Chapter 1

How the Whole Thing Began or the Logic Path Towards a Discovery

Back in 1990, the hospital of Monza, a branch of the University of Milan - Italy, sent to the Laboratory of Biomaterials of the University of Modena a vena cava filter (Montanari, 2000) which had two broken legs and that had been explanted surgically from a 62-year-old female patient (Emanuelli, et al., 1995) (Gatti, et al., 2006). The surgeon who had first implanted and then removed the filter just wanted to know why the device had failed and solving his problem was not particularly hard, as it was due to a caudal migration with the two legs trapped in a collateral vein. The filter moved downward, while the two legs could not follow the movement, broke. One of the consequences was that the fracture surfaces remained exposed to the blood and interacted with its components. What remained of the filter and the broken legs was observed under a Scanning Electron Microscope equipped with an X-ray microprobe (Energy Dispersive Spectroscope or EDS).

This analysis [A highly energetic electron beam is aimed at the sample, and that yields a number of by-products, among which X-rays. Each chemical element has characteristic energies/wavelengths which can be detected using a solid state energy dispersive spectrometer detector] (See Appendix) on this surface, where the legs had broken, revealed a relatively high concentration of Chlorine, Silicon, Phosphorus and Magnesium; all elements that did not belong either to the filter's alloy - an AISI 316L stainless steel, whose composition is Chromium 18%, Nickel 14%, Molybdenum 3%, Carbon below 0.03% and Iron to balance - or to the blood, at least not in such quantities. At that time, we could not find any reasonable explanation to what we had seen.

A couple of years later, the same hospital sent another broken vena cava filter to the Laboratory. This time the device was a temporary one (Bovyn, et al., 1997) removed from a 58-year-old male 23 days after having been implanted. Also that filter had failed, having lost one of its legs which was then retrieved from the patient's right inferior interlobar renal vein by means of a transvenous noose catheter. The scanning-electron-microscope observation of the fracture showed a few inorganic deposits that could not belong to the product's composition. As the EDS analysis proved that the filter's alloy was composed of Cobalt, Chromium, Nickel, Molybdenum, Silicon and Iron (alloy called Phynox), while Calcium and Aluminium were found in one of the deposits, Chlorine, Silicon, Potassium, Calcium, Sulphur, Aluminium, Sodium, Titanium and Magnesium in another, and single particles of Aluminium and Calcium-Sulphur were also detected. Those deposits had obviously formed in vivo, since the specimen had been carefully washed in a Potassium oxide solution, and cleaned in a Hydrogen peroxide ultrasonic bath, a treatment that destroys biofilm and leaves only insoluble precipitates strictly adhered to the surface. Some of the materials detected can be found as trace elements in ionic form or bound to organic molecules in the human organism, but what we saw was particles and the quantities we were confronted with were comparatively high. Again, no satisfactory explanation could be offered then to that phenomenon.

In December 1997 a 62-year-old patient (Ballestri, et al., 2001) (Gatti, et al., 2002) was admitted to the University hospital of Modena, where the Laboratory of Biomaterials was then located. He was affected by acute renal failure, hepato-splenomegaly and a mild haemolytic anaemia. In addition to that, for the last eight years he had suffered from a slight increase of the body temperature, a phenomenon that manifested itself in the late afternoon and remitted to a normal temperature in a matter of a few hours, without recurring to any medication. That fever followed periodic cycles, each of which lasted a few months. Among the symptoms the patient suffered from there was also a constant tiredness. Blood and urine culture had been consistently negative and, despite numerous hospitalizations, no aetiology could be determined for such collection of symptoms.

So, a percutaneous echo-assisted biopsy was performed in the liver and the lower pole of the left kidney, revealing non-caseating granulomatous areas with Langhans-type giant cells in the kidney, while the liver showed a mild fibrosis of the portal tracts filled with mononuclear infiltrates and with the presence of epithelioid giant cells. Scattered polarized light-blue particles either in the kidney and the liver were shown by polarized-light microscopy observation, often inside giant cells located in granulomatous areas, suggesting a foreign-body multi-system granulomatosis. No yeasts or alcohol-acid resistant bacteria could be identified. Clinical and immunological tests excluded tuberculosis, mycotic infection, protozoa infestation, granulomatous vasculitis or sarcoidosis, and the patient's history was negative for exposure to chemicals, drug abuse or arteriographic procedures.

