A Comparative Thrombogenicity Study of Heparin Soaked Fluoro-passivated Polyester and ePTFE Patches in Sheep

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Objectives: to compare heparin soaked fluoro-passivated gelatine sealed polyester and expanded polytetrafluoroethylene (ePTFE) patches in a sheep model of acute platelet accumulation following patch angioplasty.

Materials and methods: heparin soaked patches were placed in the carotid arteries of 9 sheep and autologous 111Indium labeled platelets were infused. The patches were explanted two hours after the injection of labelled platelets. Median specimen radioactivity was calculated as a ratio of radioactivity in explanted and in 4 ml of blood. Explanted patches were also investigated by scanning electron microscopy (SEM).

Results: platelet accumulation was significantly greater on ePTFE patches. For both materials platelet accumulation was greater at the distal end compared to the proximal (p<0.05). SEM demonstrated more platelets as well as thicker thrombus layer on ePTFE patches.

Conclusion: in sheep carotid arteries, a fluoroxygenated gelatine sealed polyester patch appears to result in less platelet accumulation when compared to ePTFE.

Introduction

Carotid patch angioplasty has been performed with a variety of patch materials. The autologous vein has long been considered the optimal choice. However, it is associated with a risk of rupture. Polytetrafluoroethylene (ePTFE) grafts have been recommended in view of their low thrombogenicity and near-zero porosity; but bleeding from suture holes remains a problem. The recently introduced fluoro-passivated polyester gelatine sealed grafts (FPG) also seem to have a low thrombogenicity.

Intraluminal thrombus deposition incorporating radiolabelled platelets detected by external gamma camera imaging, following carotid endarterectomy, has been previously reported. This study aimed to evaluate early thrombogenicity during patch repair and to compare and ePTFE and FPG patch materials in the sheep carotid arteries.

Material and Methods

All surgical procedures were performed at the Graduate School of Biomedical Engineering of the University of New South Wales with approval of local Animal Care and Ethics Committee (ACEC) and complied with the "NH&MRC code of practice".

Nine adult sheep weighing between 50 and 60 kg were induced with intravenous Nembutal (15 mg/kg) followed by endotracheal intubation and maintained on oxygen with 1.5-2.5% Halothane for the duration of the experiment. Following induction of anaesthesia, a midline neck incision was made and 50 ml of blood was withdrawn by direct puncture of the exposed external jugular vein for platelet labelling procedures.

Both common carotid arteries were exposed simultaneously and isolated. The Vagus nerves were preserved to minimise vasoconstriction of the carotid arteries. A standard dose of intravenous sodium heparin was administered (150 IU/kg) 5 min prior to proximal and distal clamping. The carotid arteries were operated upon simultaneously. Following cross clamping, longitudinal arteriotomies were made over a length of 3 cm and luminal surfaces were irrigated with heparinised saline. Patches of ePTFE and FPG were cut to the same tapered shape 30 mm by 6 mm and soaked into heparinised normal saline (10 000 IU/200 ml) for 5 min prior to implantation. The arteriotomies were closed with a continuous 6-0 monofilament nylon suture (Prolene™, Ethicon, U.S.A.) using ePTFE on one side and FPG patch on the other side. Blood flow was restored simultaneously.
Following haemostasis, $^{111}$In labelled platelets were reinjected though the external jugular vein and allowed to circulate for 2 h. Arterial diameter was recorded with callipers proximal to, distal to, and at the mid-point of the patch.

Vessels were then reclamped and the operated segments removed. The arteries were irrigated with cold Hartman's solution to remove remaining blood but not retained adherent thrombus. The vessels were opened longitudinally and photographed. The patch material was detached from resected arterial segment and divided into proximal and distal parts for gamma counting. At the same time, 4 ml of venous blood was removed for comparative analysis and the animal was sacrificed. Samples of artery, grafts and blood were immediately transferred to the gamma well counter for assessment of radioactivity.

Representative sections of artery and patch were washed in normal saline and fixed in 2.5% glutaraldehyde solution for scanning electron microscopy (SEM).

**Platelet preparation**

The platelets were labelled with $^{111}$In-oxine by the modified method of Thakur.*

Briefly, the blood was centrifuged at 200 g for 20 min at room temperature. Platelet-rich plasma (PRP) was separated from the red blood cells with a syringe. PRP was centrifuged at 640 g for 10 min and the platelet-poor plasma (PPP) was removed by pipette and saved for later resuspension of platelets. The platelet pellet was resuspended in calcium free Tyrode's solution. The labelling was done by incubation with approximately 30 mCi $^{111}$In-oxine for 1 min in the presence of a small amount of PPP and labelled platelets were resuspended in PPP for re-injection.

The radioactivity of the supernatant and labelled platelets was determined in a radiosotope well-counter (Cobra II, Hewlett-Packard, U.S.A.), followed by calculation of the "labelling efficiency (LE)" according to the equation: % LE = AP × 100/(AP + AS), where: LE = Labelling Efficiency, AP = Activity in Platelets, AS = Activity in Supernatant.

**Scanning electron microscopy**

After fixation in glutaraldehyde each specimen was dehydrated through graded (70%, 90% and 100%) ethanol and critical point dried in CO$_2$. Specimens were mounted and coated with 100 A of gold-palladium prior to examination (Stereoscan S 360, Cambridge Instruments Co., U.K.).

Representative sections of different segments were photographed at magnifications from 20 to 100 times as a visual demonstration of thrombus formation.

**Statistics**

The Mann-Whitney U-test was used for statistical analysis. A p-value of less than 0.05 was considered significant. Data are presented as median, inter-quartiles and extremes.

