# NANOPATHOLOGY AND NANOSAFETY

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## Introduction

Much of what will be illustrated in the few pages that follow may look as having little to do with the technologies that are the subject of the school.
Actually, it is not so.
Nanotechnologies are the extremely exciting, practical application of Nanoscience and are already finding - and will continue to find at an increasing speed - more and more scientific and technological applications in many fields of interest. According to The Royal Society & The Royal Academy of Engineering [1], "total global investment in Nanotechnologies is currently around € 5 billion, € 2 billion of which comes from private sources". And further on: "one widely quoted estimate puts the annual value for all Nanotechnologies-related products (…) at $ 1 trillion by 2011-2015". Other sources report much less conservative figures.
Looking at the figures, "exciting" is an adjective we may rightfully use, but Nanotechnologies are based on the utilization of nanoparticles, and we must admit that very little is known about their behaviour when they have a chance to come in touch with or even penetrate into the organism, be that entry the result of a purposeful action or be it accidental, when not unsuspected altogether.
Nanopathology is the study of the interactions between those particles and the organism, and Nanopathologies are the diseases arising from inorganic micro- and, especially, nano-particles entered the human or animal organism.
One of the key points for the comprehension of what follows is that it doesn't matter at all how and why those tiny objects, that to our tissues are foreign bodies, are generated and introduced. And it is as important to understand that the mechanisms according to which a nanoparticle behaves once it is contained in a biological tissue do not depend on the reason why it is there. So, what follows may find application in any biological environment involving the presence of such materials.
It is essential to keep in mind that what is discussed here regards exclusively inorganic materials.

## How Nanopathology started

Back in the early Nineties, the Laboratory of Biomaterials of the University of Modena directed by Dr. A.M. Gatti was asked to investigate on a broken permanent caval filter explanted from a patient. (A vena cava filter is a metal device implanted in the lower vena cava to prevent pulmonary thrombo-embolism episodes.) The question was why that device had failed after a relatively short period of implantation, since they are built to stay implanted for life. The answer was quite simple – the device had migrated distally and, on its way, one of the legs had been trapped in the ostium of a renal vein, had bent and than broke - but our analyses showed us something we couldn't understand. On the surface of the stainless-steel device, and particularly in the fractured area, there were inorganic elements such as, for example, titanium, which did not belong either to the metal the

explanted specimen was made of or to the human organism. We wrote a case report, but, being only a single case and offering no explanation to the observation, no journal accepted to publish it.
About two years later, another explanted broken vena-cava filter was delivered to the Laboratory for investigation about its failure, and, again, we found the presence of metals foreign both to the composition of the device and to the human organism.
Then, a few years later, in 1998, Dr. Gatti had the chance to see the liver and kidney biopsies from a patient who had suffered from a number of symptoms for more than eight years and no diagnosis had been issued. Much to everybody's surprise, those tissues contained ceramic micro- and nanoparticles, whose origin turned out to be a worn out, maloccluded dental prosthesis [2]. In that circumstance, we had more efficient technical equipment available than we had in the past and we could actually see and chemically characterize the particulate. What had happened was simple enough: the patient had swallowed the debris his prosthesis had lost for years due to incorrect mastication mechanics, somehow that debris had reached his liver and kidneys, and those organs had reacted with a granulomatosis, the way tissues do when matched with an invading foreign body. Thus, the origin of that particular disease was explained, the prosthesis replaced, a suitable cortisone-based therapy started, and the patient's health improved visibly.
We began, then, to search the archives of the Universities of Modena and Mainz and of the Royal Free Hospital of London for bioptic and autoptic samples from patients suffering from diseases classified as cryptogenic, mainly forms of cancer and unaccounted-for granulomatoses. In all cases, the diseased tissue contained inorganic particulate matter.
So, on the basis of the results derived from the observations described, Dr. Gatti applied for financial support of the European Community to carry on with her research, and eventually a project, called "Nanopathology", i.e. diseases caused by micro- and nanoparticles, was approved, involving also INFM (the Italian Institute for the Physics of Matter, now part of CNR, the Italian National Research Council) and the Universities of Mainz, and of Cambridge.

## The equipment

With the funds granted by the European Community, an Environmental Scanning Electron Microscope (ESEM) was purchased, equipped with an Energy Dispersive X-ray Spectroscope (EDS). The main advantage that instrument offers to our purposes is the possibility to work on biological tissues without the need to dehydrate and coat them. The elimination of any processing makes the observation of the sample free from artefacts, and the possibility to work at room conditions allows to examine biological tissues and even living cells.
The observation the equipment permits is limited to the elements heavier than beryllium.
A brief explanation on how ESEM and EDS work is the following:
A primary electron beam is delivered and hits the specimen, which causes the specimen to emit secondary electrons.
The electrons are attracted to a positively charged detector electrode.
As they travel through the water-vapour environment of the chamber where the specimen is contained, collisions occur between electrons and gas particles. The phenomenon results in the emission of more electrons and the ionization of the gas molecules.
This increase in the amount of electrons effectively amplifies the original secondary electron signal.
The positively charged gas ions are attracted to the negatively biased specimen and offset charging effects.
As the number of secondary electrons varies, the amplification effect of the gas varies accordingly.
If a large number of electrons is emitted from a position on the specimen during a scan, there is a high signal. If only a small amount of electrons is emitted, the signal is less intense.
The difference in signal intensity from different locations on the specimen allows an image to be formed.

