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# Biological properties of vascular substitutes based on an original collagenic coating

M. Y. JABLONSKI-BERNASCONI, A. PERROT MINNOT, F. ROUAIS, Ch. BAQUEY
*INSERM U.306, Laboratoire de Biophysique, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux Cédex, France*

To enlarge the field of application for polyester vascular prostheses in order to restore small-diameter vessels, original collagenic-based composites have been developed in our laboratory. The polyester knit of standard prostheses has been modified by radiochemical grafting of the Type I collagen-based matrix. In order to check the properties of this new coating, biological tests have been carried out. We noticed that on modified surfaces: fibrinogen adsorption is less important than albumin adsorption; only 50% platelets interact actively with the modified material in comparison with the other materials; the coating is more resistant to enzymatic degradation; and there is partial denaturation of the collagen but this collagen is still specifically recognized by Type I collagen antibody.

## 1. Introduction

In order to enlarge the field of application of vascular prostheses, to restore small-diameter vessels, a vascular substitute based on original collagenic composites has been developed in our laboratory. The polyester knit of standard prostheses has been modified by radiochemical grafting of a Type I collagen-based matrix [1].

To check the properties of this new coating, biological tests have been carried out. Modified prostheses have been compared with unmodified ones or with other commercial protein-coated prostheses. Plasma protein adsorption is the first step of interaction between blood and material surfaces. Two proteins were chosen, fibrinogen and albumin, for our studies, the latter because of its high plasma concentration ($40\ g\ l^{-1}$), the former because, adsorbed fibrinogen promotes platelet adhesion and activation. Such phenomena may, besides other blood material interaction events, induce blood coagulation and enhance thrombogenesis. By calculating the adsorbed albumin/adsorbed fibrinogen (Alb/Fib) ratio, it is possible to evaluate material behaviour towards the coagulation system: the higher the ratio, the lower is the amount of absorbed fibrinogen. Coagulation activation could then be less favoured. Arrangement and conformation of adsorbed proteins on a blood contacting surface determine the interaction of blood cells such as platelets and leucocytes with this surface. Platelet adhesion, aggregation and further secretion of their granular content participate in the coagulation process. Thus the haemocompatibility of a given material is highly determined by its affinity for platelets and its ability to promote their activation [2].

Because a collagenic material, introduced in blood circulation, is exposed to the potential action of various proteolytic enzymes, whether they circulate or are present in the intersticial medium, it is of prior importance to look at its biostability. Enzymes used to test enzymatic degradation of materials are unspecific digestive enzymes and a specific Type I collagenase.

The protein, calf skin collagen (94.5% Type I and 5.5% Type III, free of hexoamines), is susceptible to degradation during the chemical process, therefore antigenic reactivity has been checked in order to evince the possible denaturation of the protein.

## 2. Materials and methods

### 2.1. Plasma protein adsorption

Samples $2 \times 2\ cm^2$ are laid down on the bottom of each of the four wells of culture dishes. A 10% human plasma dilution is prepared with phosphate-buffered saline (PBS), after fibrinogen and albumin concentrations have been previously determined.

Human $^{125}I$ iodinated fibrinogen was provided by Amersham (6 Av. du Canada, BP. 144, 91944 Les Ulis Cedex, France) under the following catalogue number: IM 53P. Human $^{131}I$ iodinated albumin was provided by CIS Bioindustries (Compagnie ORIS Industrie BP 6F Gif sur Yvette) under the catalogue reference Seralb-125. The plasma dilution was added with one of these radiotracers in order to obtain a volumic activity equal to $2\ \mu Ci\ ml^{-1}$; then 2 ml of the resulting radioactive dilutions were introduced into each dish well.

Samples were incubated for 30 min at 20 °C. Then samples were washed with PBS to remove any trace of plasmatic dilution. The remaining sample activity (gross value) was then measured with a $\gamma$ counter. Radioactivity of suitable blank samples made from buffer free of labelled proteins was measured under the same conditions in order to determine the background value which had to be substracted from the measured gross values.



