At least 10 samples for each material were recovered for each time point of the study as stipulated in ISO10993-6 standard[3].

All animal experiments were conducted as per the guidelines of the Committee for the Purpose of the Control and Supervision of Experiments on Animals (Government of India). Animals were housed on anodized aluminium cages and fed *ad libitum* on commercial rabbit feed and potable water.

Gross and histopathology: At necropsy, the site of implantation was examined for haemorrhage, necrosis, discolouration and infection by a trained person. The collected samples were fixed in 10% buffered formalin. Routine procedures for processing, embedding, microtomy, and staining (haematoxylin and eosin) were done to produce 5 micron thick paraffin embedded sections. Routine histopathological observations were recorded.

Quantitative histopathology and histomorphometry: Evaluation was done as per the parameters defined in the international standard (ISO 10993)[3]. Extent of tissue response, fibrosis (fibrous capsule) and inflammation were the important histomorphometric parameters. Measurements were made against a graduated stage micrometer. Section orientation, implantation orientation and section geometry were noted as per requirement. Semi-quantitative parameters like severity of degeneration and severity of necrosis were graded depending on nuclear debris and capillary wall breaking (0 = not present, 0.5 = minimally present, 1 = mild degree, 2 = moderate degree and 3 = severe degree). Presence and distribution of inflammatory cells were evaluated; the numbers counted in each case and the severity of inflammation was graded for polymorphonuclear neutrophil, lymphocytes, plasma cells, eosinophils, monocytes and multinucleated giant cells (mean of five fields at 400x; grade 0 = 0 cells; grade 0.5 = 1-5 cells; grade 1 = 6-15 cells; grade 2 = 16-25 cells; grade 3 = >26cells). The presence of material debris, fatty infiltration, granuloma, and tissue in-growth were also graded wherever applicable (0 = not present, 0.5 = minimally present, 1 = mild degree, 2 = moderate degree and 3 = severe degree). Further, a qualitative assessment of the nature of histopathological lesions was also recorded.

Confocal Microscopy and Differential Interference Contrast Microscopy: These special microscopic procedures were performed on routine light microscopic slides with the aid of Laser Scanning Confocal Microscope (Carl Zeiss, LSM 510-META) having objectives for Differential Interference Contrast applications. Excitation was achieved with a blue-diode laser (405 nm) and the emission light was filtered using a long pass filter at 420 nm.

RESULTS AND DISCUSSION

A comparison of the histopathologic response of PVDF-coated-PET and uncoated fabric was done in a rabbit model in accordance with the guidelines of international standards[3]. Additional histomorphologic features were evaluated. The study clarified the nature of tissue response to PVDF coated fabric and the suitability of such modified fabric as a biomaterial.

Fabric is a component of many biomedical devices used in cardiac and vascular surgery. Surface modification of polyester fabrics has been found suitable for many implants[2,7]. PET is a widely used fabric as medical implant. Coating with PVDF was done as a surface modification procedure to improve the quality of the fabric as attempted previously[4,6,8]. The coating did not cause substantial changes in ultra-structural features (Fig. 1). *In vitro* experiments and tests conducted as per international norms on PVDF-coated-PET suggested that it has superior biomaterial qualities compared to native uncoated PET (data not provided here but being communicated separately). Animal experiments were necessary to evaluate the *in*

Table 1: Quantitative and semi-quantitative parameters studied for evaluating histopathology (parameters as per ISO 10993)