EDS works by detecting the X-rays that are produced by the sample hit by the electron beam. The electron beam excites the atoms in the sample that subsequently discharge the excess energy produced as X-rays. The energy of the X-rays is characteristic of the atoms that generated them, forming peaks in the spectrum. Individual elements may have more than one peak associated with them and some peaks from different elements may overlap to a certain degree. The presence of secondary peaks allows to discriminate two elements in case their main peaks overlap.
Our observations have been limited to inorganic matter, as, in the case of an organic particle, its carbon and oxygen are concealed by the same elements belonging to the biological tissue.
Thus equipped and with the new partners, a more systematic research could be started.

**The Nanopathology mechanism**

It was soon evident that micro- and nano-sized inorganic particles can enter biological tissues. According to what we observed, the most common way of entry is inhalation. (A human breathes approximately 20 $m^3$ of air per day.) Because of their size, micro- and nano-particles, no matter how they are produced, stay suspended in the air even for a very long time. From the air, they are breathed in and, once they are inside the bronchial alveoli, depending on their size, they can be phagocytized by the macrophages the way any foreign body in that anatomical district is, or, in the smaller sizes, pass directly, with a mechanism that is still to be made clear, to the blood circulation, as demonstrated also by an experimental research performed at the University of Leuven (Belgium) [3]. As we could prove, micro- and nanometric particles can be found in the blood (and cause thrombosis in predisposed subjects [4]) and nanometric debris can even enter the red cells [Fig. 1], an excellent Trojan horse to negotiate virtually any barrier.

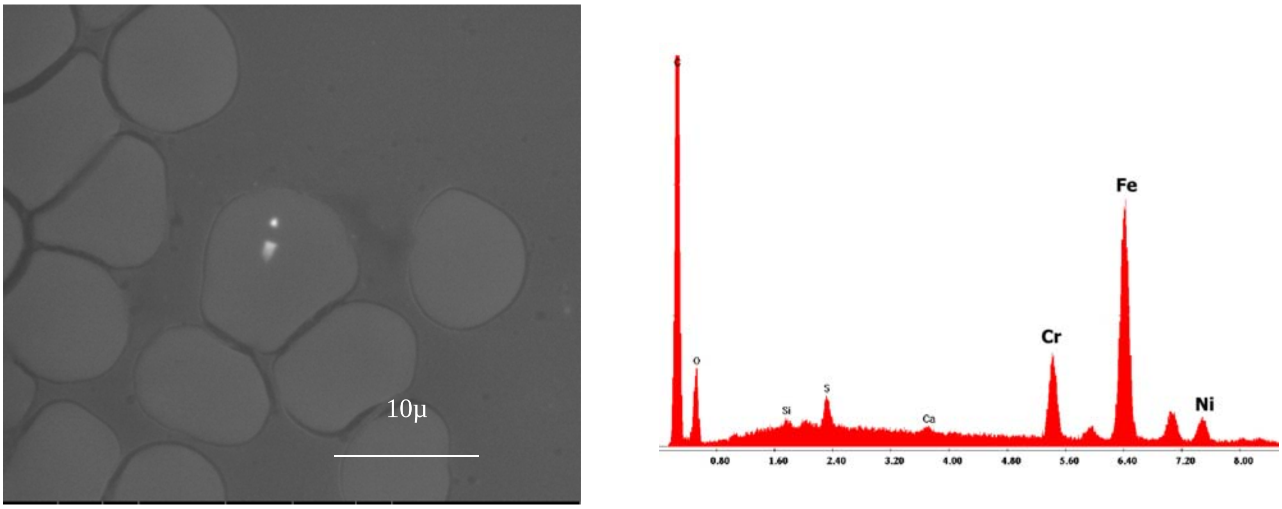


Fig. 1 - Nanoparticles in a red cell and EDS spectrum.

Whatever the modality through which they enter the blood, sooner or later those particles are sequestered by a tissue and, being not biodegradable, are impossible, or, in any case, very hard, to remove through the physiological ways of elimination. A further and certainly not negligible problem is that those non biodegradable foreign bodies are also non biocompatible. That means that, just because of their non-biocompatibility and by definition, they can induce adverse reactions, and this is what, under certain conditions, they do. As it happens with any foreign body, inflammation is how tissues generally react against that unwanted presence, and that reaction grows visible when the concentration the debris has reached is high enough. But when the particle is of nanometric size, it passes unnoticed and can enter cells, even being able to go as deep as their nucleus [Fig. 2]. It may be interesting to note that nanoparticles can pass into macrophages directly and interfere with functions as motility and ability to remove bacteria [5].