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TABLE I Enzymatic degradation

| Sample nature | Enzyme nature | Buffer nature | Percentage of coating | Code |
|---|---|---|---|---|
| Ternary copolymer | – | Phosphate | 5.7 | B |
| | Trypsin | Phosphate | 5.7 | T |
| | Collagenase | Hanks | 6.3 | C |
| | Pancreatin | Tris | 5.7 | P |
| Commercial prosthesis, Hemashield | – | Phosphate | $57.2 \pm 2$ | |
| | Trypsin | Phosphate | $57.2 \pm 2$ | H |
| | Collagenase | Hanks | $57.2 \pm 2$ | |
| | Pancreatin | Tris | $57.2 \pm 2$ | |
| Commerical prosthesis, Gelseal | – | Phosphate | $67.5 \pm 6$ | |
| | Trypsin | Phosphate | $67.5 \pm 6$ | G |
| | Pancreatin | Tris | $67.5 \pm 6$ | |

### 2.1.1. Amount of adsorbed protein

If $C_0$ is the readjusted protein concentration of the plasma dilution (mg ml$^{-1}$), $A_0$ is the activity of 1 ml of this solution (counts/min), $A$ is the sample activity (counts/min), $S$ is the sample apparent area (cm$^2$), $C$, the protein concentration on the sample, is given by

$$C = \frac{10^3 AC_0}{A_0 S} \mu g\ cm^{-2} \quad (1)$$

### 2.1.2. Platelet adhesion

The number of platelets adhering per surface unit to the material of interest was determined according to a method currently used in our laboratory [2]. Briefly, tubular samples are exposed to a flowing suspension of platelets and red blood cells. This suspension flows through a circuit made of the material tubing and medical quality silicone tubing used as control. Laminar flow conditions are created by a peristaltic pump working according to an aspiration mode. The behaviour of red blood cells on the one hand, and platelets on the other hand, is respectively monitored by suitable radioactive markers, $^{99m}$technetium-labelled red blood cells and $^{111}$indium-labelled platelets, which are prepared in order to behave as their biological counterparts, according to the previously described methods [3].

Detection and analysis by means of a gamma camera of the radioactivity expressed by the remaining cells still present on exposed surfaces after they have been rinsed, allow determination of the amount of blood cells retained by the material and their distribution. If red blood cells are supposed to have no reason to adhere to the material, they can only stay on it after rinsing if they are entrapped within the fabric network, or the macroporosity of the material. Thus, we can assume that these cells are accompanied by a corresponding volume of blood, which obviously contains an assessable amount of platelets. These considerations make possible the determination of the platelet subsets, which are readily adhering to the material among the total sets of retained platelets.

A scanning electron microscopy study is also realized with samples previously exposed to the flowing blood cell suspension.

### 2.1.3. Enzymatic degradation

The *in vitro* degradation rate of different coatings has been evaluated gravimetrically [4]. Previously weighed prosthesis segments (about 0.1 g) were exposed to different enzymes, trypsin, pancreatin, Type I bovine collagenase in solution made up from their specific buffer (Table I). For control purposes, additional segments of material were exposed to the buffer solution alone. After preset periods of exposure ranging from 1–7 days at 37 °C, the samples were removed from the solutions, rinsed with distilled water, and then freeze-dried before being weighed again. The degradation rates were calculated from the percentages of the original weights of the coatings remaining after various exposure times.

### 2.1.4. Amount of remaining coating

Let us assume $W_i$ is the initial weight of the sample before chemical modification (g), $W_M$ is the weight of the sample after chemical modification (g), $C$ is amount of coating (% wt/wt), then $W_i$ is given by

$$W_i = \frac{W_M}{1 + C/10^2} g \quad (2)$$

If $W_C$ is the weight of coating before digestion (g), we can write

$$W_C = (W_M - W_i) g \quad (3)$$

If $W_D$ is the weight of the sample after digestion (g), $W_d$, the weight of the digested coating, is given by

$$W_d = W_M - W_D g \quad (4)$$

Then the percentage of digested coating is

$$D = \frac{W_d}{W_C} 100\ \% \quad (5)$$

and the percentage of the remaining coating is given by

$$R = 100 - D\ \% \quad (6)$$

### 2.1.5. Retaining the antigenic specificity

Samples were incubated for 1 h with a 1% bovine serum albumin at 20 °C to saturate the aspecific sites. Samples were washed three times with PBS. Three series of samples were produced.