C1	C2	E1	Biological response				Number and distribution of cells						Other parameters			
			E2	E3	S1	S2	N	L	P	E	M	GC	MD	F	G	TG
One	1	6x2.5	NA	NA	2	2	1	1	0.5	0.5	2	0	2	0	0	NA
week,	2	1.2x1.2	0	1200	1	1	0.5	0.5	0	0	0.5	0	1	0	0	NA
test	3	0.5x0.5	0	500	1	2	0.5	0.5	0	0	0.5	0	0	0	0	NA
	4	6x3	NA	NA	2	2	0.5	1	0	0	3	0	2	0.5	0	NA
	5	8x2.5	NA	NA	2	1	1	3	0.5	0.5	3	0.5	2	0	2	NA
	6	3x1.5	NA	NA	2	2	1	2	0.5	0.5	3	0.5	2	0	0.5	NA
One	1	3.5x3.5	NA	NA	3	3	0.5	0.5	0	0	2	0	3	0.5	0.5	NA
week,	2	4x3.5	NA	NA	1	2	1	3	0.5	0.5	3	0.5	2	0	0	NA
control	3	1.7x1.4	NA	NA	2	2	3	2	0.5	0.5	2	0	2	1	2	NA
	4	6x3	NA	NA	2	2	1	2	0.5	0.5	3	0	2	0	0	NA
	5	6x3.5	NA	NA	2	2	2	3	0.5	0.5	3	0	2	0	0.5	NA
Four	1	4x2.5	NA	NA	3	3	0.5	3	2	1	3	0.5	3	0	3	NA
week,	2	4.5x2.5	NA	NA	2	2	1	2	1	0.5	3	0	2	2	2	NA
test	3	6x5	NA	NA	2	2	0.5	2	0.5	0.5	2	0	2	0.5	1	NA
	4	6x2.5	NA	NA	2	2	0.5	1	0	0.5	2	0	2	0	0.5	NA
	5	4x2	NA	NA	3	3	1	2	0.5	1	2	0	2	0	0.5	NA
	6	8x2.5	NA	NA	2	2	0.5	1	0.5	0.5	1	0	2	0.5	0.5	NA
	7	4.5x2.5	NA	NA	1	1	0.5	2	0.5	0.5	3	0.5	3	0.5	1	NA
	8	4.5x2.5	NA	NA	1	1	0.5	1	1	0	3	0.5	2	0.5	2	NA
	9	3x1.5	NA	NA	1	1	0.5	1	0.5	0.5	2	0.5	2	0.5	2	NA
Four	1	5x2	NA	NA	2	2	1	2	1	0.5	2	0.5	2	0.5	2	NA
week,	2	NA	NA	NA	2	3	2	3	0.5	1	3	0	2	0.5	2	NA
control	3	NA	NA	NA	3	3	2	3	0.5	0.5	3	0	2	0.5	2	NA
	4	6x2.5	NA	NA	3	3	1	3	0.5	1	3	0.5	2	0.5	0.5	NA
	5	4x1	NA	NA	2	2	0.5	1	0	0.5	1	0	2	0.5	0.5	NA
	6	4x2.5	NA	NA	2	2	1	3	1	1	0.5	0	2	0.5	0	NA
	7	5x3	NA	NA	2	2	0.5	3	1	1	3	0	2	0.5	0.5	NA
	8	7.5x1.5	NA	NA	1	1	0.5	2	0.5	0.5	2	0.5	2	1	2	NA
	9	6x4	NA	NA	1	1	0	2	0.5	0.5	2	0.5	2	0.5	2	NA
	10	5x3	NA	NA	1	1	0.5	2	0.5	0.5	3	0.5	2	0.5	2	NA
	11	6.5x4	NA	NA	1	1	0.5	2	0.5	0	3	0.5	2	0	1	NA
Twelve	1	10x6	NA	NA	1	1	0.5	2	1	0	3	0.5	2	1	2	NA
week,	2	9x4	NA	NA	1	1	0.5	1	0	0.5	2	0.5	2	0.5	2	NA
test	3	8x3	NA	NA	1	1	0.5	2	0.5	0.5	3	0	2	0.5	2	NA
	4	6x3	NA	NA	1	1	0.5	3	0.5	0.5	3	0.5	2	0.5	2	NA
	5	6.5x3.5	NA	NA	1	1	1	3	0.5	0.5	3	0.5	2	0.5	2	NA
	6	4x3	NA	NA	1	1	1	1	1	0.5	3	0	2	0.5	2	NA
	7	4x2	NA	NA	0.5	0.5	1	3	3	0.5	1	0	2	0.5	2	NA
	8	10x3	NA	NA	1	1	1	3	0.5	1	2	0	1	2	1	NA
	9	8x3	NA	NA	1	1	0.5	2	0.5	0.5	2	0	2	0.5	2	NA
Twelve	1	5x2.5	NA	NA	1	1	0.5	3	0.5	0.5	3	0	2	0.5	2	NA
week,	2	6x2.5	NA	NA	1	1	0.5	2	0.5	0	3	0.5	2	0.5	2	NA
control	3	6x2.5	NA	NA	1	1	1	3	0.5	0.5	3	0.5	2	0.5	2	NA
	4	4x2.5	NA	NA	1	1	1	3	2	1	3	0.5	2	0.5	2	NA
	5	6x4	NA	NA	1	1	0.5	3	2	1	3	0.5	2	0.5	2	NA
	6	6x4.5	NA	NA	2	2	0.5	1	1	0.5	1	0.5	2	2	2	NA
	7	4x2.5	NA	NA	1	1	1	1	0.5	0.5	3	0.5	2	2	2	NA

