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# Characterization of coatings for open-heart surgery tubing with heparin and lipid

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The aim of this study was to compare qualitative and quantitative methods for coating characterization on internal surfaces of polyvinyl chloride (PVC) tubing used in procedures of extracorporeal circulation. The methods of characterization included optical microscopy (OM) after dyeing with toluidine blue, scanning electron microscopy (SEM), atomic force microscopy (AFM) and energy dispersive spectrometry (EDS). OM after sample dyeing was excellent in allowing early detection of any absence or irregularities of coatings among the used methods. SEM was the most effective in observing the structure and thickness either in heparin coatings or in lipid coatings. AFM provided a good evaluation of the surface topography. A conjunction of all methods is recommended for complete characterization. The quantification methods based on colorimetric tests were efficient in determining the concentrations of heparin and lipid on internal surface tubes, the coating stability and the reproducibility of the results. 

## 1. Introduction

During extracorporeal circulation in open-heart surgery, the blood comes into contact with the inner surface of tubes, the area of which can sometimes be larger than 0.8 $m^2$. Several types of surface coatings with biological active substances for medical devices have been developed to reduce the trombogenicity and platelet adhesion on the surface material used in medical applications [1–8].

The aim of this study was to compare qualitative and quantitative methods of coatings characterization on coated internal surfaces of polyvinyl chloride (PVC) tubing used in the procedures of extracorporeal circulation. The PVC tubing can be used in haemodialysis, haemofiltration, autotransfusion, apheresis, cannulas and catheters. The comparison was performed between coatings with solutions of fractionated heparin-benzalkonium chloride and lipid solutions, mainly the lipid dipalmitoyl phosphatidyl choline (DPPC). Heparin coatings are intended to reduce the trombogenicity through the capacity of heparin to inhibit the trombin [9, 10], and lipid coatings are intended to reduce the trombogenicity by mimicking the lipid surface of red cells [11]. The coatings capacity to improve the biocompatibility depends on the distribution, thickness, topography, heparin, concentration, lipid concentration and stability.

## 2. Materials and methods

The methods of characterization include optical microscopy (OM), scanning electron microscopy (SEM), atomic force microscopy (AFM) and energy dispersive spectrometry (EDS).

The PVC tubing was coated with ionic binding using fractionated heparin and 0.16%–16% benzalkonium chloride (BC). Lipid coating was performed by hydrophobic interaction using a DPPC dispersion ($1.336 \times 10^{-5}$ M) suspended in 10 mM Hepes solution.

Optical microscopy was carried out after dyeing on a Reichert-Jung microscope with an Olympus camera. Heparin-coated surfaces were dyed with a solution of toluidine blue, hydrochloric acid (0.1 N) and sodium chloride (0.2%). The lipid DPPC coating could not be dyed alone; however, the association of polyethylene glycol (PEG) with dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE) lipid allowed dyeing with Comassie blue solution [12].

SEM was carried out on a Jeol JXA-840A electron microscope after gold sputter coating of the samples.

AFM was performed in a Nanoscope III microscope using a force of $10^{-9}$ N. Clean samples, free of dusty deposition, were analyzed using no contact mode. The samples were not submitted to any previous preparation.

Energy dispersive X-ray spectrometry provided a comparative analysis among elements present in both

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tubing surface and the coating. Chloride was the element selected because it exhibited the biggest difference in concentration between tubing surface (PVC) and coating layer (BC).

Heparin concentration on the PVC surface was quantified by a colorimetric method using the metacromatic properties of toluidine blue [13] with measurements of absorbance at 631 nm of the solutions before and after coating. The lipid concentration was quantified by determination of phosphate groups after oxidation of phospholipid with sulfuric acid [14].

The coating thickness was measured on the micrographs, obtained by SEM, of the transverse section of tubes fractured dipped in liquid nitrogen to avoid deformation on coated layer.

Heparin-coating stability was determined by a dynamic test, which consisted of saline solution (0.9%) pumped by a peristaltic pump inside tubing at $4\,l\,min^{-1}$ during 4 h. The removed heparin was quantified by the toluidine blue method.