Series 1: samples were incubated with 100 µl rabbit antibody solution. The antibody is directed against Type I bovine collagen ($AC_I$), and diluted to 1/500, and made up to 1 ml with PBS. Titration of

$AC_I$: 100 µl of a 1/500 diluted solution, bound 50% 15 000 counts/min $^{125}I$ iodinated bovine Type I collagen ($9 \times 10^5$ Ci mol$^{-1}$). Specificity is 100% for Type I bovine collagen and cross reactions are expressed in percentages for the following proteins: human Type I collagen 1.4%, bovine Type II collagen 0.5%, bovine Type IX collagen 0.5%, bovine fibronectin 0.5%.

Series 2: samples were incubated with 100 µl rabbit antibody solution. The antibody is directed against Type II bovine collagen ($AC_{II}$), and diluted to 1/200, and made up to 1 ml with PBS. Titration of $AC_{II}$: 100 µl of a 1/200 diluted solution bound 50% 15 000 counts/min $^{125}I$ iodinated bovine Type II collagen ($9 \times 10^5$ Ci mol$^{-1}$). Specificity is 100% for Type II bovine collagen and cross reactions are expressed in percentages for the following protein: bovine Type IX collagen 0.1%.

Series 3: samples were incubated for 2 h at 37 °C with 1 ml PBS without any antibody against collagen, in order to quantify the aspecific adsorption of antibody immunoglobulins (AIg).

After three successive washings, a $^{125}I$ iodinated donkey antibody directed towards rabbit immunoglobulin with an activity of 20 µCi µg$^{-1}$ of protein, is introduced into each tube at 0.4 µCi/tube. After a 30 min incubation period at 20 °C, samples were washed three times with PBS and counted with a gamma counter.

The results are given in ng immunoglobulin (AIg) per mg collagen, obtained by the following calculation: if $C_0$ is the concentration of an AIg antibody solution (µg ml$^{-1}$), $A_0$ is the activity of this solution (counts/min·ml), $A$ is the activity of the sample (counts/min), and $S$ its apparent area (cm$^2$), we can find $C$, the concentration of this AIg antibody on the sample

$$C = \frac{10^3 A C_0}{A_0 S} \text{ ng cm}^{-2} \quad (7)$$

Modified samples bore 2.2% (wt/wt) of coating. We made the hypothesis that 50% of this coating corresponds to collagen, i.e. 1.1 g for 100 g unmodified polyester. We determined experimentally that 15.36 cm$^2$ unmodified polyester weighed 0.3150 g

area of 100 g polyester

$$= \frac{15.36 \times 10^2}{0.3150} = 4876.2 \text{ cm}^2 \quad (8)$$

area corresponding to 1 mg coating

$$= \frac{4876.2 \times 10^{-3}}{1.1} = 4.4329 \text{ cm}^2 \quad (9)$$

$C$ concentration of AIg/mg collagen

$$= \frac{4.4329 \times 10^3 A C_0}{A_0 S} \text{ ng mg}^{-1} \quad (10)$$

## 3. Results and discussion

### 3.1. Protein adsorption

Medical grade silicone is used as control. Unmodified polyester adsorbs smaller amounts of albumin than the modified one (6 µg cm$^{-2}$) (Fig. 1). In the case of fibrinogen, the adsorbed amount on unmodified polyester is the same as the amount of albumin 2 µg cm$^{-2}$). An unmodified polyester adsorbs plasma proteins without any specificity. After chemical treatment, fibrinogen affinity is strongly decreased (0.8 µg cm$^{-2}$), Fig. 1. For medical grade silicone and unmodified polyester, the Alb/Fib ratio is about 1. Thus the adsorption of proteins is not specific for these surfaces. For the modified polyester, the Alb/Fib ratio is about 6. This material shows a relatively strong affinity for albumin and a very low affinity for fibrinogen.

### 3.2. Platelet adhesion

Medical grade silicone shows a low affinity for platelets; less than 1306 adhered platelets/mm$^2$ are present on the surface of the material. 90% of the remaining platelets are actually adhering to the silicone surface (Fig. 2). Commercial gelatinized prostheses present a poorly porous structure. The proportion of mechanically retained platelets on such surfaces is about 10%.