C1: Experimental group
C2: Number of samples recovered on which evaluation was possible as per the international standard. Samples which did not satisfy the criteria were rejected and excluded from the study.
E1: Extent of response (mm, x100)
E2: Extent of fibrosis/fibrous capsule (µm, x400)
E3: Extent of inflammation (µm, x100)
S1: Severity of degeneration (grading, determined by nuclear debris and capillary wall breaking): 0= not present, 0.5= minimally present, 1= mild degree, 2= moderate degree, 3= Severe degree
S2: Severity of necrosis (grading, determined by nuclear debris and capillary wall breaking): 0= not present, 0.5= minimally present, 1= mild degree, 2= moderate degree, 3= Severe degree
Number and distribution of cells (grading based on the number and distribution of cells); 0=0 cells, 0.5=1-5 cells, 1=6-15 cells, 2=16-25 cells, 3= >26 cells, average of five fields at magnification 400x and N – Polymorhpho nuclear neutrophils, L-Lymphocytes, P – Plasma cells, E – Eosinophils, M – Monocytes, GC – Multinucleated giant cell
Other parameters (grading based on skilled observation): 0=not present, 0.5=minimally present, 1=mild degree, 2=moderate degree, 3= Severe degree) for MD –Material debris, F- fatty infiltration, G- granuloma, TG- tissue ingrowth & NA – not applicable

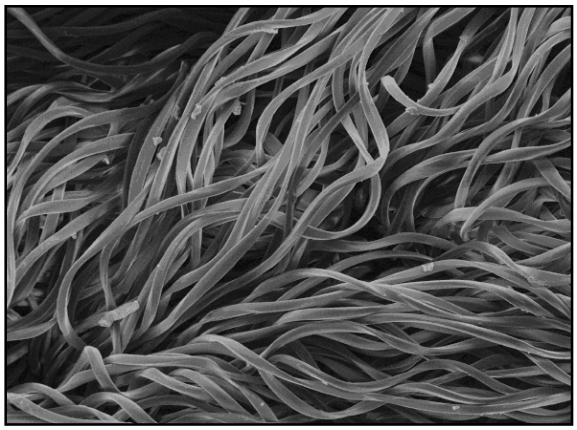

Fig. 1: Scanning electron micrograph of the coated fabric showing the organised arrangement of fibres and fibrils in the fabric. Note the space for cellular in-growth and infiltration of cells around the fibres within the fabric.

