Nanopathology : a new vision of the interaction environment-human life

By
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Summary
This article takes into consideration a new field of research called Nanopathology and describes the fundamentals of this new discipline. The paper is divided into three main sections: the in-vitro and in-vivo experiments with 5 types of nanoparticles, and the clinical pieces of evidence of the presence of particulate matter in human pathological tissues affected by diseases of unknown origin.
The in-vitro experiments verified the interaction of 5 different types of nanoparticles with endothelial, gut and liver epithelial cells and macrophages from a toxicological and immunological point of view.
The in-vivo experiments carried out on rats investigated the response to the implantation of 5 materials as nanoscaled particles and bulk disks in rats' dorsal muscles.
The clinical investigations verified the presence of inorganic micro and nano-particles in pathological tissues affected by cancer in different organs, leukaemia and lymphoma.
The main in-vitro results indicated that: a- in the presence of metallic nanoparticles (Cobalt), macrophages become unable to mount appropriate defence to bacterial challenge and danger exists of increased susceptibility to infections; b- the in-vivo tests indicated that metallic nanoparticles (and not the bulk samples) induced rhabdomyosarcoma in rats in 6 months; c- the clinical samples showed the constant presence of micro and nanoparticles in the tissues with variable chemistry, sometimes directly related to the working place exposure.
Indications of new directions of the research are discussed.

1. Preface

Objectives of the Project Nanopathology were:

1. To develop an innovative and technological method of diagnosis, capable of identifying the presence of micro- and nano-particles of exogenous nature (from the environment) in pathological processes deemed at the present time as being of unknown aetiology.
2. To employ suitable animal experimentation (nano-particles in a rat and sheep implantation model, both short- and long-term) and in-vitro models (two- and three-dimensional) to investigate pathological mechanisms of possible particle-induced disease, and assess the correlation exposure-effect.
3. To review all collected data to enable the identification of common patterns of biological reaction to the chemistry and size of micro-/nano-particles with a view to determining their pathological significance. This will form the basis of a causal therapeutic approach.
4. To improve the scientific knowledge of the role of the environment in disease development, as the micro-/nano-particles derive from exogenous sources.
5. To develop a novel diagnostic tool to detect micro-/nano-particles and thus improve patient care.

This technology was employed to check the interaction with 5 different types of engineered nanoparticles on cells and animals. At the same time, observations on pathological human tissues were carried out in order to verify the presence of micro- and nano-inorganic contaminants.
In a few circumstances, the study of the chemistry of this internal pollution allowed to identify the same pollutants in the environment and to understand the actual exposure the patient underwent.

This piece of evidence allowed to put forward possible mechanisms of dissemination of this pollution inside the body, either through inhalation and ingestion. That led to understand the possible sources of micro- and nano-environmental pollution, and its role on human and animal life. Possible mechanisms of toxicity of this nanopollution versus cells were identified and a new research project will verify the conditions for nanoparticles to trigger specific diseases like, for instance, some forms of cancer.

2. Introduction

In 2001, we coined a new word: nanopathology, meaning by that the collection of pathologies due to micro- and nanoparticles. At that time, this was a void concept, since a comparatively small number of pathologies were recognized worldwide as triggered by particulate matter. Silica particles and asbestos fibres can induce lung diseases as silicosis and asbestosis. The inhalation of dust containing particles of quartz or fibres of a silicate can also induce lethal pathologies of the lungs. It is well-known that the inhalation of mine dust or of cigarette smoke are risk factors for the onset of lung cancer. But a possible correlation with nanoscaled particulate matter was completely unknown, and that for 2 reasons: 1- in 2001, submicronic matter was recognized to be dispersed in the environment, though very few studies existed, and none of them was exhaustive, on its behaviour, but nothing had been done for nanosized particles; 2- no evidence identified nanodust as responsible of pathologies. Just micronic and (in very few instances) submicronic particles were considered as causing pathologies(e.g. pneumoconioses).