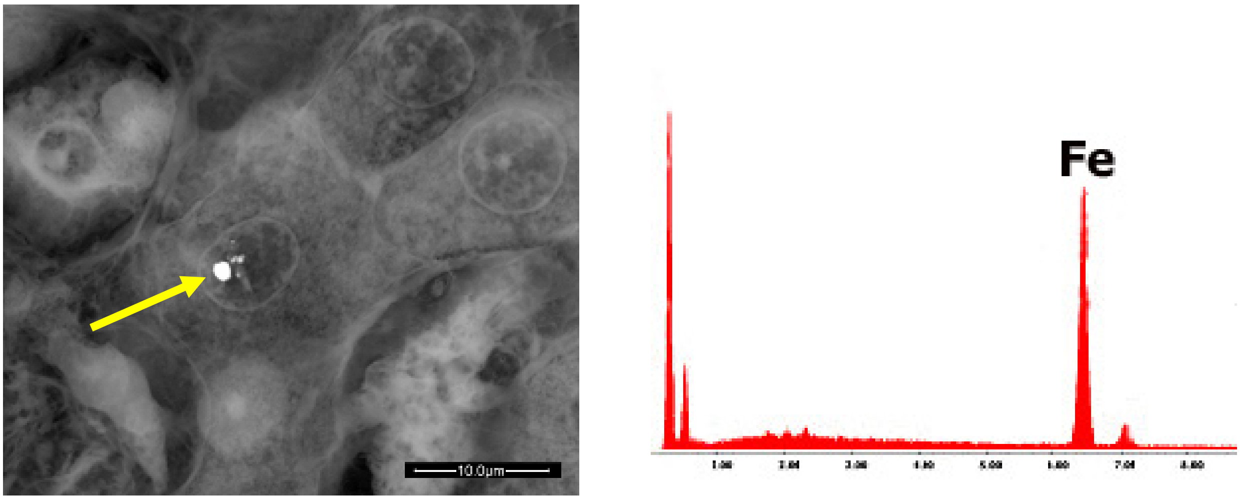


Fig. 2 – Nanoparticles in the nucleus of a liver cell and EDS spectrum.

It has been observed by other researchers that inhaled particulate matter, in that case originated by environmental pollution, causes a series of cardiovascular diseases like "thrombosis, a propensity for arrhythmias, acute arterial vasoconstriction, systemic inflammatory response, and the chronic promotion of atherosclerosis." [6] [7]

A work published in 2004 suggests that inhaled nanoparticles may reach the brain by passing along nerve axons [8], while another reports the finding of metals coming from air pollution in the brain of dogs living in urban areas [9].

After inhalation, the second most common way particles follow to enter the organism is through ingestion. In this case, larger debris, in some instances larger than 20 microns, can be found in the digestive system [Fig. 3], where neither water nor digestive enzymes nor the acidity of the stomach can solubilize the vast majority of inorganic particulate matter. Ideally, the tissues of the gastric and the intestinal wall behave like a multitude of funnels, with their lumens growing smaller and smaller. So, the smaller the particles are, the deeper they can penetrate those tissues and, if they are small enough, they get out of the "funnel" and leave the tissues to enter the blood circulation, where they meet the same fate as the inhaled particles.

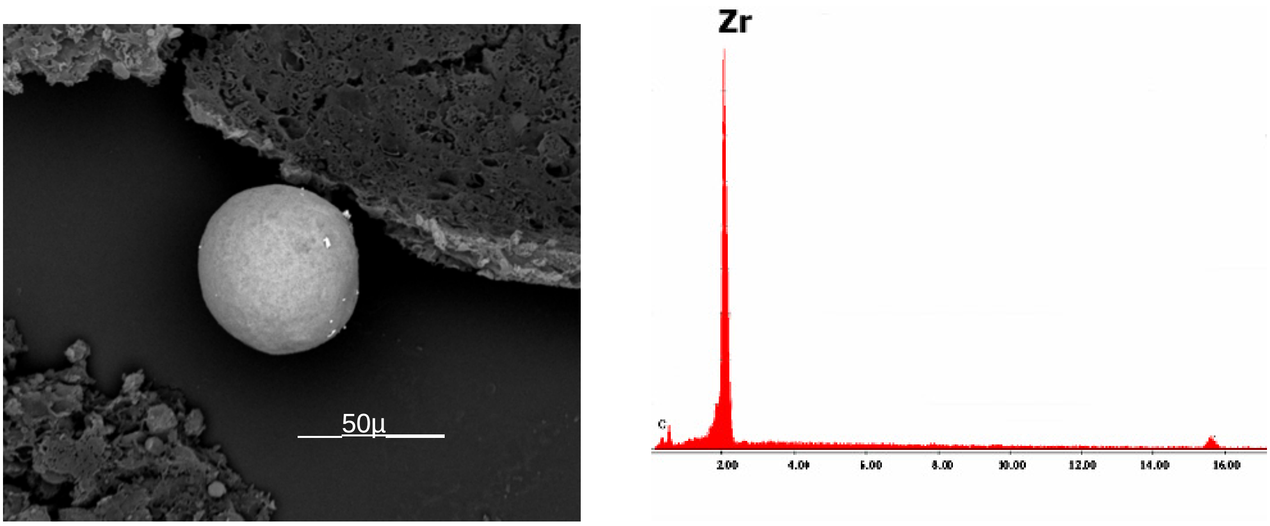


Fig. 3 – Zirconium microparticle in a stomach adenocarcinoma and EDS spectrum.

A situation where inhalation and ingestion are equally important and that may be seen as a sort of living laboratory, particularly useful for didactic purpose, is the one encountered in the areas involved in the Balkans War and in other places, which, though having nothing to do with actual war, are located in the vicinity of military firing grounds.

It is a well-known fact that the soldiers who served in that war are showing an alarming tendency to fall ill with various forms of cancers, mainly non-Hodgkin's lymphoma, and other pathologies hard to classify, particularly when they occur together. And it is another objective fact that a higher-than-usual number of civilians living in the areas where the war took place show the same problems, and that the same tendency is observed in people who had been engaged in those territories as staffers of humanitarian missions. An outstanding increase in leukaemia cases in children from all over the Yugoslavian Federation, but principally from Kladusa, Velika and Buzim, small towns close to the Croatian borderline, were denounced by Professor Edo Hasanbegovic, chief of the Paediatric Clinic of Sarajevo. In March 2000, the North Atlantic Treaty Organization (NATO) admitted the use of depleted uranium (DU) projectiles in war actions in the Balkans territories, and the next year the United Nations Environment Protection agency (UNEP) found traces of radioactivity close to Sarajevo, in a barracks at Han Pijesak and in two places inside a factory at Hadzici. After the lesson learnt from Hiroshima and Nagasaki, it is not surprising that radioactivity could be seen as the culprit of the soaring incidence of diseases sharing an up-to-then unknown aetiology. To those pathologies, some predisposing factors have been listed, among which radioactivity is one. In current military technology, besides being used to make shields for tanks, DU is employed as a component of munitions not because of its radioactive properties but because of its considerable density, hardness and pyrophoricity, qualities that make the projectiles particularly efficient as penetrators.