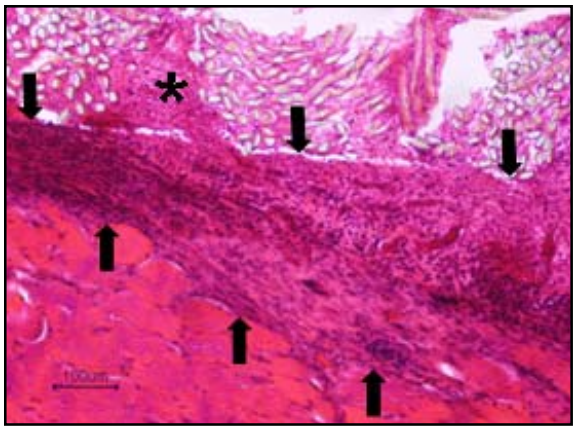

Fig. 2: At light microscopic level, fibrils of the implants appeared as retractile particles. The zone of inflammation around the implant (arrow) and its infiltration between fibres (*) are evident (PVDF-coated-PET, 7 days after implantation in rabbit muscle, haematoxylin and eosin)

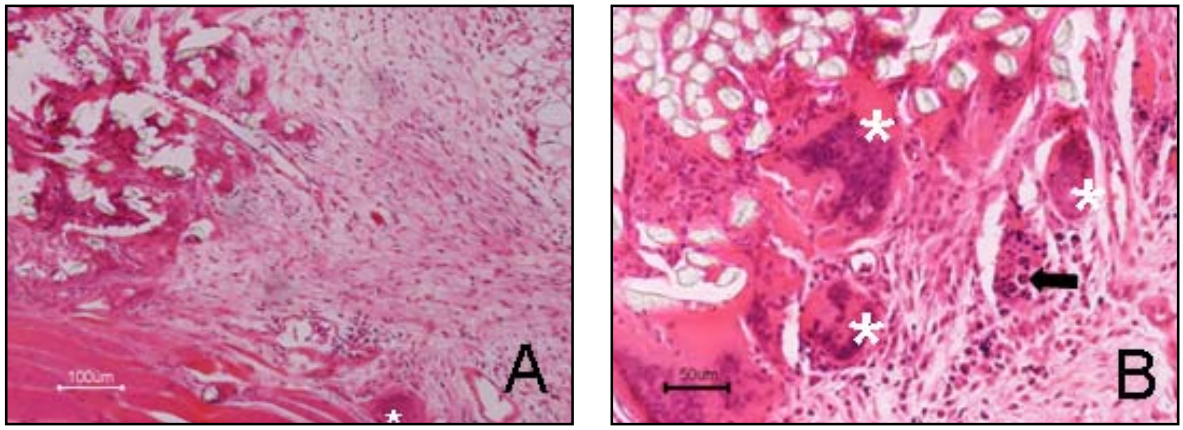

Fig. 3: Fibrosis (A) and giant cell (*) reaction (B) were common after four weeks of implantation (haematoxylin and eosin). Cell death by apoptosis (arrow) was present in many samples.

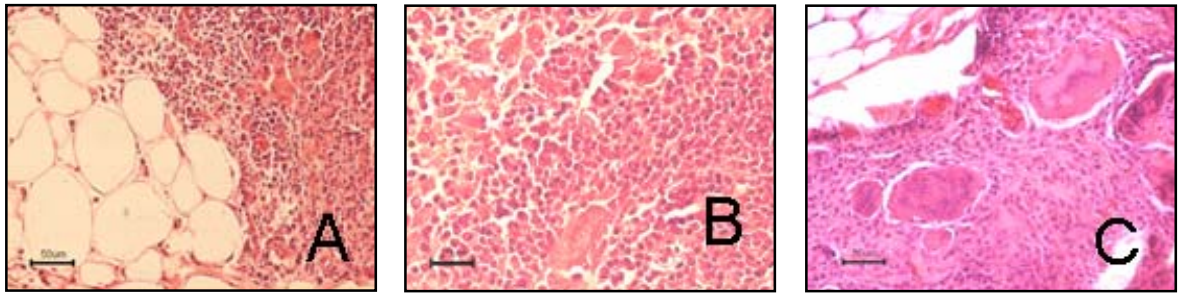

Fig. 4: Granulomatous reaction predominated by plasma cells (A), eosinophils (B) and giant cells (C) were common after 12 weeks of implantation (PVDF coated PET); haematoxylin and eosin staining.

vivo characteristics of the coated fabric.