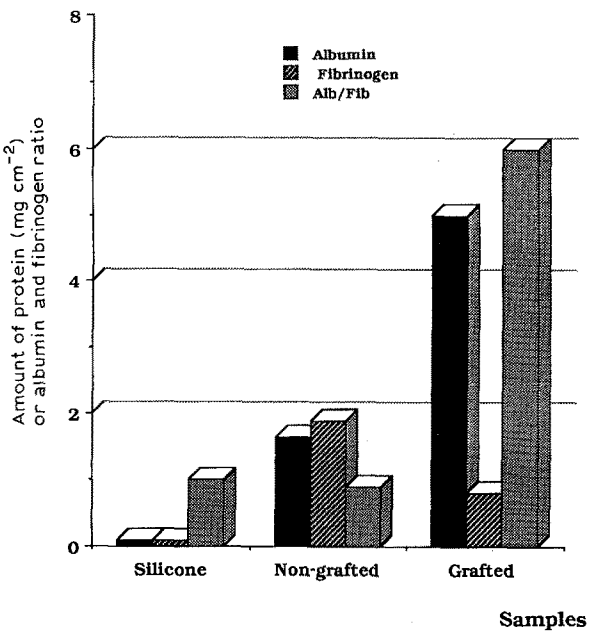


*Figure 1* Amount of albumin and fibrinogen.

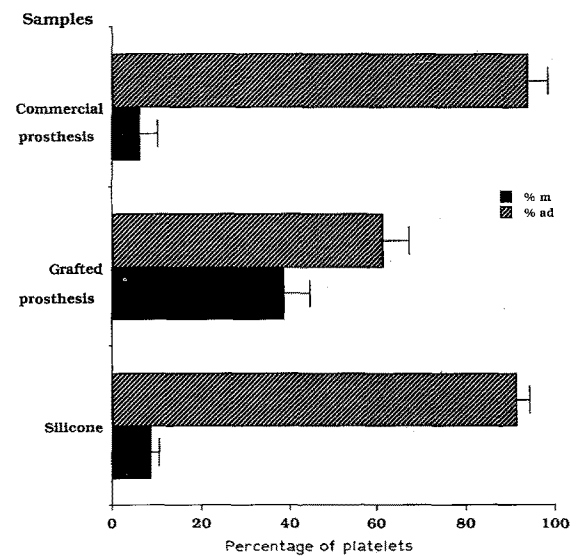


*Figure 2* Platelet adhesion. % m is the mechanically retained platelets, % ad the adhered platelets.

Modified prostheses present a microporous structure (see Fig. 3), and 45% of the remaining platelets are entrapped in related pores which have been revealed by SEM. This mechanical retention was reproducible whichever the sample under test. Only 55% platelets, which are retained on the modified material, are actively interacting with it, compared to 80%–90% for commercial prostheses and 90% for medical grade silicone. By SEM, it is possible to observe blood cell distribution along the material meshes. Most of adher-

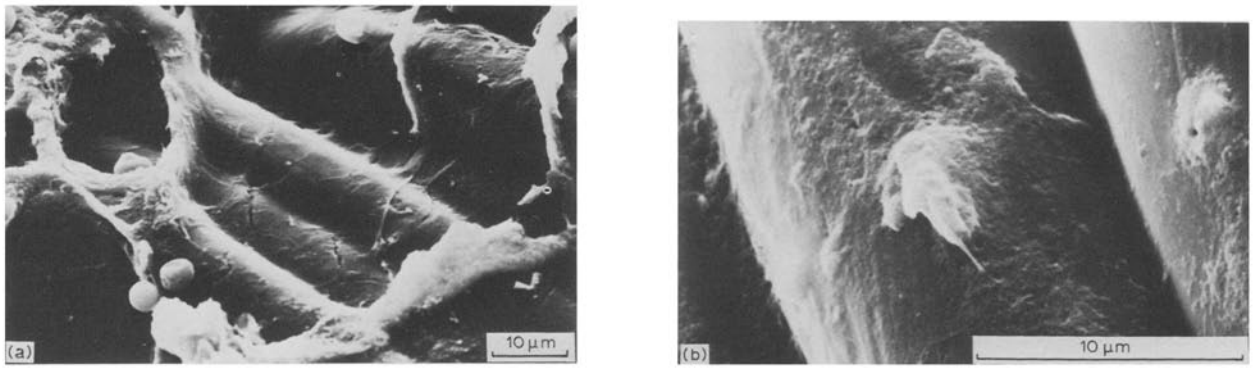


*Figure 3* Scanning electron micrographs. (a) Some red blood cells may be noticed on the collagenic coating. (b) At high magnification, an adhered platelet can be seen on a polyester fibre.