Now that nanotechnologies are a rapidly growing discipline, society is scared by possible side effects of the production, manipulation and use of nanoparticles, and nanosafety has become a priority. Many researchers are investigating the toxicity of nanoparticles towards cells, while our project has already gone further by investigating the in-vivo interaction and thir impact they have to humans.

Among the most abundant air pollutants in urban areas is particulate matter with a mean diameter < 10 µm (also called PM10 by environmental toxicologists, defined as particulate matter with an aerodynamic size of 10 µm or smaller). Over the years, it has become clear that particles with very low sizes (especially < 100 nm) are more significant health-wise than larger particles, since they have been shown to induce far more severe effects [1]. The surface/size-ratio increases exponentially with the decreasing of particle sizes, leading to enhanced surface reactivity. This enhanced surface reactivity might lead to greater biological activity per given mass, compared to larger particles, which in turn might have effects on, e.g., the internalization of particles into tissues, cells and organelles, toxicity, or the induction of oxidative stress [2]. By definition, particles with sizes below 100 nm are called nano-scaled particles (short: nanoparticles) or ultrafine particles by toxicologists.

Due to the minute size of nanoparticles, the internalization into the body's tissues appears to be extremely easy. This was shown by experiments in human volunteers with radioactive-labelled carbon nanoparticles (i.e. `Technegas`) that were shown to pass rapidly into the systemic circulation after inhalation. Radioactivity could already be detected in the blood 1 minute after inhalation [3]. Furthermore, animal studies revealed that inhaled nanoparticles were relocated into the liver [4] and the brain [5]. Thus, nanoparticles seem to be able to circumvent the tight blood-brain-barrier and possibly cross the blood-placenta barrier [6, 7]. Moreover, it has been suggested that nanoparticles are involved in thrombus formation in the blood [8, 9]. Today we know that particulate air pollution is associated with enhanced mortality from respiratory and cardiovascular diseases [10].

As the sources of internalized nanoparticles (food, air, etc.) and the location of particle detection are generally far apart, a distribution via the blood stream must have occurred. Thus, endothelial cells, which line the inner surface of blood vessels, will have direct contact with the particles. Endothelial cells are important in inflammation and wound healing. Upon pro-inflammatory stimulation of the endothelium, adhesion molecules are expressed on the cell surface, thus mediating leukocyte attachment (e.g. E-selectin and intercellular adhesion molecule-1/ICAM-1). Besides, endothelial

cells can release cytokines, such as interleukin-8 (IL-8, a key factor in neutrophil chemotaxis). Thus, these features contribute to the pro-inflammatory endothelial phenotype that permits the transmigration of leukocytes from the blood into the perivascular space [11]. The activation of IL-8, E-selectin and ICAM-1 is regulated by the same transcription factors NF-κB (nuclear factor-κB) and AP-1 (activator protein-1) [12-14].

3.Materials and Methods

The project was divided into 3 main sections: **1. in-vitro tests, 2. in-vivo tests and 3. clinical evaluations.**

1. The in-vitro tests considered the interaction of 5 different engineered nanoparticles (TiO_2, SiO_2, Co, Ni and PVC) with cells (human endothelial cells) and organs (in-vivo tests with rats and sheep). Nanoparticles of TiO_2, SiO_2, PVC, Co and Ni were examined with respect to the cellular internalisation and their influence on the cell viability, proliferative activity, and the pro-inflammatory endothelial phenotype.

SiO_2- and TiO_2-particles were produced by flame spray pyrolysis (TAL Materials Inc.). The size spectrum of SiO_2-particles was between 4 and 40 nm with 14 nm mean particle size. The size of TiO_2-particles was between 20 and 160 nm with 70 nm mean particle size. The size of Co-particles (Sigma) was between 50 and 200 nm (mean particle size 120 nm). The mean particle size of Ni-particles was about 62 nm (Nanoamor). Nanoparticles of PVC (polyvinylcloride) without phtalate were also used, but their floating in the medium due to no weight did not allow an interaction with cells. Also the aggregation of Nickel particles and their deepening in the medium did not allow a real interaction with cells, so the results, being function of a non interaction, must be fully understood.