Pathologies very similar or identical (in particular, Hodgkin's and non-Hodgkin's lymphomas) to those suffered by subjects involved in the Balkans War were diagnosed with a higher-then-expected frequency among Italian soldiers who had never been engaged in former Yugoslavia and had never come near to weapons containing uranium, but most of them had served in firing grounds. Direct and indirect evidence of the presence of uranium, especially radioactivity, was looked for either in Yugoslavia and in the Italian areas where the pathologies were reported, but, besides the three sites near Sarajevo mentioned above, nothing certain was found. While traces of uranium were detected in the United States in the urine of some ill soldiers (uranium forms metalloproteins that deposit in the kidneys and its toxicity has been known for at least two centuries), no piece of evidence was found to propose any scientifically-based theory blaming radioactivity. New tracks were then followed to find an explanation that stood. Many soldiers deployed in the Balkans War were inoculated with multiple vaccinations, and someone suggested that the practice could have been somehow responsible for the diseases, but, again, no scientific demonstration was ever given to support the thesis, especially because, among the sick, there were subjects who had never been vaccinated. So, the question remained open and unanswered: why do soldiers and people living in a theatre of war or close to a firing ground contract those diseases with such an unusual frequency?

What we found in our laboratory in the over forty cases of the so-called "Balkans Syndrome" we had the chance to investigate, is that all bioptic and autoptic specimens of pathologic tissues and biological fluids, including sperm, contained inorganic micro- and nano-particles. It may be interesting to observe that actually we could not find any trace of particulate uranium nor of radioactivity. Bone-marrow, stomach, colon, kidney, bladder and lymph-node biopsies from subjects involved in the Balkans War or living in or near firing grounds revealed the presence of micro- and nano-debris, sometimes clustered, composed of simple or combined metals: Fe-Si, Cu-Cl-Zn, Hg, Si-Ti-Fe-Al, Si-Bi, Si-Pb, Fe-Cu-Zn, Cr-Fe-Ni, Fe-Mn, and, once, Zr.

The spherical shape of so many particles among those we detected bears witness to a particularly high formation temperature, a condition compatible with that of the explosion of a DU shell. When a DU projectile hits its target, no matter what the target is, a great heat is developed and the temperature in the immediate neighbourhood involved exceeds 3,000 °C. At such a condition, solid matter volatilizes or, in some cases, sublimes and is scattered in a rather large volume of atmosphere. In the course of that short time, new, fortuitous, alloys are formed, whose composition depends on that of mark and projectile. After a comparatively short time, the temperature of that quasi-gas gets cooler, cool enough to have the matter condense and solidify again, and the new materials, suspended in the air, acquire a spherical form (often hollow inside when their size is large enough). Most of the solid particles are so small (we detected them down to $10^{-8}$ m, but that is the limit of our instrument) as to be air-born as far as wind, rain or gravity allow, before they fall slowly to the ground, and the process may take months. But once they lie on the ground, any gust of wind is enough to blow them away again, thus restarting the process. Particles coming from Kosovo were found in Greece and in Hungary, but are likely to travel undetected much farther than that. Clouds of sand coming from the Sahara Desert, in fact, are sometimes seen in South and Central Europe and even in the Bahamas, very far from their origin, though sand particles are much bigger and heavier than most of those produced by high-temperature explosions.

During the long time they remain suspended in the air, the same way as it happens with any other air pollutant, micro- and nano-particles are inhaled by whomever happens to be in the territory involved in the phenomenon. Once they have ended their journey in the atmosphere, they may settle on fruits, vegetables and grass or fall in a lake or a river or penetrate down to a water table, and then be ingested along with food and drink by men and animals alike, thus taking a further step, as an unwelcome guest, in the food chain. From the practical point of view, it must be said that in some cases even a very accurate wash cannot eliminate completely that dust from vegetables. Brassicaceae like cabbages, broccoli or Brussels sprouts, for example, because of their rough surface, are absolutely impossible to clean thoroughly, and nobody even tries to clean wheat, barley or any other cereal. And it is also impossible to get rid of particles imprisoned in the tissues of animals used as food. From this point on, it is easy to guess what happens in the light of what we learnt.

A further confirmation of the applicability of the Nanopathology theory comes from the fact that we found micro- and nano-particles compatible by shape, size and chemistry with those detected in the diseased tissues in the soil of the territories where the patients contracted their pathologies. In those very territories, traces of uranium were scanty indeed or non detectable at all.

The reason why we never found uranium in the pathologic specimens we had the opportunity to check does not necessarily mean there was none in those patients. The fact is likely to be due to the very small quantity of that element if compared with the enormous bulk of the materials that make up the targets of the shells and get volatilized. Though unlikely, the possibility cannot be ruled out that uranium is captured by organs that are not primarily involved in the disease and that, therefore, we had no chance to examine as no non-pathological sample was sent to our laboratory. As a matter of fact, Asaf Durakovic [10] declares he found traces of uranium in the urine of some subjects, but what he detected was in ionic form and was contained in a waste of the organism. It is only obvious that, materials being the same, what can be disposed of is potentially less dangerous than something that remains forever, and cannot be a chronic threat.