All the animals survived the implantation and remained healthy during the test period. Grossly, there was no encapsulation, haemorrhage, infection or necrosis around the implantation sites at one, four or twelve weeks of implantation and the biological response was similar in both the test as well as the control materials. Though, varying degrees of degeneration, necrosis, inflammation and foreign body reaction were observed at light microscopic level, the pattern of lesions were similar in both the test and control samples. The margin of the implant site was indistinct in many instances and there was diffuse tissue reaction (Fig. 2). Extensive tissue reaction and fibrosis were present in many samples. Tissue sections made from 7-days implantation period had typical hallmarks of chronic inflammation with necrosis and infiltration of various grades; mild, moderate and severe. Occasional fibrosis and granulomatous reaction were also found at 7-days but fibrosis and/or granulomatous

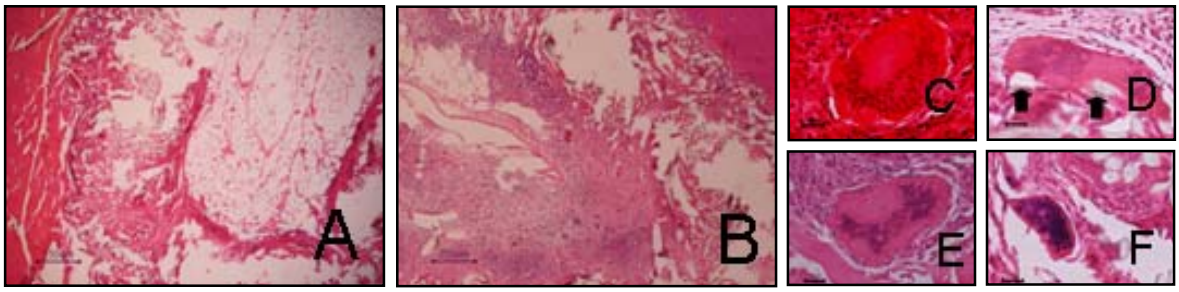

Fig. 5: Severe fatty infiltration (A) and giant cell reaction (B) in 12-week sample of coated-PET: haematoxylin and eosin staining. Giant cell characteristics with varying morphology in fabric-induced tissue reaction are presented in C, D E and F. Note that, within a giant cell, the size, number and distribution of nucleoli varied, but the foamy nature of the cytoplasm was consistently observed. The textile particles/fibrils (arrow) were seen in some giant cells.