The particles were added to the cell culture medium and tested at three different concentrations (0.5, 5, and 50 µg/ml culture medium) and the Cytotoxicity assays verified the following parameters:
Macrophage and cell survival, citokine production, modulation of TLR expression,

Also a model system that leads to the formation of in-vitro capillaries within a three-dimensional extracellular matrix (fibrin and type I collagen) after stimulation with pro-angiogenic factors was developed. This model system allowed the evaluation and quantification of pro- and anti-angiogenic characteristics of different compounds. The addition of the different particles (TiO_2, SiO_2, Ni, Co, PVC) into the three-dimensional matrix revealed that only the presence of Co particles led to changes in the in-vitro capillary phenotype. A number of abortive sprouts were formed and the developing sprouts were not so pronounced as in the positive control. However, cell viability appeared unaffected. Software-supported quantification of these Co-particle-induced changes revealed a significant reduction in the degree of angiogenesis in vitro.

2- In-vivo tests

The 5 mentioned, different materials were implanted in rats under two forms (bulk samples and nano-particulate materials). Each rat was implanted in the dorsal muscle with two similar implants. For each group, 2 rats were sacrificed after 6 months (short term), and 3 rats were sacrificed after 12 months (long term). In addition, 5 control rats were implanted with a reference material. Two of them were sacrificed after 6 months and 3 of them were sacrificed after 12 months. Five rats were fed by gavage with a mixture of nano-particles (50% Ni and 50% Zr with two different ranges) (about 0.030 – 60 mg by day): 2 were sampled after 6 months and 3 were sampled after 12 months, in order to see if the small-size particles can negotiate the bowel barrier and contribute to the pathogenesis of some diseases. Animals were sacrificed after 6 and 12 months.

After the short- and long-term implantation, samples were explanted and the gross necropsy of the animals performed. All samples were formalin-fixed and paraffin-embedded, microtome-sliced and Hematoxylin-Eosin-stained. The sections were analyzed histopathologically and under ESEM in order to verify the interface of the bulk and nanosized materials after the interaction.

3- The clinical evaluations.

More than 300 cases of tissues affected by a-cryptogenic granulomatosis of the liver; b-sarcoidosis; c- Crohn's disease; d-cancer of the liver, the lungs, the colon and the brain; e-lymphoma.
The new diagnostic tool allowed to identify micro and nanoparticulate matter inside the tissues and the Energy Dispersive Spectroscopy allowed to identify its chemical composition.
New tests were developed with body fluids like the blood, the sperm and the follicular fluid in order to verify similar foreign presences.

The project was granted also a 6-month extension as some findings needed to be investigated better.
Task 1. Analysis of pro-angiogenic M2 biasing effect of Co nanoparticles, by means of the evaluation of the angiogenic factor VEGF.
Task 2. 3D co-cultivation system of endothelial cells and macrophages. Through the quantification of E-selectin cell surface protein expression, the detection of Ki67 expression, the quantification of IL-8 release in cell culture supernatant and the quantification of E-selectin cell surface protein expression.3.
Task 3. Interaction of Co nanoparticles with endothelial cells.
Since it is known that monocytes and macrophages play a role in angiogenesis, the effects of the co-cultivation of endothelial cells together with the monocytic cell line U937 in the angiogenesis model system was tested. It was important to verify the hypothesis according to which the addition of PMA-stimulated U937 cells to the culture could lead to a distinct decrease of the length of in-vitro-capillaries and also to a reduction of endothelial cell number.
Task 4. Correlation of clinical evidence with in-vitro mechanisms The task wanted to investigate further clinical cases of different diseases and, from a certain point of view, try to identify the exposure starting from the analysis of the particles detected in the patient's pathological tissues, and from another try to correlate this possible trigger of the pathology, putting forward possible mechanisms of dissemination of the particulate matter inside the body and the pathomechanism versus cell.