A very similar scenario to the one described is offered by Afghanistan and Iraq (now and during the Gulf War of 1990-91), where soldiers and civilians fell ill with pathologies similar to those observed in former Yugoslavia. It has been reported that some veterans of the 1990-91 Gulf War showed very unusual symptoms: fatigue, shortness of breath, headache, sleep disturbance, forgetfulness and impaired concentration, all conditions that could be attributed to psychological stress; but neoplasms, in some cases appearing under different varieties in the same patient, diseases of the genitourinary system, and disorders involving the blood and the haemopoietic organs are more likely to be due to other causes. A further, apparently puzzling, problem is an increased number of birth defects in the children of the veterans. A nanopathologic origin could easily explain the presence of those otherwise inexplicable and outwardly mutually unrelated symptoms.

A third way, certainly not so important as inhalation and ingestion but still worth mentioning, particles use to enter the organism can be understood through the brief illustration of a case we dealt with.
A subject was suffering from a progressive form of paralysis which had started from his tongue, with difficulties in articulating words and swallowing. A biopsy of that muscle on which our ESEM and EDS examinations were carried out showed the presence of a particularly high concentration of zinc in particulate form. That found an explanation when the patient told us of the habit he had had for more than twenty years to use several times a day an alcohol-based mouthwash containing zinc salts. What is likely to have happened is that alcohol had been acting as a vasodilator, allowing those salts to negotiate mucosa and epithelium and penetrate the tissues of the oral cavity. Once the zinc was concentrated enough to reach saturation, it precipitated and deposited where we found it.
A like phenomenon occurs with siderosis: For want of some proteins, endogenous iron simply precipitates in the liver and takes the form of disseminated particles [Fig. 4].

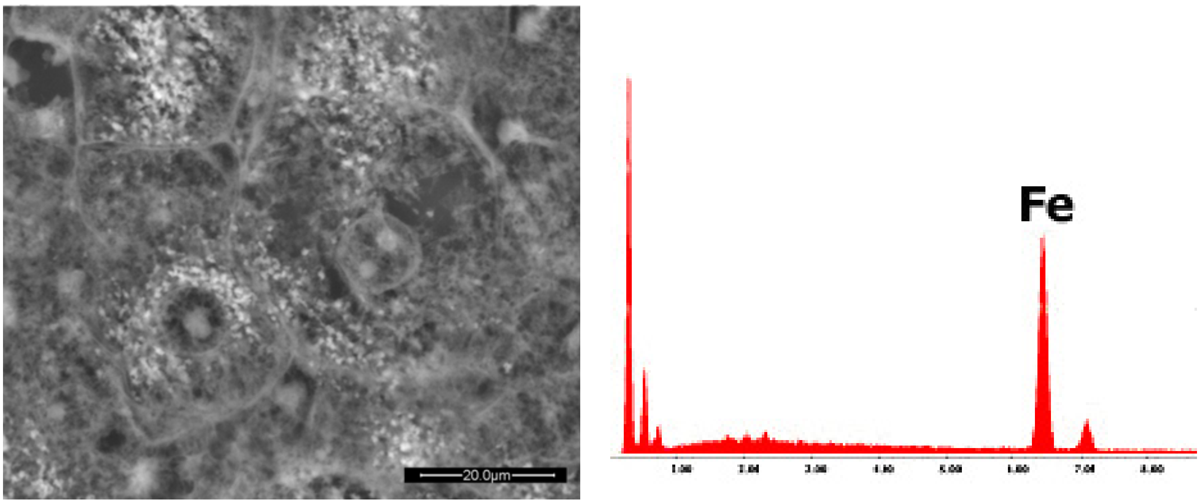


Fig. 4 – Siderosis: Iron nanoparticles in the liver and EDS spectrum.

Inhalation and direct passage through the oral mucosa are phenomena occurring together in smokers. Often tobacco leaves contain inorganic particles coming from the environment where the plantations are located or where the material is laid to dry. The cancerogenicity of particulate pollutants has been taken into account only very superficially when the much-described dangers of tabagism are listed, but that debris may be no less aggressive than the already well-known poisons tobacco contains.
Finally, there is a further route of entry to take into consideration, of which very little is known, and that is the skin. Nanoparticles are often added to cosmetics (e.g. titanium dioxide in sunscreens), but it seems that when they are applied to the skin, the particulate they contain cannot penetrate deeper than the dermis. The vast majority of the tests, though, have been carried out on undamaged epidermis and one wonders what may happen in the case of sunburns, eczema, etc. Another problem worth studying is that of iron oxide used in lipsticks, as it could be ingested rather than absorbed.

## Many questions and not so many answers

Now, a few problems arise, and the first and most obvious is: is there a cause-effect relationship between the presence of particles in the body and disease?
One of the bases of medical science is statistics, and statistics comes closer and closer to truth as numbers grow larger. Though our group keeps gathering consistent data, we do not have yet a large

enough bulk to be allowed to maintain that that relationship exists beyond any doubt, but one of the bases of science in general, when evaluating a theory, is that a theory is as good as its ability to be predictive, and every time we have the chance to come by relevant data in the field of Nanopathology, we can predict the onset of a disease and be accurate. In many instances, by the analysis of the particles detected in a patient's pathological tissue, we can travel backwards and tell what the conditions are the subject lives in, or used to live in in the past, like, for example, what the pollution in his area is or was like, and even, in some instances, what brand of cigarettes he smokes or used to smoke. Another telling indication is the possibility we experienced to induce cancer (rhabdomyosarcoma) in rats by injecting metal nanoparticles subcutaneously in their body, a reaction not obtained by implanting relatively large disks of the same material in the symmetrical anatomical site. And still a further clue is the consistent observation that debris is concentrated in pathological specimens, while the same kinds of tissue sampled from cadavers of young, presumably healthy people dead in road accidents we had the chance to check were clean. In any case, it is hard to deny that foreign bodies in biological tissues can trigger defence reactions, and that those reactions become chronic if the foreign body is neither biodegradable nor biocompatible. On the other hand, if we heed a long-established principle in medicine, non biocompatibility itself is reason enough to look at those presences as undoubtedly pathogenetic, and, on top of that, chronic inflammations have already been recognized as a condition leading to cancer [11]. And it seems as reasonable to say that foreign bodies in a tissue, including blood, in cellular nuclei or in the sperm, from where they can be introduced into an ovum when their size is small enough, are something that can be euphemistically called unhealthy.

