Coating of Polyester Fabrics with Graphite Composition to Produce Thromboresistant Vascular Grafts
Part I: Coating Technique and In vitro Study

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ABSTRACT

The purpose of this study was to develop a homogeneous and stable graphite sealant that seals polyester vascular grafts to improve the thromboresistant of grafts. Water colloid dispersion of graphites were prepared using graphite (1-3), sugar, gelatin and double distillated water. The knitted crimped polyester fabric vascular grafts with 10 and 12 mm inside diameter were coated via electrophoresis method using water colloid dispersion of graphite. Several mechanical cleaning were carried out on grafts and then the grafts were heated for 2 h at 150°C. The number of adhered platelets was determined by lactate dehydrogenase (LDH) activity measurement. Scanning electron microscopy (SEM) was performed on the graphite coated for evaluating the morphology of vascular grafts surface and also studying the morphology of adhered platelet to polyester fabric grafts. The results showed homogeneous coating of graphite on polyester vascular grafts. SEM observations showed that the platelet adhesion on non-coated vascular grafts surface were relatively high in comparison with the graphite coated fabrics. It was also observed that the graphite coating on polyester vascular grafts reduced the number of adherent platelets and prevents platelet activation and spreading on the surface. Reduction of platelet adhesion was attributed to the coated surfaces.

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Key Words:
vascular graft;
polyester;
graphite coating;
electrophoresis;
platelet adhesion.

INTRODUCTION

Polyester vascular grafts continue to be utilized in order to bypass a segment of diseased artery. These grafts are constructed by either knitting or weaving polyester yarns into a tubular form [1].

One major drawback to implanting this type of graft design is significant blood permeation through the graft wall. Initial use of these grafts required the surgeon to seal the graft matrices by infusing the patient's blood into the graft lumen, thereby forming clot within the graft wall

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prior to implantation [2].

Currently, a majority of the grafts are purchased pre-sealed with proteins (i.e., collagen, albumin, gelatin) or other materials [3-5]. Another common problem encountered with the implantation of a vascular prostheses is the failure of the graft due to of thrombosis at the site of graft. Among the causes of arterial graft failures due to arterial thrombosis are biological incompatibility and insufficient flow or velocity through the graft caused by an inadequate inflow and/or outflow and turbulence at the site of the graft. To overcome such baleful influences, it would be desirable to lend thromboresistant properties to the bloodstream graft interface [6]. Carbon has long been known to be a biologically well tolerated material from the practice of decorative tattooing and the accumulation of anthracotic material in the lungs. Experience has shown that turbostratic carbon is one of the most hemocompatible materials available, possibly because of its extreme chemical inertness, minimal platelet activation and favorable conformational changes in adsorbed proteins [7-9].

One of the carbon forms is graphite. Experience has shown that graphite placed in the bloodstream reduced thrombosis [10]. Further studies suggested that the conductivity of graphite dissipate a positive charge and that the bloodstream graphite interface possesses a negative charge. The surface of the intima of blood vessels is negatively charged (1-5mV) with respect to the adventitia. This phenomenon is associated partially with the non-thrombogenic or thromboresistant character of the intima since the formed elements of blood are also negatively charged and hence they are repelled from the surface of the intima [10-13].

On the other hand, the vascular grafts have been coated with inert materials such as carbon by depositing ULTI (ultra-low-temperature isotropic) pyrolytic carbon. The pyrolytic carbon showed an excellent compatibility and is currently most widely used to make artificial heart valve discs [14]. For this reason we have used graphite coating on polyester fabric graft, because it has negative charge as intima.

In this study, the knitted crimped polyester fabric vascular grafts were coated via electrophoresis method using water colloid dispersion of graphite to produce a homogeneous graphite coating of polyester fabrics. The hemocompatibility of the graphite coated polyester fabric vascular grafts was examined in vitro to evaluate their capability of inducing platelet adhesion in comparison with non-coated polyester fabric vascular grafts.

EXPERIMENTAL

Materials

The knitted crimped polyester fabric vascular grafts (PVG) were donated by the Technical University of Saint Petersburg of Russia with 10 and 12 mm id. Collagen coated polyester vascular prosthesis (InterGard, 8 mm id., crimped) as control was purchased from InterVascular (La Ciotat Cedex, France). Graphite powder (1-3 μm in particle size) was obtained from Merck (Darmstadt, Germany) and also gelatin powder (food grade), ethanol and ammonium hydroxide (10%) were supplied by Merck. Sugar was purchased from Pars Sugar (Ahvaz, Iran). Double distilled water (DDW) was prepared at Iran Polymer and Petrochemical Institute (Tehran, Iran).