## 3. Results

Figs 1 and 2 demonstrate some problems detected by optical microscopy in heparin and lipid coatings. The lack of heparin coating can be visualized after dyeing using toluidine blue (Fig. 1). Rose-shaped aggregates are present in the DMPE-PEG coating on the internal tubing surface (Fig. 2).

The micrographs in Fig. 3 show significant differences among the structure of the PVC tubing surface using variable concentrations of benzalkonium chloride. The coating obtained using a 0.16% BC concentration shows areas of absence on the tubing surface (Fig. 3b). A more homogeneous coating was obtained with a 1.6% BC (Fig. 3c); large aggregates could be observed on the surface when the BC concentration was increased to 16% (Fig. 3d).

SEM analysis detected DPPC lipid aggregation (Fig. 4) and an interface between coated and non-coated regions on DPPC lipid coatings (Fig. 5). The mean thickness of the heparin coating was $1.6 \pm 0.3$ μm, as determined by six different points chosen at random on the transverse tubing surface (Fig. 6). Fig. 7 illustrates the topography of a coated surface with heparin on PVC tubing obtained by AFM.

*Figure 1* Optical microscopy of heparin coating dyed with toluidine blue solution ( × 87).

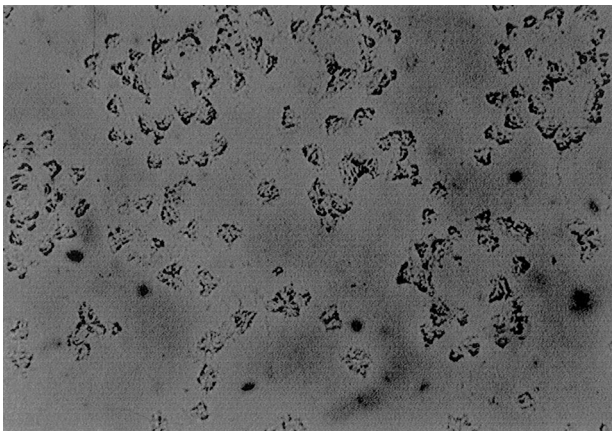

*Figure 2* Optical microscopy of DMPE-PEG lipid coating dyed with Comassie blue ( × 840).

The results of EDS microanalysis at 2.136 eV showed a difference of 55% between the chlorine amount in heparin-coated surfaces and in non-coated surfaces. The EDS did not detect differences in lipid coatings. The mean concentration of DPPC lipid on PVC tubing surface was $7.15 \times 10^{-7} \pm 8.4 \times 10^{-8}\,mol\,m^{-2}$ (Table I). The mean concentration of hepatin on PVC tubing surface was $630 \pm 16\,mg\,m^{-2}$ (Table II). The results of the stability test showed that $19\% \pm 2.2\%$ of fractionated heparin was removed after 4 h.

## 4. Discussion

Optical microscopy permits an excellent preliminary analysis of the tubing coated. The simplicity and effectiveness of this method could be useful for quality control and process validation on an industrial scale. Any lack of or irregularities in the coatings could be detected using cromatogenic compounds as observed in Figs 1 and 2. Fig. 3 shows the strong influence of benzalkonium chloride concentration on the surface structure and indicated the best range of benzalkonium chloride contents in heparin coatings. SEM analysis showed more heterogeneous surfaces were obtained with lipid coatings compared to heparin coatings (Figs 4 and 5).

The nanometric analysis performed by AFM shows that the heparin coating does not rectify the surface: the layer formed presents the same topography as a non-coated tubing.

The quantification methods were efficient in determining the concentrations of heparin and lipid on internal surface tubing, the coating stability and in verifying the reproducibility of the coating process. The standard deviation of the results indicates that the heparin coating suffers less variations than the lipid