That was indeed a very hard problem to solve through the traditional means of investigation. So, the samples were given to our Laboratory where we studied the debris by means of a scanning electron microscope (SEM XL40 by Philips) and an environmental scanning electron microscope (ESEM XL50 by Fei Company) equipped with an EDS. Both in the liver and kidney specimens, we could identify solid particles whose electronic density was higher than the biological background's. Their size ranged 6-20 microns in the liver and was smaller than 6 microns in the kidney, and the EDS elemental analyses, identical in both types of specimens, revealed that they were made of Aluminium, Silicon, Oxygen, Sodium, and Potassium or Barium, a composition that suggested that of a ceramic material (and was compatible with feldspars, non-fibrous silicates that are regular components of porcelain). In order to understand the origin of such debris, a specific anamnestic study of the patient was carried out, looking for an internal source of these debris like prostheses, implant, etc.

What we learned from the patient, was that nine years before, two ceramic bridges had been placed in his left upper and right lower dental arches. That caused immediately a marked discomfort, an uncontrolled lachrymation of the left eye, and a homolateral earache that could not be solved by the antibiotic treatments the patient had undergone. In addition to that, the patient became a bruxer.

The prostheses had been milled a little later to try and solve the problem of malocclusion and bruxism, so leaving a highly worn prosthetic surface.

So, we started to suspect that the source of the debris found in the patient's tissues could be those prostheses and had both removed. They were cross-sectioned and subjected to an Rx microanalysis that showed the same elemental composition as the foreign bodies discovered in the tissues. Ceramic particles smaller or as large as 40 microns were found in the patient's stools before the prostheses were removed, but none one month after. So the patient underwent a 6-month therapy of methylprednisolone and that induced the remission of the fever along with the reversal of hepato-splenomegaly. Inflammation, haemolysis and cholestasis disappeared, while the renal function recovered. After therapy, no steroids were administered for another six month and the clinical picture worsened. A recourse to methylprednisolone and cyclophosphamide, an immunosuppressive therapy, lead to a complete remission. Liver and kidney biopsies performed three years after the first hospitalization showed a marked reduction of the granulomatosis.

Now, the question was: how could that debris, whose origin was hard to call into question, have reached the liver and the kidneys? In our opinion, the hypothesis that those particles had been absorbed by the gastrointestinal system looked the likeliest, but no literature existed to support what we suspected. Debris of different sizes had been detected in the stools, the same kind of foreign bodies had been found in the liver and the kidneys, but those in the kidneys were smaller than those in the liver. That could suggest that the particles, undoubtedly present in the bowels, which were absolutely healthy in our patient, had passed through the intestinal mucosa and had been cleared by the liver before entering the general circulation and, from there, the kidneys.

Such passage was not described in the then current handbooks of Physiology and sounded hard to believe.

Professor Peter Revell of the Free Royal Hospital of London gave us then four bioptic samples of liver granulomatosis whose origin had been declared to be unknown, but viruses, bacteria and parasites had been ruled out as possible causes. In three of them we found evident traces of surprisingly enough but undoubtedly environmental dust, while in one we detected particles of a metal that had been used for therapeutic purposes. Gold nanoparticles had been injected in the patient's knee joint to treat an arthrosis and it was that Gold that we found in the liver tissue.

That made us understand that solid environmental pollution could be suspected as being able to penetrate the body and settle in the liver. How, it was far from clear, but the most reasonable ways of entry seemed us to be the digestive system – for which we had already a piece of evidence with the case of the dental prosthesis - and, perhaps - but it was just a guess - the respiratory system.

In the meantime, we had received more samples both from our University Hospital and the Istituto Tumori (Cancer Institute) of Milan – Italy, and they regarded cases of colon cancer and Crohn's disease. In all instances, inorganic dust was present in considerable concentrations and variety. In one case we could even detect 15 different chemical elemental compositions in the same cluster in less than 1 cm^2 of tissue.

Thanks to our experience in caval filtration and the new availability of filters that could be explanted even after a long time, we started to take into consideration the blood clots that were regularly found stuck to the device when it was removed from the patient and the thrombi that the device had trapped. If inorganic dust, especially composed of chemical elements that should not be present in the organism, had been detected, that would have meant, without any possible doubt, that that dust had an exogenous origin. As a matter of fact, as will be illustrated in detail later in this book, all samples showed that presence.

Finding once unexpected inorganic particulate had become a daily experience, but we could say very little or nothing at all about its way of entrance.

So, we started to look at some pathological lung sample we got from our University and the University of Siena (Italy), and among them there were also some cases of pneumothorax. Then, we studied cases of cystic fibrosis, sarcoidosis and of different forms of lung cancer.

Inorganic micro- and nanodust was to be found with a great frequency.