Water-Colloid Dispersion of Graphite Preparation

The procedure for preparing 1 L of water-colloid dispersion of graphite was as the follows:

3 g of gelatin was completely dissolved in 100 mL of DDW at 60 C. To this solution, 3 g of sugar was added and the mixture was stirred using a magnetic stirrer until the sugar was completely dissolved. This solution was gently poured into a 2 L beaker containing 400 mL of DDW. A total of 90 g of graphite powder and 1.5 g of ammonium hydroxide (10%) was added at three stages while stirring the solution (30 g graphite plus 0.5 g ammonium hydroxide (10%) at each stage). The solution was stirred for 10 h at 50 C until a uniform dispersion of graphite was obtained.

Coating Process

Coating process of PVG with colloidal graphite was carried out at three steps:

1. Degreasing of PVG samples

PVG samples were washed in the soapy water at 20 C, followed by washing in hot (90 C) DDW. The samples were then left in ethanol for 10 h, dried in air and weighed.

2. Application of graphite coating by electrophoresis technique.

A schematic coating of PVG samples by electrophoresis technique is shown in Figure 1. In this process a voltage of 100 V was applied for 3 min. The

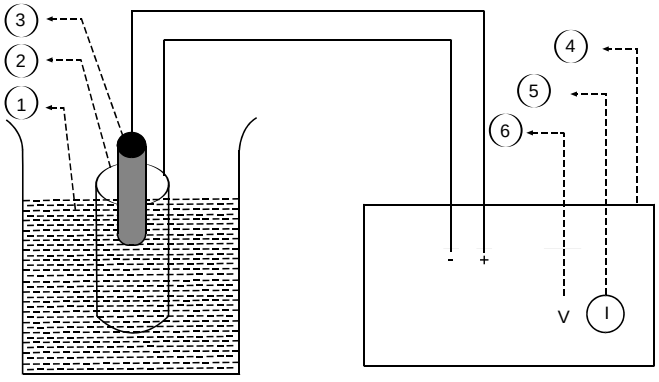


Figure 1. Schematic diagram of the device for applying of graphite coating on PVG samples: (1) graphite colloidal solution (2) auxiliary electrode (3) PVG sample (4) power supply (5) amperemeter (6) voltmeter.

amperage was set for 300 mA. Upon application of the coating, a process of drying-washing-drying was repeatedly carried out on the coated samples. The weight of the sample was recorded after each washing-drying step.

Investigation of Surface Morphology

The surface morphology of coated and non-coated samples was evaluated using scanning electron microscopy (SEM) (Philips model-XL-Cambridge-360S) after sputter coating the samples with gold.

In vitro Experimental

The experimental procedure employed here in the platelet adhesion studies was similar to that of used by Tamada et al. [14]. Human blood collected into a 250 mL blood bag containing 35 mL of CPDA-1 anti-coagulant (100 mL anticoagulant contains 327 mg of citric acid monohydrate, 2630 mg of sodium phosphate dehydrate, 2900 mg of dextrose anhydrate, and 27.5 mg of adenine). The blood was centrifuged to obtain platelet-rich plasma (PRP). The PRP concentration was determined by a Cobas Coulter counter (type 4) and adjusted to 300,000 platelets/mm^3 by adding phosphate-buffered saline (PBS).

The PRP was placed on PVG sheets (1 x 1 cm) and kept for 1 h in an oven at 37 C. Then the sheets were taken out and dip-rinsed twice with PBS, to remove the platelets that were not attached to the sheet surfaces, and treated overnight with 2.5% (v/v) glutaraldehyde at 4 C. The samples were washed with saline and subjected to a drying process by passing them through a series of the graded aqueous alcohol solutions and dried to the critical point. The dried samples, after gold coating, were examined by SEM.