Results

1- The in-vitro results verified that:

Whereas the ceramic particles of TiO_2 and SiO_2 did not show significant cytotoxic effects, the nanoparticles of Co and Ni induced a significant, concentration-dependent impairment of cellular viability. This impairment was observed at different levels (i.e. decrease of cell number, protein expression of the proliferation marker Ki67, and the metabolic activity).
A pro-inflammatory effect in HDMEC occurred after exposure to SiO_2-, Co-, and Ni-particles and was apparent by an enhanced release of IL-8. Only higher particle concentrations (25 and 50 µg/ml) induced this increase in IL-8 release. The E-selectin protein expression was enhanced by high amounts of Co-particles whereas Ni-particles induced no protein expression of E-selectin. In contrast to the particles, divalent Co- and Ni-ions induced the expression of all pro-inflammatory markers tested (i.e. IL-8, E-selectin, ICAM-1).
In detail:
a- The investigated nano-particles do not affect cell survival or proliferation. Only Co nano-particles are toxic at >100 mg/10^6 cells. They inhibit the expression of LPS receptors TLR4 and CD14 and induce a down-regulation of mRNA expression for TLR4 and CD14 (The two receptor chains which recognise bacterial lipopolysaccharide and activate macrophage defence functions).
An interesting result is that Co nano-particles impair macrophages activation by bacterial LPS, in fact a negligible cytokine production was detected. These results mean that in presence of these metallic nanoparticles the macrophages become unable to mount appropriate defences to bacterial challenge. That means that danger exists of increased susceptibility to infections. These very interesting results were not obtained with Nickel nanoparticles since, due to their forming micrometric aggregates and sinking in the medium, they did not interact with the cells.
b- The Cobalt nanoparticles with endothelial cells showed an angiogenic behaviour,