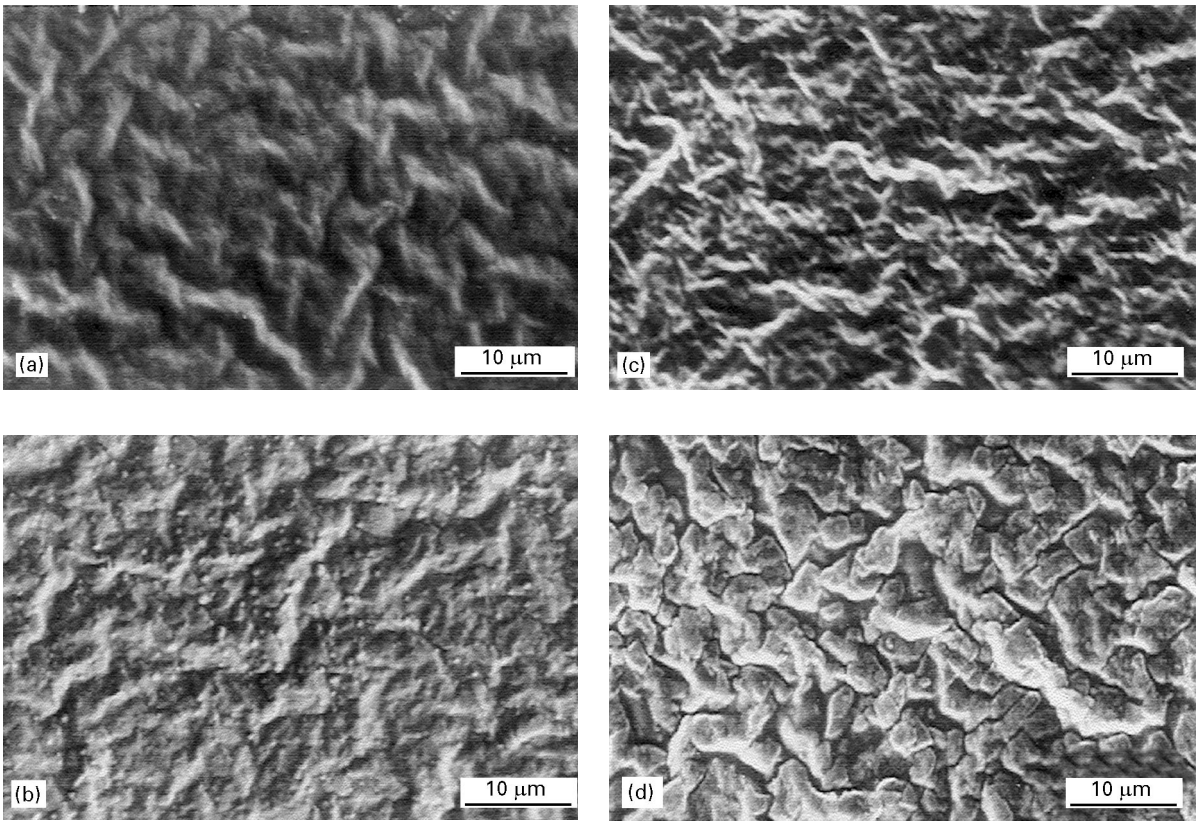


*Figure 3* Scanning electron micrographs of (a) non-coated tubing surface, (b) coated tubing surface with 0.16% BC, (c) coated tubing surface with 1.6% BC, and (d) coated tubing surface with 16% BC.

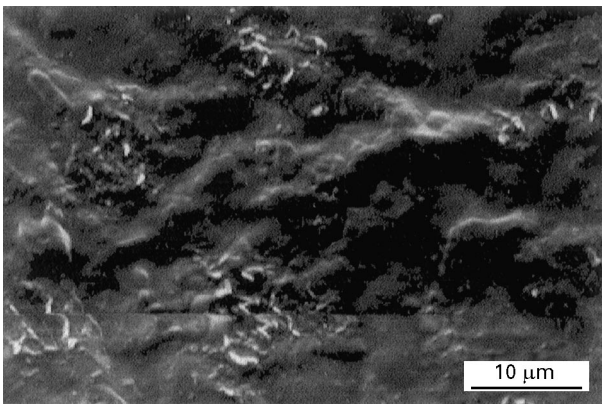


*Figure 4* Scanning electron micrograph of DPPC lipid-coated surface.

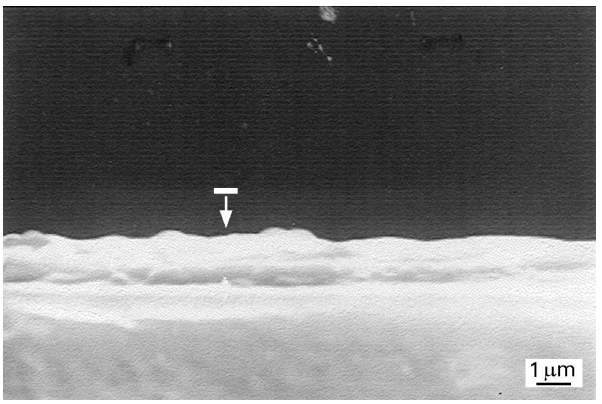


*Figure 6* Scanning electron micrograph of a transverse section of heparin-coated tubing.

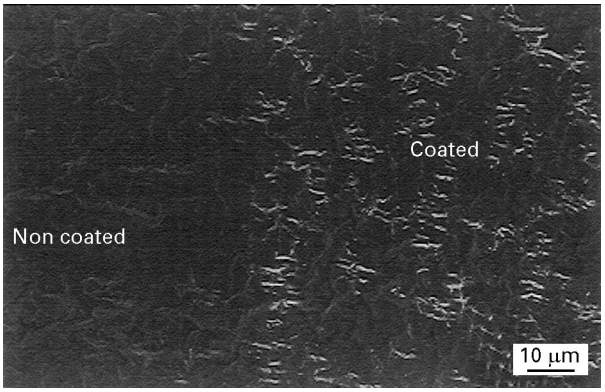


*Figure 5* Scanning electron micrograph of a heterogeneous surface in DPPC lipid coating.

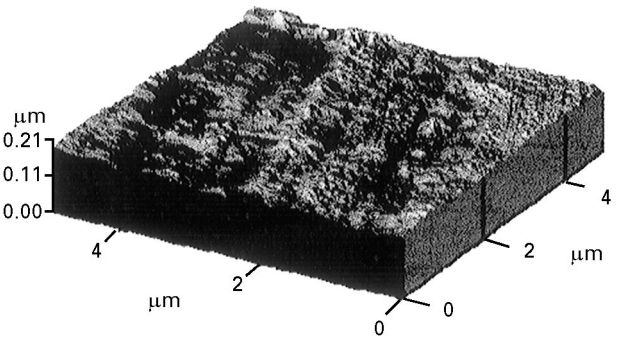


*Figure 7* Atomic force micrograph of a surface coated with heparin.

TABLE I DPPC lipid adsorbed on PVC tubing. Initial solution concentration = $1.336 \times 10^{-5}$ mol, coated area = 0.0004 $m^2$.

| | Sample | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| DPPC on coated surface (mol) | $3.06 \times 10^{-6}$ | $2.96 \times 10^{-6}$ | $2.46 \times 10^{-6}$ | $3.26 \times 10^{-6}$ | $2.56 \times 10^{-6}$ |
| Concentration ($mol\,m^{-2}$) | $7.65 \times 10^{-7}$ | $7.40 \times 10^{-7}$ | $6.15 \times 10^{-7}$ | $8.15 \times 10^{-7}$ | $6.40 \times 10^{-7}$ |

TABLE II Fractionated heparin on coating. Initial solution concentration = 5 $gl^{-1}$, BC concentration = 1.6%, coated area = 0.0004 $m^2$.

| | Sample | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Heparin on coating surface (mg) | 0.252 | 0.262 | 0.242 | 0.255 | 0.250 |
| Heparin concentration ($mg\,m^{-2}$) | 630 | 655 | 605 | 637 | 624 |

coating, probably due to the stability of the initial solutions. The heparin removal (less than 20% after 4 h) determined by the stability test is a reasonable result for heparin coatings to extracorporeal circulation applications, considering that the duration of the procedure is usually less than 2 h and that the heparin liberation could be beneficial to maintain controlled blood anticoagulation.

SEM was the most effective and precise method used to observe the distribution and thickness, either in heparin coatings or in lipid coatings however, the utilization of other methods is important to complete and to confirm the results.

Alterations in coating process parameters can cause significant differences on the surface and consequently give different biocompatibility results. The main parameters which affect the coating structure are drying temperature and drying time, contact time of material with the coating solution, chemical products origin, storage conditions of solution and product, the reservoir materials used, solution preparation methods, cleaning before and after coating, pre-treatments, etc. These characterization methods are important tools to compare different coatings and to confirm the reproducibility of defined processes.

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