Another foundation of science is the evaluation of the validity of a model, and a model is as good as its capability to explain phenomena. If we look at facts with an unbiased eye, we will immediately see that the nanopathological theory explains simply and clearly the aetiology of a number of "cryptogenic" diseases.

We may discuss the mechanisms involved in connecting cause to effect and that is for sure a very interesting and important issue into which interdisciplinary groups of scientists must go deeply, but the attitude of considering micro- and nanoparticles biologically harmless just because their lot in the organism is largely ignored is like burying one's head in the sand and seems ingenuous, to say the least, if one considers the admittedly not numerous but always consistent data already available. What cannot be set aside at this stage is a serious and exhaustive study about how cells, tissues and the whole organism behave when confronted with objects of such a size, where the "old" laws of biology seem to lose their validity. In fact, trying to apply those laws when the orders of magnitude are those of the nanometres is like thinking in pure-mechanics terms when trying to describe what happens within an atom. And it must be understood that nanoparticles do not behave like ions either, and it would be a big, though far from uncommon, mistake to try and assimilate the two behaviours.

## Factors influencing the pathogenicity of micro- and nanoparticles

As particles grow smaller, the ratio surface to volume increases. That means that a nanoparticle has a much grater surface area per unit mass than a larger particle. Since catalytic reactions, particularly evident with transition metals, occur at surfaces, a given mass in nanoparticulate form will be far more reactive than the same mass of material made up of larger particles.

A further, well-known property of nanoparticles is their quantum effect. As their size approaches the smaller end of the nanoscale, the effect on their electric, optical and magnetic behaviour becomes more and more visible.

Finally, the ability nanoparticles, being on the same scale as cellular components, have to cross cell membranes and evade natural defences is a peculiarity that deserves the greatest attention.

The properties briefly summarized above, probably among other that are not as well-known or are still ignored at all, are of the utmost importance to understand why nanoparticles behave in such a distinctive way and can interact so oddly with cells and organisms, from humans down to bacteria.
To assess the noxiousness of those particles, a number of factors must be taken into consideration. Probably, the most important of all is their being a foreign body, which the organism regards as an outsider or even an enemy to be somehow eliminated or, failing that, as far as possible, safely isolated; all reactions that may be unsuccessful and trigger the onset of a pathological condition. As already emphasized, it should always be kept in mind that none of the micro- and nano-particles we come across are biodegradable or biocompatible, with all the consequences those two characteristics imply. We have often observed giant cells phagocytizing inorganic particles as an immunological reaction, but once the cells die, as they are destined to do, and their organic matter is decomposed and physiologically eliminated, the particle remains in the organism unaltered, with the exception of those the giant cells had carried up along the airways and eliminated through cough and expectoration. So, phagocytosis may just mainly result in displacing inorganic debris from an anatomical district to another, so the fact that in some instances inhaled particles are not found in the lungs and do not cause pathologies directly there is misleading as to their pathogenicity.
Obviously, as a pathogenic factor, chemical nature is of the highest importance: that chromium is more harmful than iron, or antimony than titanium needn't be explained, and, according to our observations in animal models, ceramic materials are much less aggressive than non ceramic ones.
Though we have never clearly observed the phenomenon, it is not impossible that particles lodged in a tissue can corrode the way metal implantable devices do, and so alter their chemical toxicity. What we have seen is that some nanoparticles can form bonds with proteins and that is likely to be a crucial clue to explain much of the biological and pathological mechanisms we still ignore.
As to solubility in water, it has an influence on toxicity. Particles such as soluble salts may dissolve before being able to start an adverse reaction.
Than the size of the particle must be considered: large, micrometric particles are often isolated from the surrounding tissue by granulomatous and mild inflammatory reactions, while nanometric ones can either form clusters and behave in a way which is similar to microparticles' or worm their way into cell nuclei, thus, at least in theory, having the possibility to interact with DNA both physically and chemically, thus giving an explanation to some forms of cancer and foetal malformations.
A further factor is concentration: As we could see in our experience, particles, particularly the small ones, tend to gather and coalesce, and, as soon as a certain concentration threshold has been exceeded - how to quantify it, we still don't know - a pathological condition is triggered. This may not be valid for nanoparticles that have entered a cell nucleus, from where they may set off a pathology even if the cells involved are in a very low number or, we suspect, even alone.
Intake velocity does also influence the onset of a disease, as the faster the debris is taken, the highest its pathogenicity is. People who were close to the New York Twin Towers when they collapsed or were engaged there in rescue operations inhaled a large quantity of mostly high-temperature-generated dust in a very short time and, as expected, a considerable number of them contracted pathologies. Though it is sad, it is not hard to foresee that many more people living in New York or involved in the rescue operations will show symptoms of nanopathologies in the next few years.
Shape has some importance as well, as it seems that needle-shaped particles like asbestos' are particularly penetrating. And, as far as shape is concerned, as mentioned briefly above, the higher the surface area of the particle or the cluster is, the higher its chemical reactivity is and the higher its pathogenicity seems to be.
The health condition of the subject or that of one of his organs in particular are also to be taken into consideration. A compromised health makes an organ or a whole organism less resistant against the action of micro- and nano-particles, especially if the disease the subject already suffers from is a nanopathology. A pre-existent condition of inflammation or vasodilatation seems to make the passage of particles easier inside a tissue.