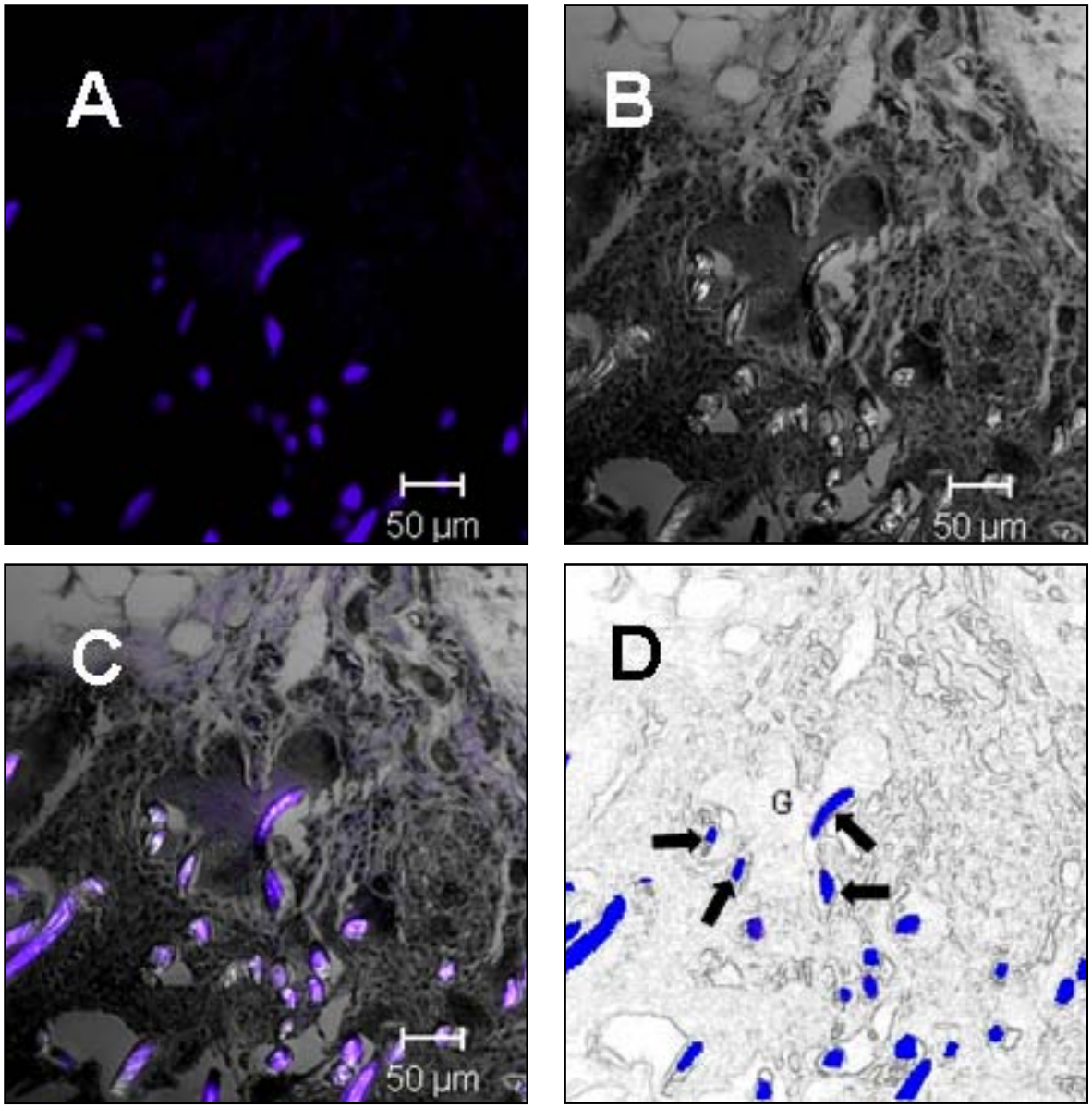

Fig. 6: Confocal image of implanted PVDF-coated-PET, captured at 405 nm blue diode laser with a 420 nm long pass emission filter (A) and a differential interference contrast image (B). Super imposed image (C) of the above confocal and differential interference contrast images clearly indicated the relative location of cells participating in inflammation and the fibrils of the implant. A large giant cell (G) which is probably attempting to engulf fibrils (arrow) can be appreciated on the cartoon format (D).

reaction predominated in all samples at 4-weeks (Fig 3). These reactions persisted after 12 weeks but many of them had progressed to frank granuloma with either plasma cells eosinophils or foreign body giant cells as the principal cell types (Fig. 4). Giant cell reaction and/or fatty infiltration, as expected were the significant observations after 12 weeks (Fig. 5; A and B). The size and morphology of giant cell were highly variable parameters, but most of them had a foamy cytoplasm. The size and number of nuclei also varied considerably (Fig. 5; C, D, E and F). Many giant cells were seems to contain fabric particle but the fabric in histology sections were restricted along the periphery of giant cells. Probably the giant cells are circling around the long fibrils in an attempt to engulf them. The pattern of tissue response was similar to fabric studied in other species and animal models[1,8] but no specific study was available for comparison of coated fabric in the rabbit model.