We decided, then, to present a project to the European Community, a project we called "Nanopathology", by that neologism we had created meaning the pathologies due to micro- and nanodust, and by that project intending to study if what we had observed for a few years had a scientific basis.

1.1 Introduction

Man has only recently landed on what to him was a new planet: Nano. But Nature has always been there. Just to give one example, Nature uses a "nanocode" called genome to preserve all the information about the species each individual, be it a man, a virus or any other living being, belongs to, and that code is extremely complex, sophisticated and efficient. Genome, in its turn, gives all the necessary instructions, many of which we are not aware of, to build the (nanosized) proteins necessary to live, and does that at a nanoscale.

We are accustomed to interacting with objects the size we can handle and/or see, and have also been accustomed for a relatively long time to investigating and trying to understand how atoms and molecules behave; but Nano is something different from both worlds. Nanoscale systems are too big to be considered molecules, but definitely too small to be understood in terms of macro, and attempting to extrapolate their behaviour starting either from the smaller or from the larger would lead us astray. Neither quantum nor classic physics can be fully applied.

Of this sort of "mesoworld" we know very little, but what has immediately become visible is that the properties of those objects are extremely interesting from a scientific point of view and, in addition to that, offer many prospective possibilities to be exploited to our benefit. Right for that latter reason, an enormous throng of scientists, technicians, industrialists and financiers flung themselves, and are still doing with growing enthusiasm and hopes, headlong into that field, the way pioneers did in the past when a new land was discovered or gold was unexpectedly found somewhere.

More than a couple of decades ago, in 1986, Kim Eric Drexler, an American engineer, published *Engines of Creation*, a book describing nanomachines capable of reproducing both themselves and virtually any material object, extending life duration, curing diseases, in short, working all sorts of wonders and, at the same time, reducing pollution. Many scientists ridiculed such outlook, and nevertheless the book exerted a strong influence on many people. And many people means good business. Now, after more than twenty years have elapsed, we are still far from what Drexler we do not know how rightfully anticipated, but it is a matter of fact that nanotechnology is finding more and more applications and the limit seems to be imagination.

According to Forbes, (web ref. 1) now the most common applications of nanotechnology are in sports goods, ski wax, tennis racquets and tennis balls being the top three, but some medical applications are already in use and many more are being attempted, cosmetics, photocatalists and computer chips are already available and what are called "smart" surfaces, by that meaning hydrophobic, self-cleaning, anti-bacteria, anti-mould treatments are best sellers. Just as a brief observation, the adjective "smart" sounds very à-la Drexler.

So, after having landed in what if not a promised is at least a promising land, it would be fool not to advance and explore it with the purpose of exploiting it. But like any unknown territory, also this can hide unexpected dangers and, for that reason, using prudence and exercising some patience may look advisable. Who carried out exhaustive studies about the impact those technology and, in particular, handling nanodust may have on human and animal organisms? How can we know how long those entities will take to interact with environment and organisms, if ever they will interact in a perceptible way? Is there any long enough experience in that field? No satisfactory answer can be given to those questions.

What we can do is extrapolate.

Micro- and, much more rarely, nano-scale particles have always been produced by a number of natural sources: belching volcanoes, forest fires, rock erosion, and airborne desert and beach sand, but the greatest quantity of such dust, particularly the smaller, comes from man. Much of it is the undesired by-product of combustion, a process that has started to be used on a large, industrial scale only a couple of centuries ago, i.e. roughly 1/10,000 of the time man has spent on the Earth. Generating heat at temperatures higher than that of burning wood has always been difficult and, when the concept of selling value was introduced, expensive. For those reasons, materials such as, for example, glass have been rather precious for a long time. But when we started to exploit fossil combustibles, heat has grown cheaper and cheaper and easy to come by, and now very high temperatures can be attained without any difficulty and at a low cost. All that creates dust, and, as a rule of thumb, the higher the temperature, the tinier the dust.

So, foundries, cement plants, incinerators and internal-combustion engines, among an indefinite number of other sources, produce particles, and their quantity is constantly on the increase, particularly as regards nanoparticles, since industrial filters are not efficient enough to trap them and the increase in temperature makes microparticles rarer and nanoparticles more common.

Particulate matter is released in the atmosphere and behaves in a way that is very similar, or all but identical in the case of nanosize, to gas. Some of those particles, especially the small ones, tend to form clusters and, for that reason, making a clear distinction between micro and nano is not always possible and often not even meaningful. However, in any case the structure of a nanoparticle remains the same.