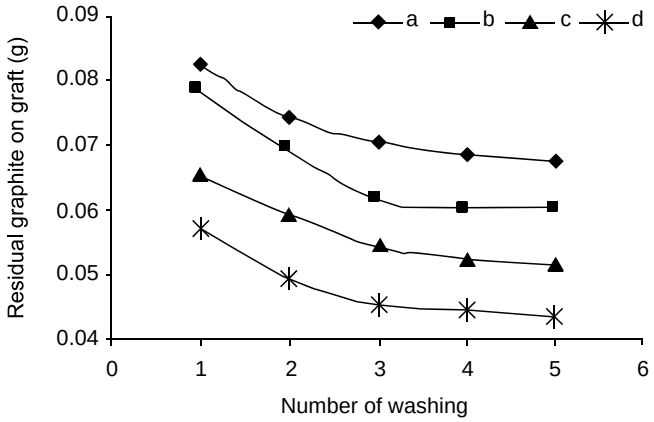


Figure 2. Residual graphite on polyester vascular grafts (PVG) after five steps of washing at 90°C for 1 h (a)PVG with 12 mm (id) and initial weight of 1.068 g (b)PVG with 12 mm (id) and initial weight of 1.349 g (c)PVG with 10 mm (id) and initial weight of 0.778 g (d) PVG with 10 mm id and initial weight of 0.780 g.

The number of adhered platelets was determined by the lactate dehydrogenase (LDH) method [14]. The sheets were put into 2 mL of PBS containing 1% Triton-X100 for 1 h at room temperature to lyse the adhered platelets. The LDH activity of the lysate was measured with an enzymatic method in which the adhered platelets were counted using a calibration curve of platelet counts. The change in ultraviolet absorption at 340 nm was measured using a Pharmacia Biotech spectrophotometer (model Novaspec II, Cambridge, England). The experiment of platelet adhesion was repeated five times using different PRP. Results are the mean value of five determinations ± SE.

Statistical Analyses

Analysis of variance and unpaired Student's t tests were performed using Microcal Origin 3.5.

RESULTS AND DISCUSSION

In Figure 2 variation of graphite content left on PVG, is plotted versus the number of washing time. As it is evident, after three times of washing the curves reach a plateau state, which means that the graphite is fixed on the PVG and it is no more washed away.

In Figure 3, SEM micrographs of coated and non-coated prosthesis can be seen. Figure 3(a) shows formation of graphite coating on PVG. Researchers have shown that graphite coated surfaces have high level of thromboresistance. This is because of physical and chemical nature of carbon [17].

(a) (b)

Figure 3. Scanning electron micrographs of (a) surface of non-coated PVG and (b) surface of graphite coated PVG. Original magnification x 100.

Carbon does not affect albumen of plasma. This is an important matter and necessary for good compatibility of carbon and blood cells [17]. There exists no problem of decomposition in the organism when chemically inert carbon surfaces are used compared to the polymers implanted in organism. The major problem with compounding, painting and dipping methods for graphite coating is non-uniformity of the coating and poor adhesion to the graft surface [17]. This results in graphite to be washed away with blood flow which in turn causes blood clotting on the prosthesis surface. By using electrophoresis technique for coating, these problems can be overcome.

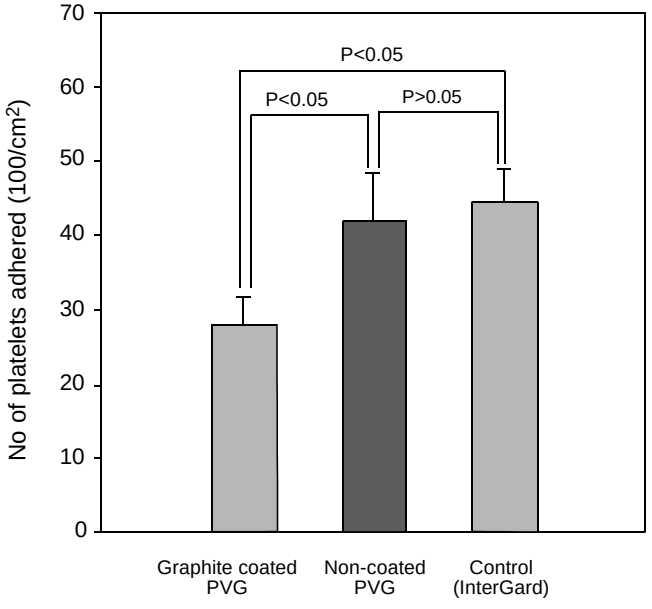
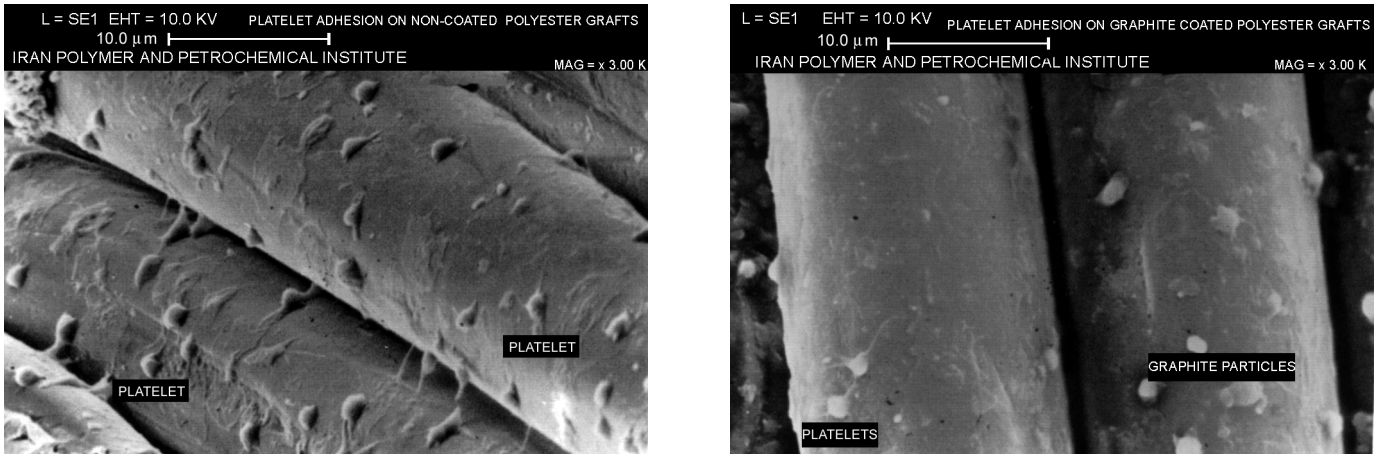