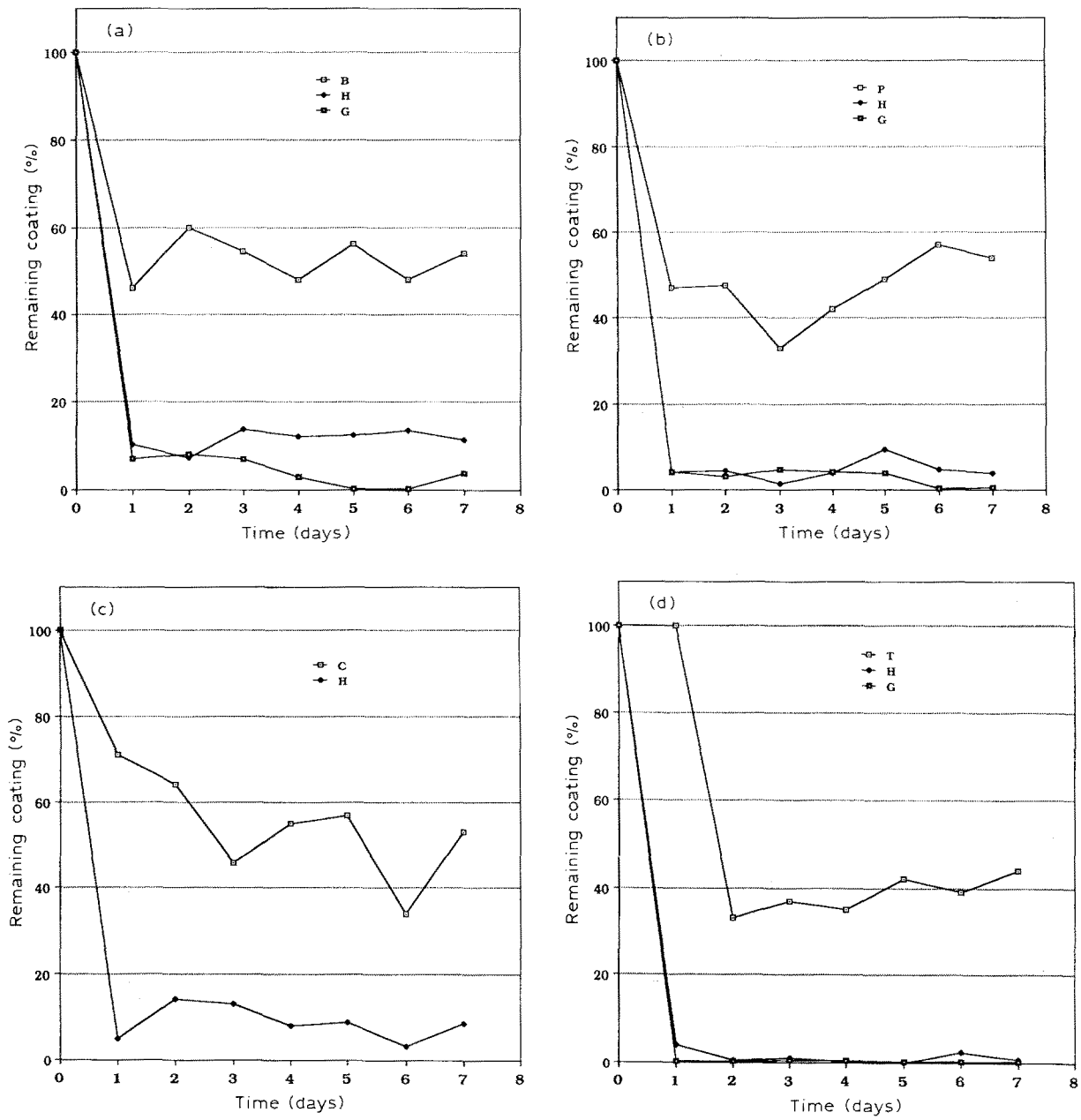


*Figure 4* (a) Degradation in PBS, and enzymatic degradation by (b) pancreatin, (c) collagenase, and (d) trypsin.

ing cells present the usual morphology for adhered platelets. Few of them still retain a round shape (Fig. 3).