The dust we deal with is inorganic, most of the times crystalline, insoluble in water or other common, natural solvents, and non biodegradable. Being so small, pressure and thermal gradients and wind carry it virtually everywhere, and when it eventually falls to the ground, a gentle breath of wind can lift it up again, thus restarting the cycle. That way, those particles can travel very long distances and stay with us forever.

Besides, when we look at those inorganic particles from the pollution point of view, we must not forget that they are often the carriers of organic pollutants like, for example and among many others, dioxins, furans or polycyclic aromatic hydrocarbons. And we must also remember that in a number of environmental conditions, even inside biological tissues, those particles tend to coalesce and, for that reason, differentiating micro and nanoparticles is not always possible and in some instances could be meaningless. Another important point is that spherical particles generated by combustion, especially the larger ones, are often hollow and very fragile, so, when they break, they create fragments which are obviously smaller and, as a consequence, microparticles can create nanoparticles.

From the compositional point of view, the nanoparticles we deal with are very often alloys fortuitously created by the fact that the chemical elements that make them up were present in the materials burnt and combined to form an alloy that in most cases is not to be found in any metallurgy handbook. So, their elemental chemical composition can be a telltale indication that allows to identify the source.

But there are also cases when those particles come from wear and friction of machines or industrial working, and heat is not involved in their formation. In that case, their composition is the same as that of the original material.

An important issue regards mass and volume. If, for the sake of simplification, we assume that particles are spherical (which is often the case with the particles we deal with, when created at high temperature), we can easily calculate their volume and their mass, which depends, obviously, on the matter that object is made of. Let's suppose that we have a sphere whose diameter is 10 µm, i.e. that of a coarse particle. In that case, its volume is $4\pi r^3/3 = 523.3\ \mu m^3$. Now, let us suppose that we wish to make spheres with a diameter of 2.5 µm, i.e. ¼ of the one of the former ball. In that case, the volume of each sphere will be $8.0593\ m\mu^3$, i.e.64 times as small. That means that, using the same amount of matter necessary to make a particle whose diameter is 10 µm, we can create 64 particles whose diameter is 4 times as small. And, continuing, we can create 1,000 particles with a diameter of 1 µm or 1,000,000 with a diameter of 0.1 µm. This is particularly important if we look at this simple geometric fact with the nanopathologist's eye, since the same amount of matter, if burnt at different temperatures, can give origin to particles whose effects on the organism is very different [see also Chapter 6 – Introduction]

On that dust we collected some experience and thanks to that we can try and extrapolate about nanotechnological dust and its biological behaviour.

Small particles are classified in a number of different ways, according to the classifier and his discipline. Physicists, chemists and biologists do not seem to be always agreed. We are not much interested in academic classifications and we do not really know where micro ends and nano starts in the organism. It is highly probable that a threshold exists below which natural, physiological barriers are ineffective and even one below which cells do not oppose any resistance to the entrance of foreign bodies. Our studies do not allow us to quantify that barrier nor to say if and, in case, to what extent that threshold is influenced by size, shape, surface/volume ratio, chemistry, state of aggregation or other factors. Ours is a work in progress.

1.2 Bibliography

Ballestri, M., Baraldi, A., Gatti, A. M., Furci, L., Bagni, A., Loria, P., Rapanà, R. M., Carulli, N. and Albertazzi, A., (2001), Liver and Kidney Foreign Bodies Granulomatosis in a Patient with Malocclusion, Bruxism and Worn Dental Prosthesis – Gastroenterology,121:1234-38

Bovyn, G., Gory, P., Reynaud, P. and Ricco, J. B., (1997), The Tempofilter: A multicenter study of a new temporary caval filter implantable for up to six weeks – Ann Vasc Surg;11:520-25

Emanuelli, G., Gatti, A. M., Cigada, A. and Brunella, M. F., (1995), Physico-chemical observations on a failed Greenfield vena cava filter – J Cardiovasc Surg;36:121-5

Gatti, A. M. and Montanari, S., (2006), Retrieval Analysis of Clinical Explanted Vena Cava Filters – J Biomed Mat Res Part B: Appl Biomater 77B:307-314

Gatti, A. M., Ballestri, M. and Bagni, A., (2002), Granulomatosis associated to porcelain wear debris – American Journal of Dentistry, Vol. 15, No. 6

Montanari, S., (2000), Malattia tromboembolica e filtri cavali - Ed. C. Rabbia, G. Emanuelli – 90-140 Minerva Medica – Turin

Ref.web 1
[http://www.forbes.com/investmentnewsletters/2005/01/12/cz_jw_0112soapbox.html]