Quantitative assessment of the tissue reaction was also done[4]. ISO 10993 provided a standard and quantitative basis for the comparison of tissue response to the implanted fabrics. The summary of histomorphological lesions is presented in Table 1. Essentially, the nature and severity of response were similar. The data also indicated that the PVDF-coated-PET is a biocompatible biomaterial similar to the uncoated-PET and therefore useful for fabricating vascular graft. It may be recalled in this context that assessment of biocompatibility is usually performed for specific applications. Therefore the comparison made here suggests that coated PET is as good as uncoated PET used for fabricating clinically acceptable vascular graft.

When studied with Laser Scanning Confocal Microscope, the implanted material appeared to have fluorescence when exited with a blue-diode laser (405 nm). The emission wavelength could be captured with 420 nm long pass filter (Fig. 6). Combined with differential-interference-contrast application, the confocal microscopy facilitated viewing of embossed three-dimensional appearance of the fibrils and giant cells in histology sections.

In conclusion, the tissue response of rabbits to PVDF-coated-PET fabric was similar to PET used for fabrication of vascular graft. Since the PET used as the control is an accepted biomaterial, the PVDF-coated-PET was also deemed to be a biocompatible material. The surface modification considered in this study was aimed at enhancing the biomaterial property and the coating procedure did not affect the biocompatibility, as per the relevant international standard, when examined by rabbit implantation studies. The authors have presented pre-clinical data for assessing the clinical use of coated PET.

ACKNOWLEDGEMENTS

The authors thank the Director, SCTIMST for providing facilities for conducting the study. The research project received financial assistance through a TDF-funding.

REFERENCES

1. Bull B and Brunwald NS (1971). Human histopathologic response to completely fabric-coated heart valve. *Ann. Surg.* **174**: 755-761.
2. Chinn JA, Sauter JA, Phillips RE, Kao WJ, Anderson J M, Hanson SR and Ashton TR (1998). Blood and tissue compatibility of modified polyester: thrombosis, inflammation and healing. *J. Biomed. Mater. Res.* **39**: 130-140.
3. International Standard, ISO 10993-6 1994 (E): Test for local effects after implantation.
4. Margovsky A, Parsson H, Chao A and Lord RSA (2002). A comparative thrombogenicity study of heparin soaked fluoro-passivated polyester and e-PTFE patches in sheep. *Euro. J. Vasc. Endovasc. Surg*. **23**: 39-43.
5. Naylor R, Hayes PD, Payne DA, Allroggen H, Steel S, Thompson MM, London NJM and Bell PRF (2003). Randomised trial of vein versus Dacron patching during carotid enarterectomy: long-term results. *J. Vasc. Surg.* **39:** 985-993.
6. Pedrini L, Dondi M, Magagnoli A, Magnoni F, Pisano E, Giudice ED and Santoro M (2001). Evaluation of thrombogenicity of fluoropassivated polyester patches following carotid endarectomy. *Ann. Vasc. Surg.* **15:** 679-693.
7. Ratner BD, Hoffman AS, Schoen FJ and Lemons JE (2004). *Biomaterials Science: An introduction to materials in Medicine*, 2nd ed. Amsterdam Elseviers Academic Press. Pp. 86-100.
8. Soares BM, Guidoin RG, Marois Y, Martin L, King M W, Laroche G, Zhang Z, Charara J and Giarad J (1996). *In vivo* characterisation of a fluoropassivated gelatine impregnated polymer mesh for hernia repair. *J. Biomed. Mater. Res*. **32:** 293-305.