Last, but certainly not least among the factors to be considered, is the individual response to a stimulus. It is a well-known fact in human Medicine that single subjects react in a more or less different and variable way to a certain situation - different from one another and variable according to time and circumstance in the same individual - and that is one of the problems that exist also for Nanopathology and that make Medicine such a difficult and, in a way, unscientific discipline.

**And now?**

Now the critical, urgent question is: can we get rid physiologically of those particles stored in our tissues?
As far as we could see, the answer is No or, better, Perhaps Not. We could not clearly make out any such mechanism, but that does not of necessity mean that there is none or that an artificial one cannot be invented. If we go back to our first patient, he had undoubtedly accumulated a large quantity of ceramic particles in his liver and kidneys, but his health improved visibly when his maloccluded prosthesis was removed, thus eliminating the source of pollution. Whether that means that his organs managed to eliminate at least a fraction of those particles, we are not in a position to say, as no biopsies were taken immediately after his prosthesis was replaced nor a few months later, when his health had already improved. On the other hand, our present technique does not allow to quantify the concentration of particulate matter contained in an organ.
As mentioned above, a large number of people are already suffering from the nanopathological effects of having inhaled and ingested the dust produced by the 9/11 disaster of the New York World Trade Center (by the way, a consequence we had foreseen much in advance), and our group has been asked to help solve the problem of finding a method to treat that apparently unprecedented set of pathologies. Some preliminary experiments have already yielded encouraging results, but it goes without saying that, in order to accomplish such a task, time, men, equipment and money are needed. Such an investment is certainly worth the risk, since, if an efficacious method were found, its applications would be very numerous.
The next question is: where do particles come from?
Sources are all but endless, a few of them have already been mentioned, and new ones are being added every day. Some are natural but most are not.
As regards quantity, combustion processes are the main responsible for the generation of micro- and nano-debris, and, with the exception of volcanic eruptions and fire, no high-enough temperature is naturally attained on the Earth. So, industrial or ecological processes are to be blamed, and, among them, those used in foundries, power stations and incinerators are the commonest, while domestic heating and especially car traffic are often the greatest responsible in urban areas. It has already been observed by many researchers that when the concentration of particulate pollution increases in the air, there is an exacerbation of heart and lung disease in vulnerable people. According to The Royal Society & The Royal Academy of Engineering [1] “rises of only $10\mu g/m^3$ are consistently associated with an increase in cardiac deaths of about 1%”. An increase in cot death and stroke is also being suspected and is the subject of a few epidemiological studies in progress. The objection according to which Man has always been exposed to nano-pollution (fire, volcanoes, rock erosion, sand storms, mines, etc.) and must have grown accustomed to it may be easily refuted with the fact that the environmental concentration of nanoparticles of the XXI century coming from high-temperature processes is not comparable with that of the past, nor is nowadays’ particles’ average size the same, being it definitely smaller and, as a consequence, producing more harmful effects. The next answer to the objection is that the aetiology of some diseases that were (and in most circumstances still are) classified as cryptogenic and, therefore, not attributed to particulate pollution, is now clear in the light of Nanopathology. And the final one is that a non negligible number of affections, especially of neurologic origin, that we now recognize as nanopathologies was not diagnosed at all.

To a lesser scale, but very important to people who are assigned to that particular kind of operation, micro- and nano-pollution is the matter-of-course consequence of high-temperature welding. In many ways, generation and, in particular, behaviour of particles thus created are similar to what can be observed in war theatres. The effects on health are not so immediately visible, because the concentration of pollutants and the velocity at which that concentration is reached are much lower. Also in this case, as a rule, the smaller the size, the more aggressive and dangerous the particles are, and the smaller the size, the longer they stay suspended in the atmosphere, being also able to travel far and thus coming in contact with a more numerous population.

Especially in the past, nanoparticles were inhaled by people who worked with asbestos, but are still regularly inhaled by people who live in rooms where old linoleum floors exist and people who work where talc is used, a material not too dissimilar from asbestos.

An indirect source of particulate pollution is to be looked for in food. We completed an investigation on 135 samples of wheat-based products (bread and biscuits) coming from 10 countries worldwide, and the result was that about half of them were polluted by inorganic micro- and nano-particles. Some of that debris was clearly of environmental origin, as witnessed by its shape and chemical composition, while other, still judging from the same parameters, might have been introduced by the machinery used to grind that wheat or knead that dough.

Other food contamination of environmental origin was found by our group in vegetables, two instances of which may be of some interest because of their oddity. One regards leaves of wild chicory picked not far from our city (Modena – Italy), which had particles containing uranium compounds on their surface, the only explanation of that being an origin from a close-by ceramic industry using uranyl compounds for their coloured enamels. And the next case is a cabbage coming from the slopes of Mount Etna in Sicily and picked when that volcano was erupting, on whose rough and impossible-to-wash surface was a great quantity of nano- and micrometric basalt. In the former case, the same kind of particles we found on the leaves were detected in the cancerous tissue of a patient affected by peritoneal mesothelioma who had eaten that food for decades.

But sometimes particles are purposely introduced into food or products that are not too dissimilar from food. Some chewing-gums, for example, contain silica and zinc oxide in particulate form added by the manufacturer as an abrasive to help clean the teeth.