**Results**

The mean common carotid artery diameter was $7.2 \pm 0.8$ mm (mean±SD).

A thin coating of red thrombus material was observed on materials at 2 h. The patch material was clearly visible through the thrombus on the majority of FPGP implants (Fig. 1) whereas the e-PTFE patches were entirely covered with red thrombus.

**Radioactivity of platelet deposition**

The labelling efficiency showed low variability ranging from 62% to 71% with a mean of 66 ± 3% (Mean±SD, $n=9$).

Platelet deposition on the e-PTFE patch was significantly higher compared to FPGP (p=0.024) (Fig. 2A). For both materials platelet accumulation was greater at the distal, compared to the proximal, part (p=0.018).

Platelet deposition on the artery surface was similar, regardless of patch type used. Platelet uptake was significantly larger on the FPGP patches as well as on ePTFE patches compared to corresponding segment of the arterial wall (patch/wall uptake ratio). This ratio was higher on ePTFE patches compared to FPGP patches (p=0.040 (Fig. 2B).

**Scanning electron microscopy**

No lesions of arterial endothelial segments opposite to the patch surface were found. SEM images showed a smooth arterial intimal surface with minimal platelet adherence.

SEM examination demonstrated thicker thrombus material deposited at the distal end on all patches.

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Additionally, there was a tendency for ePTFE patches to have slightly more thrombus coverage compared to polyester patches.

Scanning electron microscopy of FPGP revealed typical filament knitted structure of the graft material, however, platelets did not cover the entire surface of patches. Formed focal thrombosis especially on the suture-line of the patches was noticed (Fig. 3A).

Similar appearances were found in ePTFE patches, covered by numerous aggregated platelets together with red blood cells (Fig. 3B). Deposition of platelets was seen on the entire surface rather than on small islands. The platelets were especially noticeable around needle holes and sutures.

All visualised segments showed signs of platelet metamorphosis with enlargement and appearance of pseudopods.

Discussion

In this study, acute thrombus formation was investigated on FPGP and ePTFE patches. Quantification of platelet deposition on luminal surfaces by \(^{131}I\) labelling of autologous platelets showed that fluoropassivated polyester patches had lower levels of platelets deposited over two-hour exposure periods. Previous studies both in the laboratory and in clinical investigations after carotid reconstruction have shown that radioactivity platelets are a reliable marker of localized thrombosis after carotid reconstruction and for examination of thrombogenicity of biomaterials. Both static and dynamic techniques have been used and the present study measured the activity on patches explanted after 2 h of perfusion. This should reflect the maximum platelet uptake since previous dynamic
studies have demonstrated maximal uptake and steady state after 60–120 min of perfusion. 

Fig. 3. (A) Scanning electron microscopy (SEM) of a representative FPGP patch with partial cover of the surface with red blood cells and platelets. (B) SEM of a representative ePTFE patch with almost complete cover of thrombus material. Note the textures in the distal end of the patch.

The ability of prosthetic material to accumulate platelets could be crucial in that early post-operative platelet deposition may be responsible for the formation of thrombosis, which has an impact on post-operative outcome. 

Early thrombus formation might also predispose to late intimal hyperplasia. 

Previous experimental and clinical studies have suggested that ePTFE grafts are less thrombogenic compared to untreated Polyester. 

No significant differences in early platelet attraction between the two materials were found in our previous study comparing standard gelatin coated polyester and ePTFE. The FPGP prosthesis combines the porosity of the gelatin coated polyester with the surface properties of ePTFE. The process known as "fluoromer passivation" enhances the water-repellent and antithrombogenic properties of polyester by coating it with a thin layer of ePTFE like fluorinated polymer before gelatine sealing.

One of the main limitations in using labelled platelets as a marker of thrombogenicity is the limited control of labelling efficiency, which may have impact on the results. In this experiment the labelling efficiency was consistent throughout all procedures. Guidon et al. investigated the healing response of fluoro-passivated and gelatine sealed polyester (GSP) in a canine thoraco-abdominal model, using 111In platelet radionuclide technique. No significant differences in platelet and fibrin uptake between the two types of grafts were found. The histologic and scanning electron microscopic observations of the FPGP-grafts were found to be similar to those of the GSP control graft. Our own previous study reported no significant differences between polyester patch material and ePTFE. 

The current study demonstrates a reduced acute platelet uptake for FPGP patches compared to ePTFE patches. Although the present study demonstrates a reduced platelet deposition in the FPGP specimens, this is not a proof of better clinical results, only a "soft" indicator of such an effect.

Many studies have demonstrated that postoperative thrombosis in animals can be reduced by the use of intra-operative heparin. However, despite a therapeutic dose of intravenous heparin (100 IU/kg) used in our previous study, which prolongs the activated partial thromboplastin time to 4 to 5 times, we found extensive platelet deposition on patch material as well as on the arterial surface. This observation raised the question of the adequacy of this dose. In our preliminary study (unpublished data) we found that usage of 150 IU/kg as an initial bolus followed by 50 IU/kg after 2h, produced more adequate results obtaining similar level of partial thromboplastin time for the entire experiment.

There is considerable clinical and experimental evidence that post-operative thrombus formation and occlusion occur very early after blood flow is resumed through an artery injured. Much of the platelet deposition occurs within minutes. Thus this animal model may be useful for direct assessment of therapies designed to limit platelet deposition following arterial repair. At the same time this may have applications in evaluating thrombogenicity of different prosthetics in other animal and human models.

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References


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