### 3.3. Enzymatic degradation

Modified prostheses have been compared to commercial prostheses, either collagen-coated ones (H) or gelatin-coated ones (G) (Table I). After 7 days incubation in a buffered solution free of enzyme, 95% of the coating of commercial prostheses is degraded for G, and 90% for H. Under identical conditions, the amount of coating for modified prostheses is stabilized around 60% of its initial value (Fig. 4a). The action of trypsin induces a larger degradation. At day 7, less than 5% coating remains on commercial prostheses compared to 40% for the modified ones (Fig. 4b). The action of collagenase seems to be aspecific as far as the collagenic coating is concerned. Its action can be compared to that of pancreatin (Fig. 4c and d). Both these enzymes are less effective than trypsin.

### 3.4. Retaining antigenic specificity

Control samples were prepared by adsorption of native solubilized collagen on unmodified polyester. The amount of adsorbed collagen was calculated in order to be equivalent to that of the collagen-modified samples (Table II). This control is morphologically equivalent to the sample. Controls, firstly reacted with the $AC_I$ antibody, are able to bind the second antibody at a significant level (20 ng $ml^{-1}$ collagen), nevertheless such samples demonstrate an aspecific binding of this antibody (8 ng $mg^{-1}$ collagen) when no incubation with the $AC_I$ antibody is performed (Fig. 5). No specific reaction is obtained with the $AC_{II}$ antibody. The amount of adsorbed AIg antibody after a first incubation with $AC_{II}$ antibody is equivalent to the amount adsorbed when the sample is directly incubated with AIg. The amount of bound $AC_I$ antibody for modified samples, is lower than for samples on which collagen had been previously adsorbed (Fig. 5), as can be deduced from the amounts of AIg, respectively retained by the grafted samples, on the one hand, and the adsorbed samples, on the other, after a first incubation in the presence of $AC_I$. This difference can be explained by a partial denaturation of collagen upon treatment. Furthermore, no specific reaction has been detected after use of $AC_{II}$, which was the type of sample considered.

TABLE II Antigenic specificity

| Sample nature | Series |
|---|---|
| Grafted sample | With $AC_I$ |
| | Without $AC_I$ |
| $G = 2.2\%$ | With $AC_{II}$ |
| | Without $AC_{II}$ |
| Non-grafted | With $AC_I$ |
| | Without $AC_I$ |
| polyester | With $AC_{II}$ |
| | Without $AC_{II}$ |
| Non-grafted | With $AC_I$ |
| polyester adsorbed | Without $AC_I$ |
| with collagen | With $AC_{II}$ |
| | Without $AC_{II}$ |

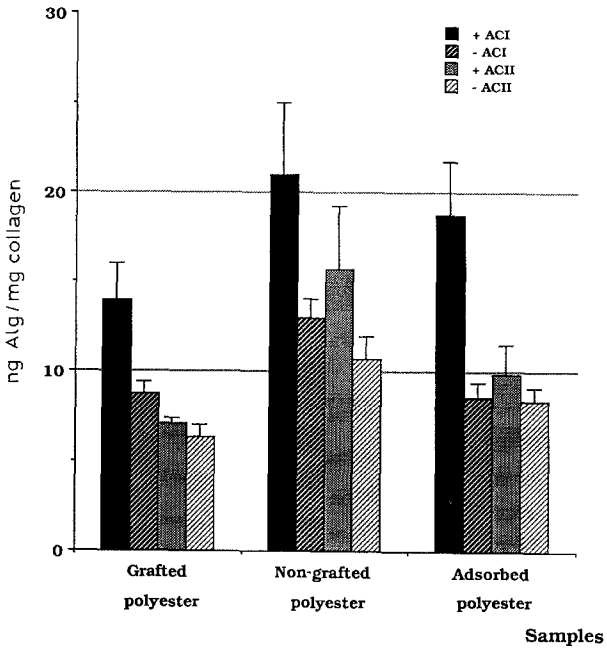


*Figure 5* Antigenic specificity.

## 4. Conclusion

Modification of polyester prostheses by covalent binding of bovine collagen improves polyester behaviour towards some tests of haemocompatibility. We compared these modified prostheses with other commercial protein-coated ones. It is possible to observe that the modified prostheses present equivalent or better properties than the commercial one.

We noticed that on modified surfaces: fibrinogen adsorption is less important than albumin adsorption; only 50% platelets adhering to the modified material interact actively with it, while this figure may reach a level of 90% for other materials; the coating is more resistant to enzymatic degradation; and there is a partial denaturation of the collagen but this collagen is still specifically recognized by Type I collagen antibody.

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