Figure 4. Number of platelets adhered onto graphite coated and non-coated PVG in comparison with InterGard as control. Values are means ± SD for n = 5.

Platelet Adhesion

To evaluate the thrombogenicity of the PVG surface the LDH method has been used [14]. This method provides a quantitative determination of the number of platelets adhering to the surface that are providing procoagulant site [14].

Figure 4 shows the number of platelets adhered to surface of graphite coated PVG and non-coated PVG in comparison with InterGard as control. The data obtained from LDH activity measurements indicated that the platelet adhesion has been reduced as result of graphite coating. The graphite particles may prevent the protein molecules and platelet cells from direct contact with the graft surface owing to negative charge. Because cell-surface interaction is a very complicated phenomenon, it is not clear which property is really dominant for cell adhesion on the surface. Although the platelet attachment studies have been carried out with the LDH method, a comparison of the morphology of platelets attached onto different surface under the same



(a) (b)

Figure 5. Scanning electron micrographs of adhered platelets onto (a) non-coated PVG and (b) graphite coated PVG.

experimental conditions provides information about the potential ability of the surfaces for platelet activation.

Figure 5 shows the typical scanning electron micrographs on the adhered platelets on graphite coated PVG compared with non-coated PVG. As shown in this figure, the platelets adhered to the non-coated PVG were relatively high in number compared to the graphite coated PVG. The platelets on the non-coated PVG extend long pseudopods, leading to their complete spreading, whereas the platelets on graphite coated PVG retain their discoid shapes. Therefore, graphite coating on PVG reduced the platelet adhesion and prevented platelet spreading. These observations are in agreement with the data obtained from the LDH activity measurements.

It is recognized that adhesion and proliferation of different types of cells on polymeric materials depend on surface characteristics such as chemistry, charge, roughness and rigidity [17,18].

Some proteins in serum, like fibronectin and vitronectin, are well known to play an important role in cell attachment onto the substrates [17]. These proteins are absorbed more on positively charged surfaces than on negatively charged ones [19-21]. This is because of negative charge of the blood components, which tend to adhere to positively charged surfaces. Since graphite-coated PVG have negative charge on their surface, therefore platelets have a little adhesion to them in comparison with the non-coated PVG. Therefore, in vitro blood compatibility appears to be better when the surface has a negative charge and also it can be concluded that the chemical and physical properties of the graphite PVG surface, including charge and chemistry affect the platelet adhesion and activation.

Another virtue of the graphite coating are well endothelialization and improving biodegradation resistant of the graphite coated fabric which have extensively been studied using in vivo model and will be published separately.

CONCLUSION

In summary, graphite coating of PVG can be done by electrophoresis method using water colloid dispersion of graphite.

Results showed that it is possible to apply an uniformed stable coating of graphite on polyester prosthesis. The results from in vitro studies showed that platelet adhesion and activation onto the PVG was drastically reduced because of the graphite coating. We conclude that the platelet adhesion and activation on the PVG surface is influenced by the surface charge of the graphite coated fabrics.

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