As reported by The Royal Society & The Royal Academy of Engineering and as we ourselves observed, “Other organisms such as bacteria and protozoa may take in nanoparticles through their cell membranes, and thus allow the particles to enter a biological food chain” [1].

A further form of particulate pollution we found, but have had no chance to study in the details it certainly deserves, is that in pharmaceutics. Micro-particles of titania, alumina, silica or talcum, not to name but a few chemicals, are traditionally considered biocompatible and liberally used as excipients in tablets, without anybody having ever fully checked their effect on chronic consumers. That they are harmless is an act of faith destitute of any observational foundation. As illustrated, those particles, especially when taken for a long time by a patient, have the possibility to induce adverse reactions and, as a consequence, the very concept of biocompatibility needs to be revised and supplemented in the light of the new knowledge achieved. In doing that, the concept of size must be introduced, since cells and organs demonstrate toxic responses even to substances that have always been considered as non-toxic but have entered the organism in a sufficient quantity in the nanometric size range.

And then there is the problem of more or less involuntary pollution in drugs, which might be particularly important in connection with the debate involving vaccines, in a sample of which we detected Zn-Al-S-Ba under particulate form.

Tobacco smoke, as already mentioned before, is also a remarkable source of particle pollution, especially because starting point and goal are physically very close, just the length of a cigarette, and there is no room enough to dilute the particles contained in smoke in a large volume of air. As a consequence, smokers inhale them in a concentrated form.

Among other causes, small enough debris is also produced by industrial cold-working procedures: the construction of roads and buildings or the slow crumbling of masonry due to ageing, though, in general, the quantities involved are often far from being like those generated by high-temperature processes.
But nowadays, nanoparticles can also be produced on purpose. The object of nanotechnology companies is, in fact, manufacturing them or, in any case, exploiting their wonderful properties. At the moment, this production is quantitatively negligible if compared with the huge mass of micro- and nano-debris generated otherwise, but the potential danger inherent in that technology must be taken into consideration both as far as working procedures and use of the final products are concerned. Inorganic nanoparticles used in a number of medical applications should be studied more carefully and more honestly, as their dynamics after completion of the task they are called to carry out inside the organism is of the greatest importance. As emphasized above, our studies could not discover any physiological mechanism to eliminate those particles once they have reached the blood circulation, and pretending they simply disappear as by magic is a totally unscientific attitude. Putting aside medical applications, among other phenomena, ageing in surfaces treated with inorganic nanoparticles is a phenomenon that should not be disregarded, since that could be a source of nano-debris available to enter the human organism. Believing as an act of faith that nanoparticles are harmless just because that complies with our wishes may show sorely disappointing in a few years, when their use has grown much more widespread than today and when time enough has elapsed to allow possible pathologies they may have originated to become visible. It must not be forgotten that mesothelioma, the fatal cancer typically caused by asbestos, can take as long as forty years before growing apparent.
On the other hand, there is a loud movement of opinion that is inclined to demonize nanotechnology and to hamper their use without the support of a scientific consciousness, much the way it happens with genetically modified organisms.
So, if we don't want to create in a hurry a big machine that will be compelled to stop abruptly as soon as we realize that we have no means to drive it safely - and that may happen suddenly, too late, and with catastrophic economic effects - or, on the contrary, if we don't want to give up extraordinary opportunities just because of fear induced by ignorance and superficiality, it is imperative that a serious plan of nanosafety study be started without delay, and Nanopathology is by far the most urgent among the many disciplines nanosafety is composed of.
The last is a crucial question: what can be done?
Two are the possible answers: Nothing and a lot.
Nothing if we keep playing the ostrich and make all possible efforts to minimize the significance of the problem, or even go as far as to pretend that the problem does not exist at all. Analogies can be made with plastics, a few decades ago regarded as the smart and cheap solution to many problems, but which have proved to have adverse effects on people and the environment. Unfortunately, and for reasons this is not the right place to discuss, pollution in general, and, as mentioned in the introduction, this variety in particular, involve huge economical, political and military interests. Nature has already started to send signals, but they are mostly ignored. Many scientists and technicians are busy inventing and implementing novel technologies working wonders, but care only superficially and far from effectively for safety to which they pay just lip service. In that they find an excellent alibi in the attitude of Medicine that, with only rare exceptions, recommends a non specific "prudence" and continues to demand more and more repetitive data on the effects of nanoparticles, devoting itself to what are now minutiae, before deciding that the problem is a problem indeed, probably without understanding that this is not prudence but its opposite, and the problem involves the whole Planet.
Energy demand is skyrocketing and in most cases this coincides with a steeply increasing side-production of particulate pollutants. Progress is growing more and more exciting and the outlook promised by many novel technologies is extremely alluring. Among them, Nanotechnologies, the technologies that make use of the fantastic properties of nanoparticles, are certainly a horse to lay a

stake on for the next future. But, like any tool, nanoparticles are neither good nor bad per se: it all depends on how one makes use of them. In any case, besides unquestionable advantages and once unimaginable possibilities that are now at hand and that would be fool not to pursue, using nanoparticles requires the consciousness of their potential short- and especially long-term consequences. Of course, achieving that consciousness costs money and seemingly slows down the speed of progress. But that is a price that must be paid and that, in the long run, will certainly be worth the sacrifice, if only in terms of health. And health is a blessing as great to nanotechnologists, industrialists, politicians, soldiers, financiers as to anybody.

One thing must not be forgotten: inorganic nanoparticles are easy to create but extremely hard to get rid of.

So, unprejudiced research and a real, efficacious implementation of nanosafety procedures are necessities that cannot be set aside, if we don't want to pay too dear for our naive lack of wisdom. And this is the lot that can be done.

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