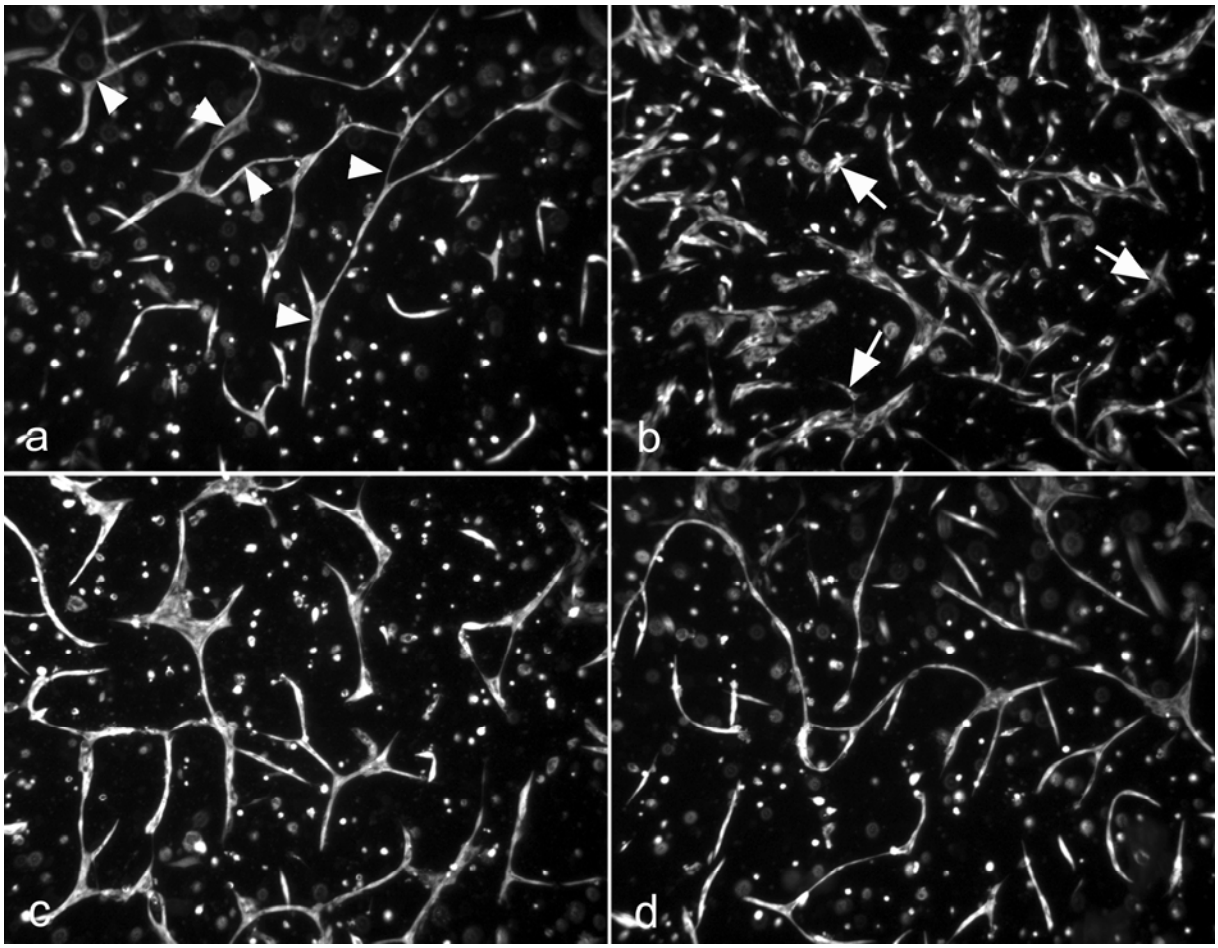

Fig. 1: 3D-angiogenesis a) untreated control (arrowheads mark a contiguous in vitro capillary-network consisting of a number of endothelial cells), b) Co-particles (arrows: abortive sprouts), c) SiO_2-particles, d) ZrO_2-particles (fluorescence images of a vital staining)

The analysis of images by an optical phenotype evaluation revealed that only Co-particles induced an impairment of angiogenesis in vitro (Fig. 2b). This impairment was characterized by the occurrence of a number of abortive sprouts (arrows). Furthermore, the developing sprouts were not as pronounced as in the positive control (i.e. shorter sprouts with a larger diameter). However, the cell viability appeared unaffected, as the cell number was not reduced. All other particles (TiO_2, SiO_2, ZrO_2, Ni, PVC) did not induce apparent deviations (2c - SiO_2-particles, 2d - ZrO_2-particles) in comparison to the untreated control (Fig. 1)

Our study has shown that human endothelial cells possess a large capacity for the internalization of nanoparticles. All nanoparticles tested were taken up by the endothelial cells and to a major extent into vacuoles

The Cobalt nanoparticles showed an angiogenic behaviour, that can trigger a carcinogenic reaction.

The results during the extension of the project induced to put forward the hypothesis that the observed pro-inflammatory activation after Co-particle exposure may be attributed to a release of divalent Co-ions by the particles, since the exposure of endothelial cells to these ions leads to the impaired endothelial viability and pro-inflammatory stimulation. This contrasts with the effects of the Ni-particles. Here, the suggestion of a Ni-ion release by the particles resulting in an induced

pro-inflammatory stimulation is not congruent with the pro-inflammatory effects induced by the respective ions, since Ni-ions induced both an increase in the release of IL-8 and the protein expression of endothelial cells adhesion molecules (i.e. E-selectin and ICAM-1), whereas Ni-particles induced only an increased IL-8 release and the expression of adhesion molecules was not initiated. This indicates an activation mechanism for the Ni-particles that deviates from the Ni-ion-induced activation that is shown to occur via a cooperation of the above-mentioned transcription factors NF-κB and AP-1. Since oxidative stress is also a relevant aspect in the mechanisms of (Ni-) particulate matter-induced effects [32], this mechanism of differential activation of pro-inflammatory gene promoters might play a role. Thus, it can be suggested that the Ni-ion release by the nanoparticles remains under the critical limit for pro-inflammatory activation but further Ni-nanoparticle induced effects (possibly oxidative stress) are responsible for the enhanced IL-8 release. Also the tests with the co-cultures (macrophage-endothelial cells) indicate a deep interaction of Cobalt nanoparticles with the cells. The addition of PMA-stimulated U937 cells to the culture led to a distinct decrease in the length of in-vitro capillaries and also to a reduction of endothelial cell number. In addition to that, the number of U937 colonies within the 3-dimensional extracellular matrix rose.

These in-vitro results indicate some mechanisms of actions of nanoparticles with cells and explain the in-vivo results and the clinical evidence.

2- In-vivo tests

Our animal studies verified that the metallic nanoparticles were cancerogenic, while the bulk material induced just a fibrotic capsule with a granulomatosic reaction

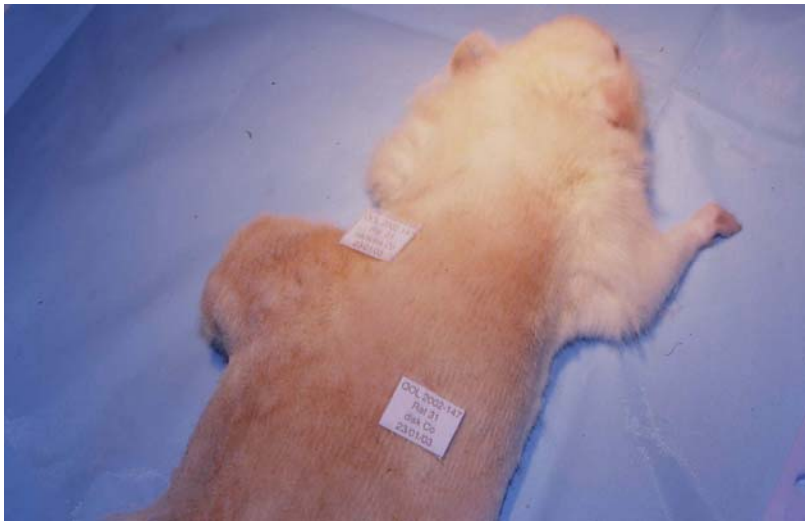

Fig.2 Cobalt and Nickel nanoparticles induced rhabdomyiosarcoma in the rats' back . The metallic disks induced only fibrotic capsules.

The other materials (PVC, Silica, and Titania) developed only a fibrotic capsule or a granulomatous tissue. The reason could be the agglomeration of the nanoparticles that transform them to micrometric debris. In this case, the body reacts to the size of particulate matter, as the materials are chemically inert.

The feeding of rats with Zirconia and Nickel showed that there is a passage to the internal organs for Zirconia, but not for Nickel. The ESEM analyses carried out in the internal organs verified only the presence of few particles of Zirconia. The Nickel particles were not found . A possible explanation is that their aggregation formed microparticles eliminated through the faeces.

The observation of the section of the site of implantation of nanoparticles in the sheep's knee joint was negative, since the nanoparticles were not found in the implantation site. That was the demonstration of their dissemination in other parts of the body due to the extracellular fluids.

3- Clinical observations.

The study of our series of pathological samples showed the following interesting findings:

1- In the cases of biological reactions typical of some diseases like cryptogenic granulomatosis, sarcoidosis and Crohn's disease, foreign bodies were always present inside the granuloma. In some cases, the chemical composition of the particulate detected made it possible to identify the kind of exposure the patient underwent. Just as an example, the case of a dentist who had developed a lung sarcoidosis showed a close correlation between the subject's disease and the powder he had used to bleach his patients' teeth. In 20 years he had employed about 300 kilograms of such powder, a part of which he had certainly inhaled and whose concentration in his organism had probably exceeded the tolerable threshold concentration, and that had triggered the disease.

2- Micro and nanoparticles were always present in cancerous tissues. It must be emphasized that this constant presence was due to a pre-selection of the cases we observed. In a number of patients suffering from cancer we had the chance to check outside the scope of this project, no particles were found. This is only natural, as predisposing and causing factors exist, such as genetics, radiations, exposure to organic solvents, etc. In the cases investigated in this project, those inorganic foreign bodies were concentrated at the interface between primary cancer and healthy tissue. This specific location, never discovered before, seems to be meaningful in understanding the mechanism of the onset of cancerogenicity. In two cases of liver cancer, particles were detected inside the nucleus of living cells.

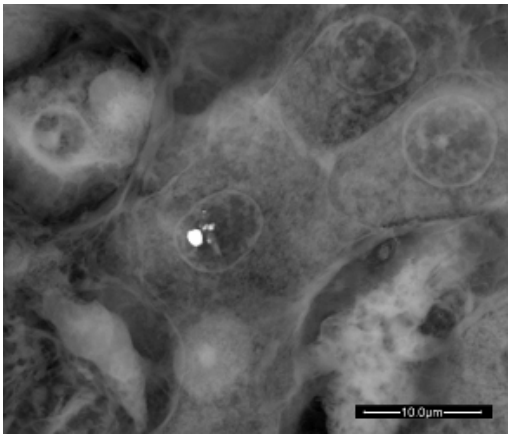

Fig.3 ESEM image of a cancerous tissue of liver with a living cell containing nanoparticles in the nucleus

3- Specific observations were carried out in soldiers exposed to high-temperature combustion processes involving the blast of Depleted Uranium weapons. Our original aim was to find Uranium nanoparticles in the bioptic samples we received, but what we found instead of Uranium was metal dust coming from target and bomb volatilized by the heat generated by Uranium and re-condensed under nanoparticle form. Inhaled and ingested nanoparticles can negotiate either the alveolar and the digestive system walls, migrate to the blood and be carried virtually to any organ. We had the chance to check some forty Italian cases, three French and one Canadian. In addition, we examined ten cases of civilians from Sarajevo and as many cases of sick civilians living in the vicinity of a firing ground. By using a Field-emission Environmental Scanning Electron-Microscope, we could detect 10-nanometer Br, Cl and Sb particles.

4- Sperm samples belonging to dead soldiers who were active in war territories were referred to our laboratory and we developed a novel technique to be able to test them. In all cases, nanoparticles were present, while nothing was found in similar samples coming from healthy subjects we used as reference.
5- Having found dust dispersed in the sperm, we checked if malformed human and animal foeti contained that particular form of pollution. We had the possibility to test two malformed lambs whose parents grazed close to a firing ground and eight livers from foeti affected by Neu-Lexova syndrome we received from Malta. As a matter of fact, inorganic particulate matter was visible.
6- An American scientific society had become aware of our studies and asked us to help them find a way to detoxify the subjects who contracted diseases for having inhaled dust during the collapse of the World Trade Center of September 11 in New York or during the rescue operation. To do that, we developed a particular technique to analyze the patients' sweat.
7- Having found evident traces of inorganic particulate pollution in some pathologies of the digestive system, we checked if food could be the carrier of such a contamination. So, we tested some 200 samples of food and in about half of them micro and nanoparticles were found coming either from environmental sources or from the wear of the machines used to work it.
8- The reference standards we used as healthy tissues were the organs of young people dead in road accidents. