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Biocompatibility of micro- and nano-particles in the colon. Part II

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Abstract

Pathological colonic tissues were investigated with an Environmental Scanning Electron Microscope technique to verify the presence of inorganic, non-biodegradable pollutants, i.e. micro- and nano-debris of exogenous origin, after debris in liver and kidney had been discovered.

In all, 18 samples of colon tissues affected by cancer and Crohn's disease were evaluated and found in all the cases to contain micro- and nano-particles. Their chemistry, detected with an X-ray microprobe, indicated a heterogeneous nature, whereas the size of the particles was homogeneous. Three control samples of healthy, young, cadavers were analysed and showed the absence of debris within the normal, healthy colon mucosa.

The study reveals the presence of particulate debris, generally considered as biocompatible, in pathological specimens of human colon. The findings suggest a possible link between the presence of such particles and the underlying pathology in the cases analysed.

Keywords: Biocompatibility; Nano-particles; Silicate; Wear debris; Nano-pathology

1. Introduction

Former investigations [1,2] on hepatic and renal tissues affected by cryptogenic granulomatosis through a scanning electron microscopy technique and X-ray microanalysis allowed us to correlate the presence of inert, non-biodegradable, exogenous micro- and nano-particles with diseases that traditional histopathology could not account for.

It was already well known that debris produced by the wear of hip prostheses could induce an inflammatory reaction and a local foreign-body granulomatous reaction [3,4]. Recently, their migration and dissemination in other parts of the body, far from their origin, has been documented [5,6], with its possibility of causing further pathologies. However, we now know that there is no efficient gastrointestinal barrier for inert, particles with a diameter below 20 μm [2]. Such a finding attracted the attention to those particles, and more than once, when the patient's collaboration could be obtained, the source of those slight foreign bodies was found to be dental materials like porcelain [7] or over-worn alloys like gold/ruthenium [8]. In addition to that, finding in a liver barium sulphate particles, a very common contrast medium used in gastroscopy, was a further indication that all those debris can cross the intestinal barrier [9].

The maximum size of the particles found in the liver was 20 μm, while that of the ones in the kidney was below 6 μm.

The correlation between the presence of those normally considered to be non-irritant from a chemical point of view particles and the granuloma due to a so-called "foreign body" and the occasionally unexpected chemistry of some particles induced the investigator to retrace the possible path the debris had followed.

The next step was to look for particles in diseased colons, to verify the hypothesis of their passage through the bowel or their entrapment and permanence in the colon mucosa [10]. Obvious ethical reasons prevented further invasive investigations on the patients; so cases of pathologies of unknown origin were selected, such as Crohn's disease (since those patients are likely candidates to bowel cancer [11]), and colon cancer or ulcerative colitis.

This paper deals with 18 cases investigated with an Environmental Scanning Electron Microscope (ESEM)

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technique and an Energy Dispersive Spectroscopy to assess the chemical composition of the particles.

2. Materials and methods

In all, 16 patients suffering from colon cancer or ulcerative colitis and two from Crohn's disease were studied. Their clinical files reported no surgery for knee or hip joint prostheses, but no details were reported about their dental prosthetic situation. Three bulk samples of colon were taken from healthy people (two young men who died in a road accident and an elderly subject, died from a heart attack,) treated the same way as the fresh (not processed) pathological samples and kept as reference to show that healthy, normal tissues did not contain any inorganic particle (see Table 1). The samples were divided into two categories: those collected fresh at the time of surgery and the paraffin embedded samples obtained from archival tissue material.

Sections (20-μm thick) were cut (microtome by Leitz, Germany) from the paraffin blocks containing the colon biopsies or the surgical samples coming from archives of histopathology departments. The slices were suspended in warm water and deposited on a clean rectangular sheet (4×2 mm) of acetate. The samples were covered with some drops of xylol and 98% alcohol. After a few seconds, the excess of liquid was slid along the sheet edge and absorbed in blotting paper. Every sheet was glued with a carbon disc on a 4-mm diameter aluminium stub and, after having been coated with a conductive layer of carbon or gold/palladium, observed first under a Scanning Electron Microscope (SEM XL30, Philips, the Netherlands) and then under an ESEM (Quanta, FEI-Company, The Netherlands). All the preparation procedures described above are no longer necessary when employing the latter new piece of equipment, as it can work at room conditions and allows to observe wet and oily materials without any need of dehydration and of making the specimen electro-conductive. The ESEM we used was equipped with a tungsten filament, an ionization secondary electron sensor and an Energy Dispersive System (EDS by EDAX) for the chemical analysis that detects all the elements except Hydrogen. The observations were carried out in different modalities: high vacuum (10^{-6} Torr), low vacuum (0.2–1.2 Torr) and air; secondary and backscattered electron mode; energy varying from 25 to 12 kV; spot size varying from 6.0 to 4.0. This specific, new instrument was deemed necessary, as being able of observing unprocessed samples is a guarantee that no foreign pollutants have been introduced after the explantation.

The fresh tissue was obtained from surgically explanted samples and, after a half-hour's passage in formalin, they were sectioned with a scalpel in 2×1-mm samples with parallel surfaces in order to get a flat surface of observation. Some of them were subjected to a critical-point dehydrating process in order to verify the difference in the sensitivity of the instrument.

3. Results

Table 1 shows the list of the cases investigated, with the chemistry and size of the debris found in the biopsies and in the surgical samples. Particulate debris that varied considerably in size were found in all samples, except in the control ones and sometimes particles of different chemical composition co-existed in the same, small sample.

Fig. 1 shows large debris on the colon mucosa affected by cancer (Case 6). The wrinkled strip as well as the mucosa were submitted to the EDS microanalysis to try to identify their composition.

The EDS spectrum (Fig. 1a) of the tissue after the fixation and the dehydration processes indicates a composition of carbon, oxygen, sodium and calcium, while the debris are composed only of carbon (Fig. 1b). The peak of gold, visible in both spectra, is due to the first electro-conductive coatings necessary for the SEM XL30. A sample, made only of carbon, could indicate just carbon or a polymeric material. That was deduced to be a plastic fragment, either from the morphology or the elements of the EDS spectrum, polymers not being easily identified with this technique.

Another sample showed a high concentration of micro- and nano-sized particles of silicon, aluminium, calcium, sodium, sulphur, potassium, iron, titanium (the elements are listed in order of intensity of the EDS peaks) (Case 15) (Fig. 2). The debris look whiter than the tissue since they are composed of atoms denser than those in a normal tissue, as shown by the EDS spectrum (Fig. 2).

In another bulk biopsy, many particles of different size are visible. The sizes range from 30 μm to some hundred nano-meters. They contain carbon, sulphur, oxygen and calcium (Fig. 3), a composition identifying a ceramic material, probably a "dehydrated" calcium sulphate, i.e. gypsum (Case 5).

Case 13 revealed the presence of particles entrapped in the colon tissue. The mucosa surface appeared flat (no microvilli) and dense (resistant to cutting). The tissue was full of debris also with a geometric shape (rectangular). The particles showed different spectra containing silicon and calcium, and ceramic materials, most likely a calcium-silicate (Fig. 4).

In a 4×4-mm tissue sample of colon tumour that was obtained from a young female patient (Case 18), a high concentration of particles of iron, chromium and nickel was found (Fig. 5). The composition suggests that the particles are metallic, probably stainless steel. The

Table 1
List of the investigated cases with the size and chemistry of particles found in the pathological tissues

Case	Tissue specimen	Frequency[a]	Chemistry of debris[b]	Debris size in μm	Type of sample/disease (F = fresh, P = paraffin embedded)
1	Colon	H	SiAlMgFe, SiMg, Al, CaSiPS	<100 <100	P/cancer
2	Colon	L	CaSiAlMgNaPFe, CaAlSiFe, Ag*	20 7	P/Crohn's disease
3	Colon	H	FeCr,FeCrSi, FeNa, MgSi, AlSi, NaPZn, Al Si,	20 3–0.5	F/cancer
4	Colon	H	FeSiCl ClSiCaAlNaF SiCaFe, FeCrClSiZn, etc.	3 4 3,12	F/cancer
5	Colon	H	AlSiK, NaPCaSMgAl, FeCrSi SiNaCa, SiMg, SiCa, Sca, etc.	22 10–30	F/cancer
6	Colon	H	Zr,ZrFeCr AgMoCl AlPbCl	20,30 10 10	F/cancer
7	Colon	M	Ag* FeCr, Fe SiMg	20, 18 30 12	F/cancer
8	Colon	H	AgCl Cluster Ca, PcaFeZn FeCrS, CaSiNaK	10 5–10 25 20	F/cancer
9	Colon	H	PcaAlSiFe, FeNiSi SiAlTiK, PcaFeZn	>20 10	F/cancer
10	Colon	M	FeNiSi Pal	10 5	F/cancer
11	Colon	H	SiNaSPkCa, Al SiAl, AlSiSNaMg, AlSiS	2 6–10	P/adenocarcinoma
12	Colon	M	FeSiS, SiAlKNa	3, 50	P/ulcerative colitis
13	Colon	L	AgS Cluster SiCa	20 10	P/adenocarcinoma
14	Colon	M	CaSiKAlFeTi, PcaSiAlS	30, 5–20	P/cancer
15	Colon	H	CaPFeSiMgNaS, AlSiCaFeZn	15 20	P/cancer
16	Colon	L	Zr, Fe, Al	12, 20, 10	P/cancer
17	Colon	H	FeCrNi, ZrSiNa, NaClSiFeCrNi	70–30 2.5–1	P/adenocarcinoma and Chron's disease
18	Colon	L	FeCrNi	20	P/adenocarcinoma
19	Control specimens[c]	A			No particles
20					
21					

[a] Frequency is an arbitrary score (L = low, M = medium, H = high, A = absence) given trying to define the debris distribution at 300× magnification, but at that magnification nano-particles are not visible.

[b] The sequence of the elements has been listed according to their EDS intensities (from the highest to the smallest).

[c] The chosen control specimen the only possible ones we had at our disposal, showed immediately the problem of not being homogeneous with the samples. In fact the delayed fixation altered the morphology of the biological components and made them not really comparable with the pathological samples. A great obstacle was also the dirtiness of the mucosa surface due to the permanence of faeces that demanded numerous washings before fixation; instead the pathological samples are excised after an accurate 24-h patient's preparation.

* see Fig. 7.

presence of nano-particles of zirconium, silicon and sodium was also detected in another area.

The author examined different specimens that were sampled from various locations at the site of tumour in each case: Multiple sampling has verified that the concentration and the chemical composition of the particles were not consistent among the samples as evident from the discrepancies in the values obtained.

Nano-particles of zirconium were found entrapped in another tissue of colon cancer together with other debris of iron and aluminium (Case 16) (Fig. 6).

In four different cases the presence of silver or silver compounds was found (Fig. 7) (Cases 6–8, 13). The particles were found aggregated in few-micron clusters and the size of a single debris ranged 200–300 nm.

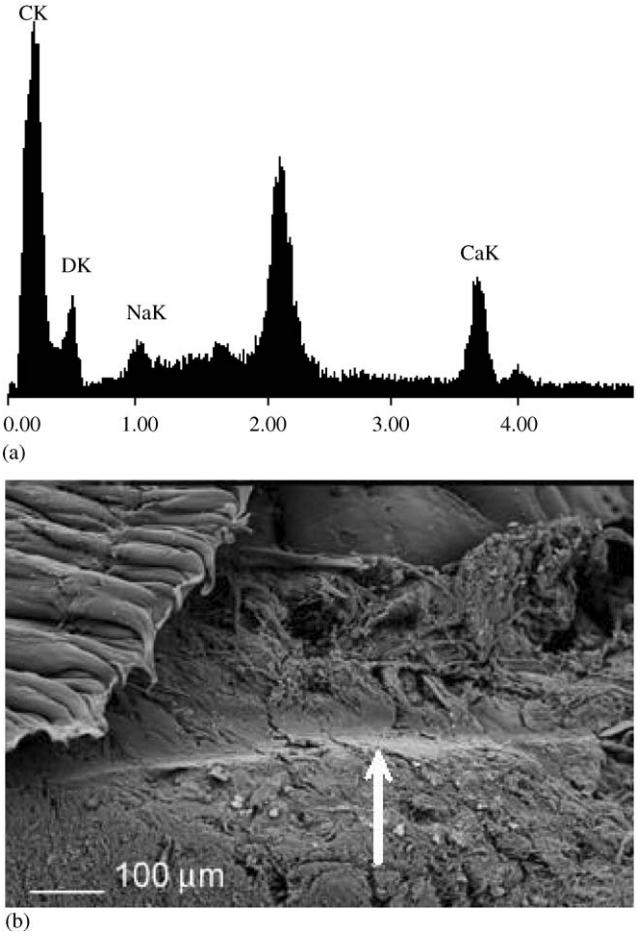


Fig. 1. Gold-coated bulk sample of colon cancer where a wrapped strip of polymeric material is visible (left upper part). The EDS spectra were carried out on the biological tissue (a) and on the debris (b). The strip appears lifted from the surface for the fixation, dehydration procedures, but it is still visible its impression on the tissue (arrow).

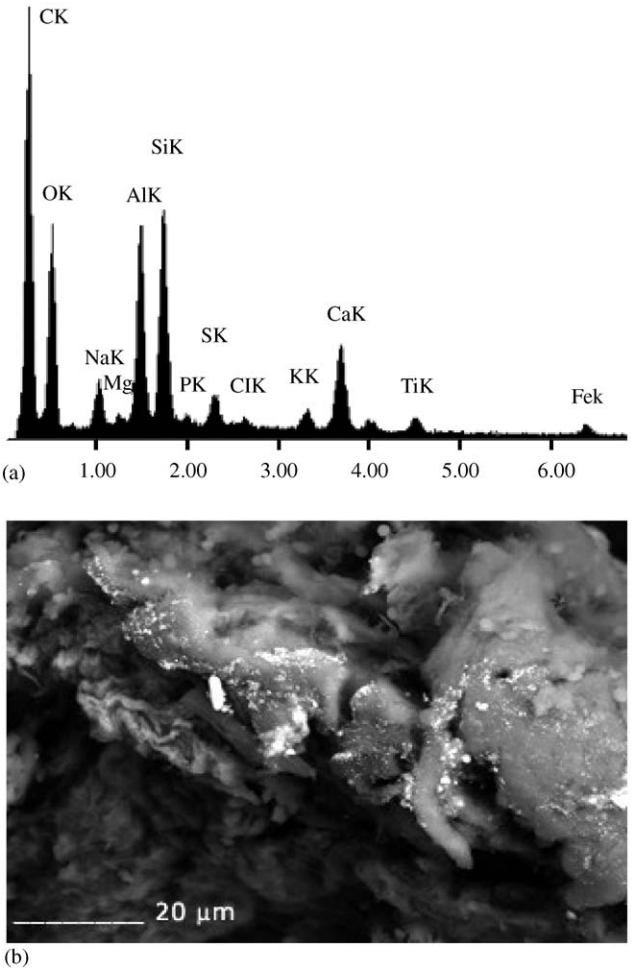


Fig. 2. Bulk colon cancer biopsy with micro- and nano-debris entrapped in the tissue. The EDS spectrum identifies them as ceramic particulate called filosilicates.

Scattered silicate particulate debris were detected within the mucosa of the colon tumour in Case 1. As demonstrated in Fig. 8 large number of these particles appear at different focus together with two well-preserved red blood cells (erythrocytes) that exhibit greater size (approximately 7 μm) than the particles: The well-preserved morphology of the erythrocytes indicates little or no significant cellular interaction with the particles and suggests a possible transportation of the particles entrapped within the cavity of the red cells.

4. Discussion

The constant presence of debris in all the investigated cases adds new parameters to be taken into consideration when approaching the study of these pathologies, to date considered as of unknown origin. There is clearly an exogenous aetiology, since some elements such as silicon, aluminium, zirconium, silver, etc. are not present in the composition of the human body. The presence of so many different kinds of debris (in one case, in a few mm^2, 14 different chemical compositions were detected) suggests that the colon mucosa is not impassable to them. It is possible that they induce a "*physical*" *toxicity*, i.e. adverse reactions caused by the very presence of solid particles larger than a hypothetical "threshold size" whose effects are not yet being investigated at present.

In the case of the plastic debris, it can be observed that the debris clinging to the mucosa surface hampers the passage of nutritional components in that area, thus probably causing a local mal-absorption problem, but that can be caused also by a transformation of the mucosa into a fibrotic tissue (visible histologically) for an inflammatory reaction due to the presence of "foreign-bodies".

The finding of silver debris is particularly puzzling as to its origin. Silver is a common element in dental amalgams, but it is always present as an alloy together with tin. The absence of any signal for tin was sufficient to reject the hypothesis of a dental origin. Silver is a

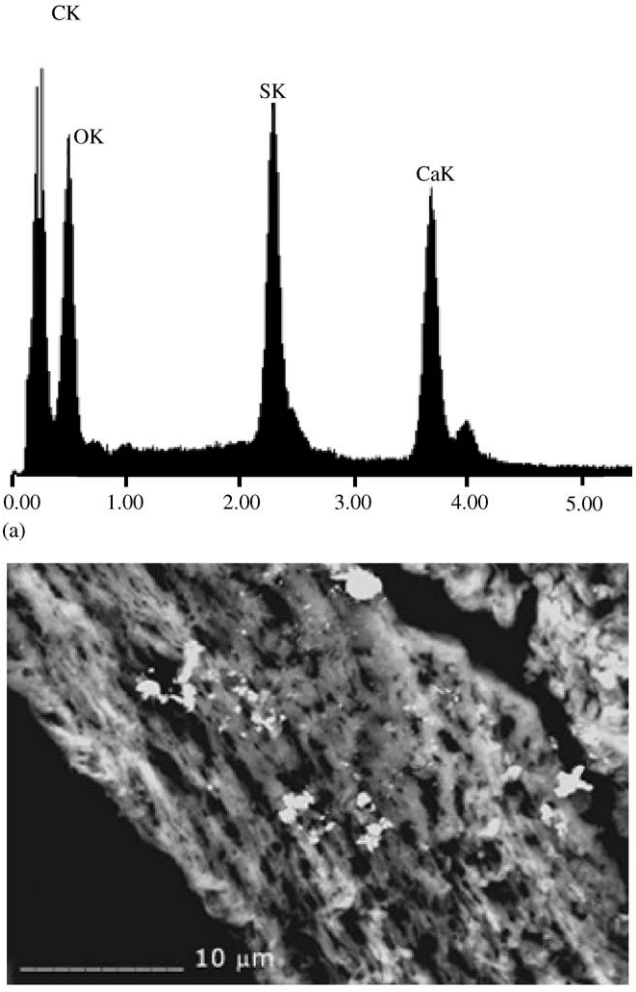


Fig. 3. Colon cancer sample with particles of Sulphur and calcium.

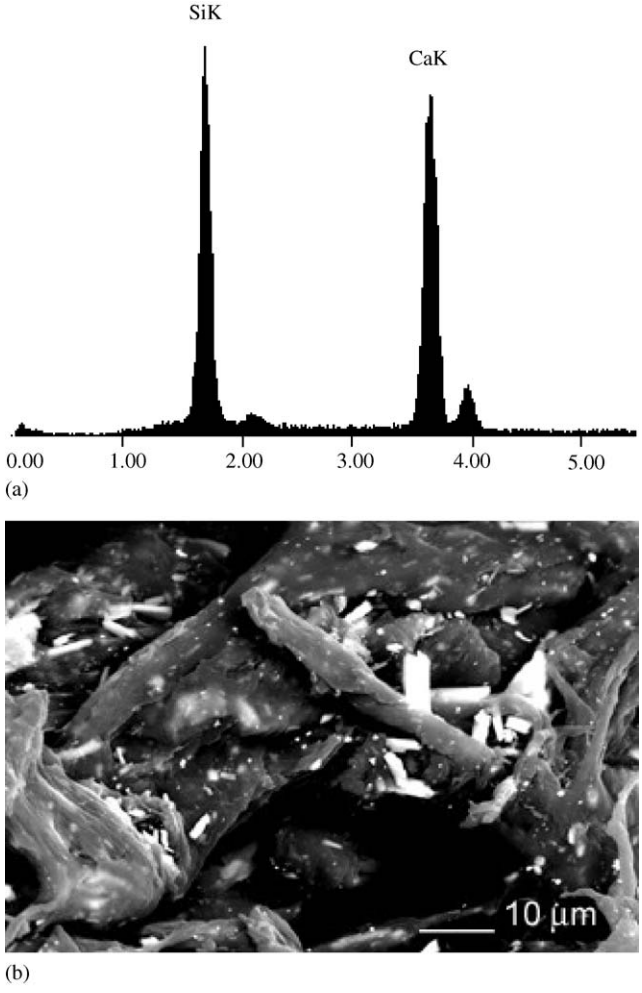


Fig. 4. Rectangular-shaped particles of different size and morphology. The composition is homogeneous namely calcium and silicon.

component of some homoeopathic therapies, but no patient reported any voluntary intake. Silver can also be a hygienic coating of toothbrushes (silver oxide is a bactericide) and its wear is an obvious phenomenon, but no confirmation of such an origin was possible. Many other speculations or logically possible explanations can be put forward.

The size of debris varies from some 100 μm down to 50 nm. In general, it was observed that the farther the distance from the entry point of the debris (mucosa surface), the smaller the particle size. Another important observation concerns their localization: In the three cases of primary cancer observed, the debris were consistently found at the interface between the healthy and pathological tissue, while the cancerous tissue was free from them.

The presence of zirconium debris may be explained as due to the wear of dental porcelain or to filling particulate of restorative composites, but no evidence of such dental prostheses was found in the clinical report, since the status of the teeth is usually considered to be irrelevant in the patient's anamnesis and the author had almost no chance of seeing the patients. It is well known that dental materials wear [12–15], but until now their elimination with the faeces was taken for granted, though there has never been a demonstration of that. (*Note*: At present no quantification of particles was attempted because of the dis-homogeneity of the samples (fresh, fixed, planar (sections) and three-dimensional (bulk)). The fixation itself induces also a shrinkage of the tissues, absent in the fresh tissue, that does not allow a comparison among these samples. Due to the involved magnifications ($300\times$ up to $30{,}000\times$), and the differences of particulate diameters (from 30 μm up to 50 nm) a specific software and a motorized system of the sample holder for the $x-y$ scanning must be developed).

The chemistries of all the particles identify them as inorganic, inert, not biodegradable, not chemically toxic. Studies are in progress to detect the possible sources in dental materials, food and drugs.

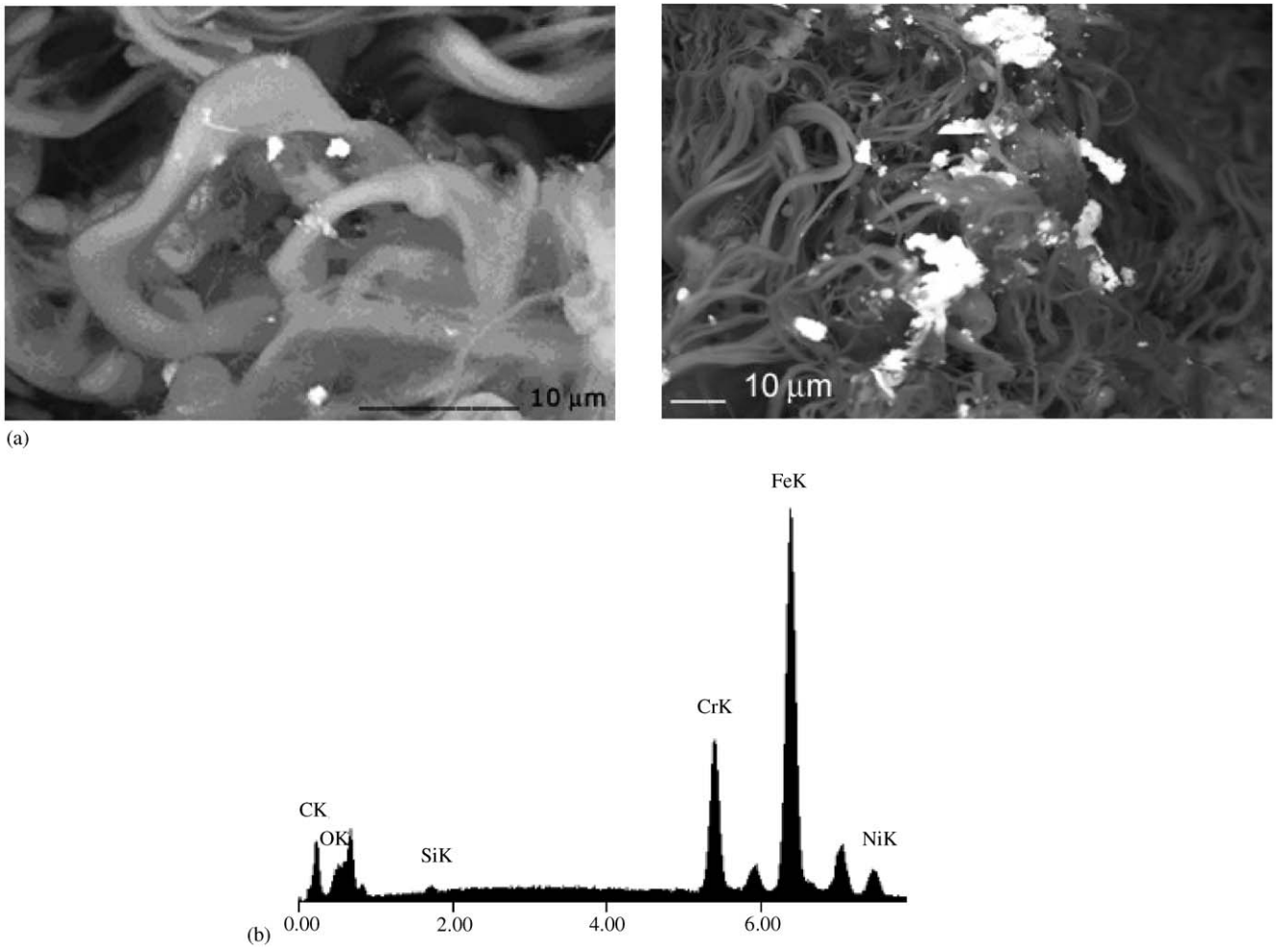


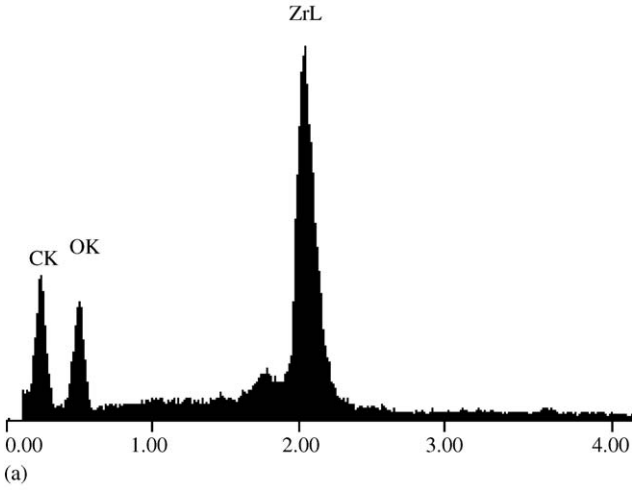
Fig. 5. Low and high magnification of a colon tumour from a young female patient shows particulate debris of stainless steel.

The concept that no fully biocompatible materials exist and that the biocompatibility is a dynamic concept (changeable in time) was already considered by Black [16] in 1984. Possible metabolic, immunologic, carcinogenic effects of implants, especially from orthopaedic prostheses, have already been discussed on the basis of some clinical evidence [3,5]. The failure of those implants, in some cases after years, was related to the onset of some systemic diseases in the patients. Allergenic as well as carcinogenic reactions due to foreign bodies were related to the corrosion debris and ions of implant materials, but at that time no evidence of their presence in tissues was found.

The present study identifies particles inside pathological tissues, but in no case was their origin ascribable to worn orthopaedic prostheses. The constant presence of particulate material in cases of colon cancer and Crohn's disease, only occasionally coming from dental prostheses, is an evidence that calls for further and systematic investigations both as far as the pathological tissues and the source of the particles are concerned. That need is urgent since, in another field, the correlation between a long exposure to fine-particulate air pollution and lung cancer has been already fully demonstrated [17]. Other authors have demonstrated the correlation between wear particles from joint prostheses and the onset of various forms of cancer [18–20].

If the particulate matter is responsible for causing pathologies, other parameters such as particles' size and morphology, their concentration and times of exposure must be taken into account in the study of those pathologies. An in vitro test on the biological activity of polyethylene wear debris from hip joint prostheses showed that particles "in the size range 1–10 μm were five times less active than the 0.1–1 μm particles, and the particles in the size range 10–100 μm were 25 times less active than the particles in the smallest size range" [21].

Particles with a size, smaller than that of the cells might represent a physical "quantum" capable of triggering yet unknown biological phenomena. A non-toxic, but not biodegradable particle with a size similar



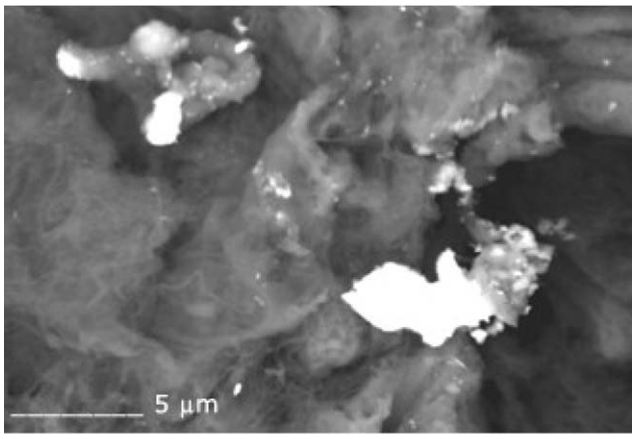
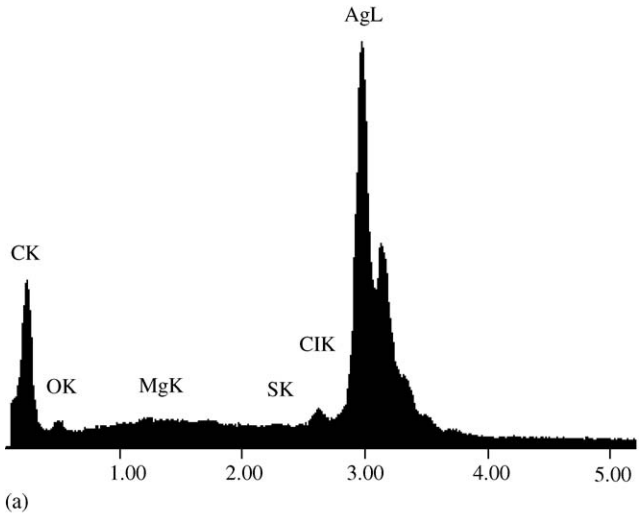


Fig. 6. The image shows a large particle of silicate and small particles of zirconium.



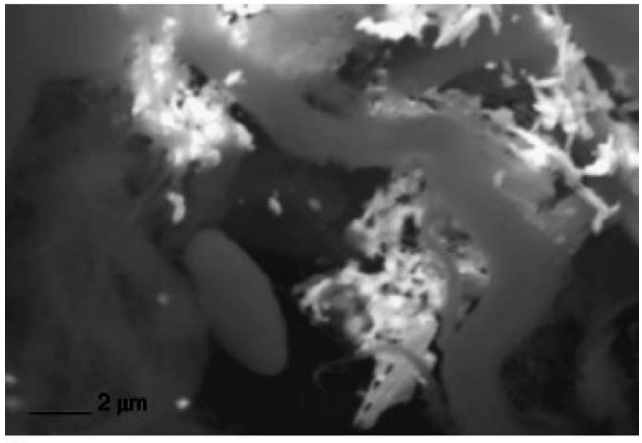


Fig. 7. Nano-particles of silver (*see Table 1) in a colon cancer sample.

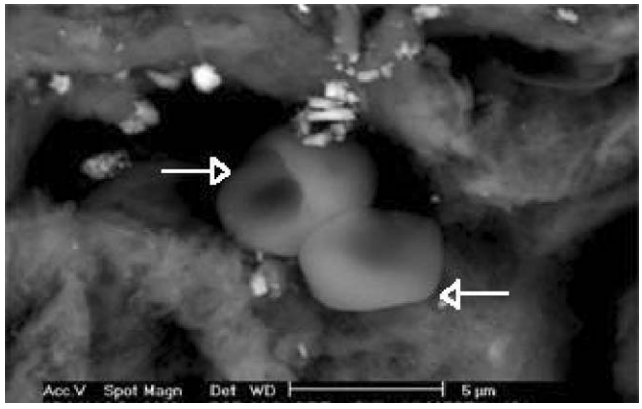


Fig. 8. Colon cancer sample with silicate debris and two red cells (arrows).

to or smaller than that of the surface "sensors" may be likely to induce non-specific, not yet investigated, irreversible reactions. To identify these new aspects of pathological interaction between micro- and nano-particles with cells, a new word was coined: "nano-pathology".

5. Conclusions

The new technique, applied to human samples allowing to see and identify micro- and nano-sized debris, gives a new point of view of the considered pathologies and opens new perspectives in the study of diseases of unknown origin. New "physical" parameters, as debris concentration, particle size, velocity of accumulation, probably can be correlated the disease and its evolution. The different found chemistries witness their different origin (food pollutants, drug matrix, worn dental materials, etc.), and induce us to consider, one time more, that the environment can represent a risk for the human health. In vitro and in vivo studies are in progress in order to determine the biocompatibility of micro- and nano-sized physical stimula and to assess the risk of "dust".

References

[1] Gatti AM, Ballestri M, Baraldi A, Rapanà R. New aspects of the degradation of porcelain in dentistry. In: Ogushi H, Hastings GW, Yoshikawa T, editors. Bioceramics, vol. 12. Nara, Japan: World Science Publishing Co.; 1999. p. 29–32.

[2] Ballestri M, Baraldi A, Gatti AM, Furci L, Bagni A, Loria P, Rapanà R, Carulli N, Albertazzi A. Liver and kidney foreign bodies granulomatosis in a patient with maloocclusion, bruxism, and worn dental prostheses. Gastroenterology 2001;121:1234–8.

[3] Revell PA, Al Saffar N, Kobayashi A. Biological reaction to debris in relation to joint prostheses. Proc Inst Mech Eng 1997;211:187–97.

[4] Al-Saffar N, Revell PA. Pathology of the bone–implant interfaces. J Long Term Effects Med Implants 1999;9(4):319–47.

[5] Urban RM, Jacobs JJ, Tomlinson MJ, Gavrilovic J, Black J, Peoc'h M. Dissemination of wear particles to the liver, spleen and abdominal lymph nodes of patients with hip or knee replacement. J Bone Jt Surg 2000;82-a(4):455–77.

[6] Shea KG, Lundeen GA, Bloebaum RD, Bachus KN, Zou L. Lymphoreticular dissemination of metal particles after primary joint replacements. Clin Orthop 1997;338:219–26.

[7] Gatti AM, Rivasi F. Biocompatibility of micro- and nanoparticles: Part I. In liver and kidney. Biomaterials 2002;23(11):2381–7.

[8] Gatti AM, Ballestri M, Bagni A. Are the dental porcelain debris safe? Am J Dent 2001;121:1234–380.

[9] Gatti AM, Revell PA. Micro and nano biomaterial inducing liver granulomatosis. Eur Soc Biomater Proc Lond 2001;T3:3.

[10] Gatti AM. Impact of micro and nanoparticles in inducing biomaterials systemic pathologies Transactions of the 28th annual Meeting in Biomaterials, Tampa, FL, 2002. p. 616.

[11] Ekbom A, Helmick C, Zack M, Adami HO. Increased risk of large bowel cancer in Crohn's disease with colonic involvement. Lancet 1990;336:357–9.

[12] Leinfelder KF. Evaluation of clinical wear of posterior composite resin. In: Vanherle G, Dennis CS, editors. Posterior composite resin dental restorative materials. The Netherlands: Peter Szulc Publ; 1985. p. 501–8.

[13] Eick JD. In vivo wear measurement of composite resins. In: Vanherle G, Dennis CS, editors. Posterior composite resin dental restorative materials. The Netherlands: Peter Szulc Publ; 1985. p. 351–64.

[14] Derand P, Vereby P. Wear of low-fusing dental porcelain. J Prosthet Dent 1999;87(4):460–3.

[15] Sulong MZ, Aziz RA. Wear of materials used in dentistry: a review of the literature. J Prosthet Dent 1990;63:342–9.

[16] Black J. Systemic effects of biomaterials. Biomaterials 1984;5: 11–8.

[17] Pope III AA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston G. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 2002;287(9):1132–41.

[18] Nyren O, McLaughlin JK, Gridley G Ekbom A, Johnell O, Fraumeni JF, Adami HO. Cancer risk after hip replacement with metal implants: a population-based cohort study in Sweden. J Natl Cancer Inst 1995;87:28–33.

[19] Gillespie WJ, Henry DA, O'Connell DL, Kendrick S, Juszczak E, McKinneny K, Derby L. Development of haematopoietic cancers after implantation of total joint replacement. Clin Orthop 1996;329:S290–6.

[20] Peoc'h M, Ducros V, Barnoud R, Pasquier D, Laverriere MH, Pasquier B. Foreign body histiocytosis reaction after hip replacement with concomitant metastatic adenocarcinoma in the same lymph node. Hum Pathol 1998;29:95–8.

[21] Fischer J, Bell J, Barbour PSM, Tipper JL, Matthews JB, Besong AA, Stone MH, Ingham E. A novel method for the prediction of functional biological activity of polyethylene wear debris. Inst Mech Eng 2001;215(H):127–32.