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Detection of micro- and nano-sized biocompatible particles in the blood

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The research deals with new scanning electron microscopic evaluations of the interface between blood and explanted temporary vena cava filters from patients affected by blood disorders. The biological tissues adherent to the filter and the small thrombi formed *in vivo* were detached from the metallic structure of the device, fixed, dehydrated and prepared for the histological and the electron microscopy. The analyses showed that both samples (thrombus and newly formed tissue) contained foreign, in some cases nano-sized, bodies. The chemistry of these particles was different and varied, and unusual compounds containing non-biocompatible elements like bismuth, lead, wolfram, tungsten were also detected.

The interaction between these debris travelling in the blood stream and the blood itself leads to suspect that the formation of the thrombus can originate from these inorganic and inert foreign bodies that act as triggering agent of the blood coagulation.

Introduction

The interaction of the blood with biomaterials represents a still unsolved problem, since the blood is circulating in a closed system and every measure device, causing the opening of the circuit, influences the meaningfulness of the blood parameters to be measured. The European standard (EN 30993-4 [1]) suggesting some simple tests to verify the haemocompatibility of a material employs heparinised blood to avoid the coagulation, but this addition changes dramatically the boundary conditions, thus upsetting the results.

Vena cava filters are devices that prevent pulmonary embolism by entrapping and breaking up the floating thrombi, thus avoiding their reaching the lung arterial circulation. The observation of their surface after implantation can allow the direct study of the thrombi captured in humans and the punctual interaction between blood and device.

A new device with its until now unique feature of being removable even after a long time, gave us an excellent opportunity to verify the interaction of blood and blood components with the metallic structure of the filter. Explanted vena cava filters from patients affected by blood disorders who needed the device temporarily to prevent pulmonary thrombo-embolism were sent to the Laboratory of Biomaterials from a number of Italian hospitals and analysed in order to determine the blood–device interaction.

Materials and methods

The analysed vena cava filters (ALN-Implants Chirurgicaux, France) are entirely made of AISI 316L stainless steel. Its shape is that of an umbrella skeleton, 5-cm long, and is composed of nine 0.3-mm diameter arms, the shorter six with a distal fixation hook and the longer three without any fixation device. The longest arm has a small ring at its distal tip that serves to facilitate the femoral-approach implantation. The arms are crimped together at the vertex of the structure inside a small ogive. Fig. 1 shows an X-ray photograph of the filter at the implantation time.

Fifteen vena cava filters were explanted from humans who received the device as a prophylaxis before (mainly orthopaedic) surgery. All patients were judged by the clinicians to be at risk of pulmonary embolism. The filters remained *in situ* for periods ranging from 18 to 210 days, as shown in Table I.

At the explantation time they were immersed in 4% formaldehyde in phosphate buffer and sent to the Laboratory of Biomaterials for evaluation. After a gross observation of the metal structure under stereo-microscope (Nikon, Japan), samplings of the biological materials adherent to the filter (tissue or thrombus) were taken and embedded in paraffin. Sections were cut with a microtome (Leitz, Germany). Some of them were haematoxylin–eosin stained for the histo-pathological observations, while others were mounted on 4-mm

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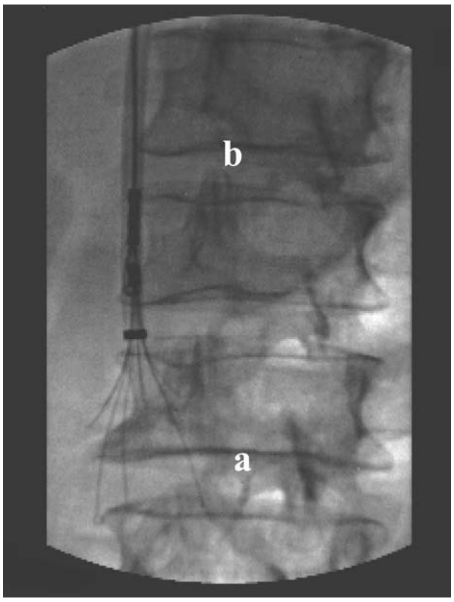

Figure 1 X-ray image of the implantation of an ALN filter. Note (a) the filter and (b) the catheter that released it in the vena cava.

diameter aluminium stubs for the scanning electron microscopic evaluations (ESEM-Quanta 200, FEI Company, The Netherlands). The instrument was equipped with an X-ray microprobe for the elemental analyses by means of an energy dispersive spectrometer (EDS by EDAX).

The morphology of the tissues was investigated, but the presence of foreign bodies was particularly looked for, since previous works [2–4] showed that micro- and nano-sized foreign bodies were present in internal organs like liver, kidney and spleen affected by pathologies of unknown origin such as cryptogenic granulomatosis, cancer, sarcoidosis, etc. When debris were detected, a microanalysis was carried out to check their chemical compositions, and, at the same time, under the same working conditions, the inorganic chemical content of the biological tissues surrounding these particles was investigated and correlated to the chemistry of the particles.

Results

The ESEM observations showed that in all the analysed specimens the presence of foreign, in many cases nano-sized, and variously concentrated, bodies was found. They were contained in a fibrous tissue that sometimes, especially in the long-term implantations, coated a long stretch of the filter's prongs, but also inside the small thrombi that sometimes were detected, in the majority of cases entrapped in the vertex of the filter.

Fig. 2 presents, (a) the low-magnification histology of the tissue, (b) an ESEM view and (c) the EDS spectrum carried out on the biological tissue after the chemical process of fixation, dehydration and paraffin embedding. Foreign bodies composed of elements other than carbon, oxygen, sodium and sulphur, appear whiter, since the atomic density of their components are higher than that of the biological tissue.

Fig. 3 shows debris found in the biological tissue, they are highly concentrated and are composed of carbon, oxygen, chromium, iron and nickel. (The elements are listed as per increasing energy values.) They identify probably a stainless steel.

Also in the 97-day implantation case, many debris remained entrapped in the fibrous tissue; one (800 nm size) was composed of carbon, oxygen, aluminium, silicon, sulphur and potassium; they identify a ceramic material, probably a filosilicate (Fig. 4).

Fig. 5 shows a cluster of nanoparticles composed of carbon, oxygen, lead, calcium and copper. In the same area particles of different compositions coexisted like, for example, silver, zinc, silicon, sulphur, phosphorus and calcium. The particles' compositions are rather unusual and their compatibility with the blood is unknown. No

TABLE I List of the cases of patients who had vena cava filters for different periods

Case	Time (days)	Pathology	Debris chemistry
1	18	Second hip joint prosthesis; prophylactic	Si; Si Mg, Cr Fe Si S Na; S O Ba Na; Fe Sb S P Si Na
2	19	Protection for loco-regional thrombolysis	Fe O S Si Cu Ca; Ni O S Na, Zr O S Na; W S O P Na Cu, Bi Cl S O Na Cu
3	47	Prostate cancer; prophylactic	Pb O P Cl Al Ca Si Na Cu; O S Ag Zn P Si Ca; S Fe O Cr P Na Ca Ni Zn
4	62	DVT, stroke	Sn O Si Ti Sb S P Al Na; S Ba Ca; Fe Cr Ni
5	65	DVT, PE	Ba Cl S; Si C Al O Na Ca; Zr Si Cl Al; Pb Cl Cr Si Al Ca Cu
6	75	Hip joint prosthesis, prophylactic	C Fe S; Co S Ba; C W O S P; Cr O S Ca Fe P Na; Au O S Ag Ca K Cl Na; C Ti O S
7	97	DVT	Fe Cr Ni W P Cl S Al Fe Cr; Cl Zn S Ca Si Al Fe P; Si Al Na Mg P S Cl K Ca Fe
8	156	DVT, polytrauma	Fe O Cr S Ni P; C Sb O; Si
9	106	Femur fracture; prophylactic	Sb S; W O S P Fe; Bi S Cl Si Na Ca; Pb Si Al Cr Fe Mg Ca
10	108	DVT, hepatitis	S O Ba; Fe O Sb S Na P Si Mn; Fe Cr O Ni S Si Ca; C Cr O S; Zn O Ca P S; Cu Cl O lS Ca Si K Fe
11	156	DVT, polytrauma	Fe O Cr S Ni P; C Sb O; Si
12	126	Patella fracture; prophylactic	Ag O S Fe Cr Si P Al Mg Cl Ni Cu; Fe O Cr Ni Mo Si P Ca Cu Na; Fe Ti O S Ca; C Zr O S
13	188	DVT, polytrauma	Fe Cr Ni; Ti O Ca S P Al Fe; Fe Ti; Zn Fe Mn
14	197	DVT, PE	Al Si C K O; Co Si Fe S P Ca; Si O C; Ca S C Al O Si Cl P K Na
15	210	DVT, PE	S Cl Ca Bi P Na Mg; Ag O S Ca P Si Mg; Au Cl S O Ca Ag Na Si Mg Cu Fe

DVT – Deep vein thrombosis.
PE – Pulmonary embolim.

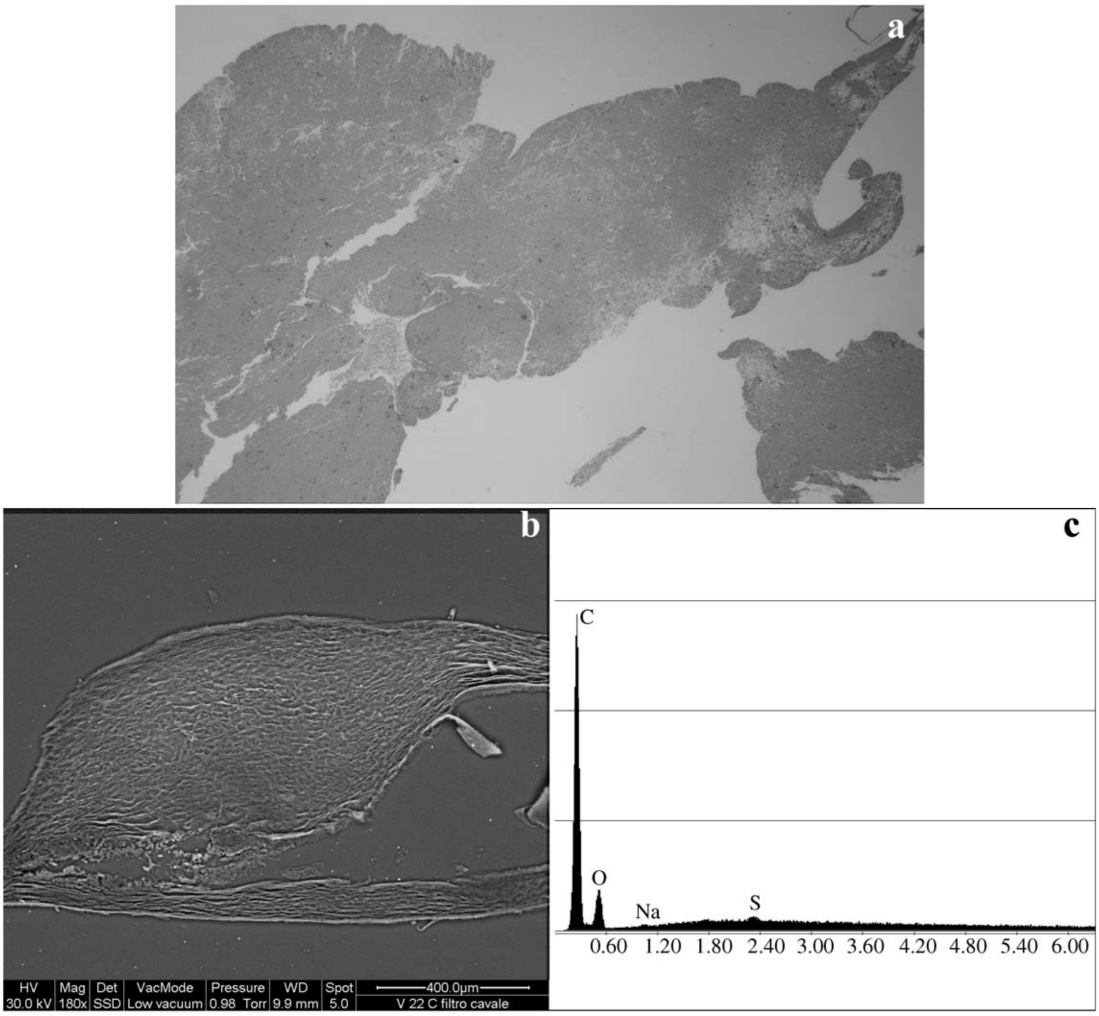

Figure 2 (a) Histological view of the tissue detached from a filter prong after *in vivo* implantation, (b) SEM microphotograph of the same tissue of (a) and (c) EDS spectrum of the biological tissue after the fixation process.

existing literature takes into account the biocompatibility of such particles with cells, tissues or organs.

Generally, the particles show complex chemistries, containing also elements like gold, silver, cobalt, titanium, antimony, tungsten, wolfram, nickel, zinc, mercury and barium. Few showed simple chemistries as iron, chromium and nickel, or silicon, oxygen, namely glass, or silicon, magnesium, namely talc or barium and sulphur probably barium sulphate: a radio-opaque material. Different patients with the same pathology (blood disorders) show blood clot entrapping particles of different chemistry. The common

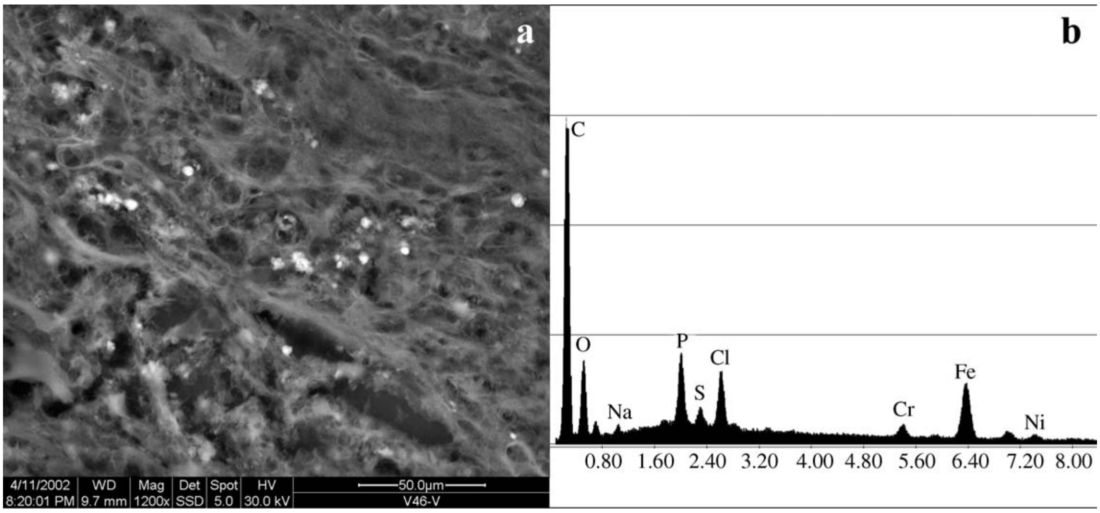

Figure 3 (a) Debris of a iron–chromium–nickel and (b) compound entrapped inside the tissue formed around the filter after a 156-day permanence.

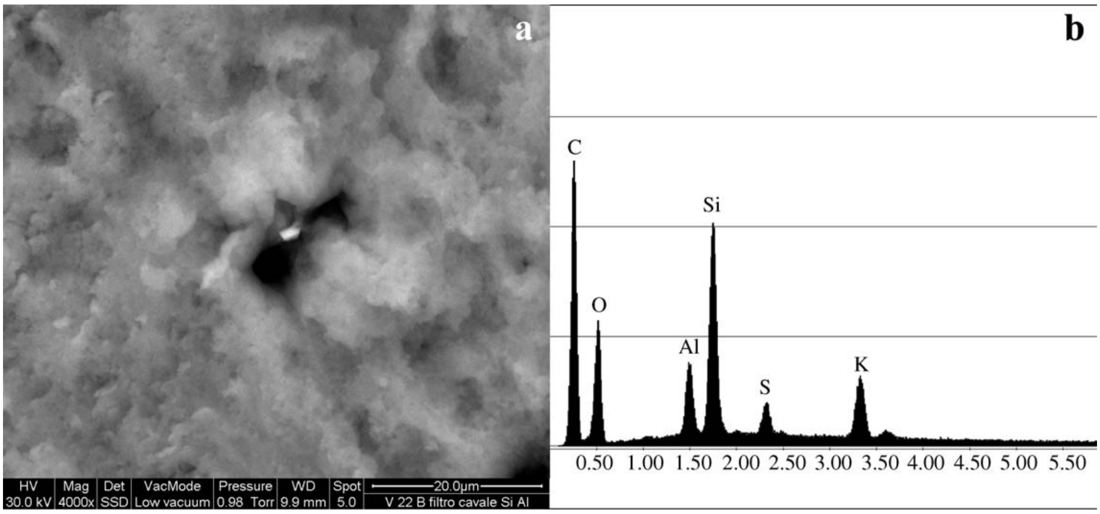

Figure 4 (a) Particles of an aluminium–silicate compound found inside the thrombus that was entrapped in the filter after a 97-day permanence and (b) its EDS spectrum.

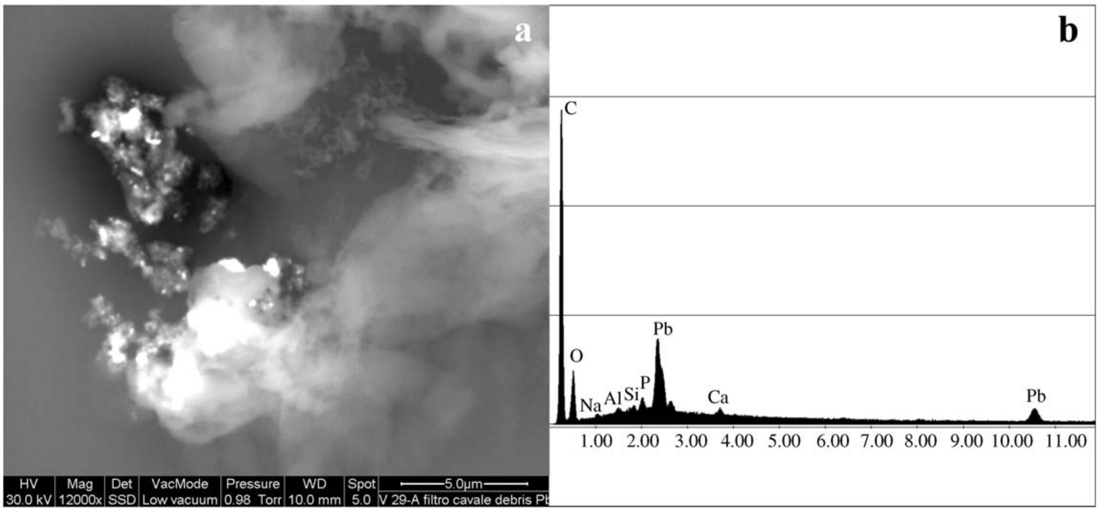

Figure 5 (a) Cluster of nanoparticles (b) of lead–copper compound found after a 47-day permanence of the filter in the human body.

factor is the size, that can vary from few microns to tens of nanometers.

Conclusions

The study showed the presence of particles in the human blood. They have an origin outside the body, from the environment and probably from environmental pollution. Nothing can be said about the ''door'' of entrance. The lung is the most probable, but also the colon mucosa is a possible access. A research work from Nemery *et al*. [5] demonstrated in humans that radiolabelled nanoparticles, inhaled, can reach the blood in 60 s and the liver in 60 min. Other articles by Revell and Urban [6, 7] demonstrated a movement of microparticles from hip joint prostheses to the liver and the spleen.

Our previous works demonstrated that the colon mucosa can give way to particles with a size up to 20 µm. It is also possible that these particles be responsible for the blood clotting, acting as a nucleating agent for the thrombus. In this hypothesis the Virchow's Triad must be modified in order to include the foreign bodies as a fourth factor of coagulation.

Acknowledgments

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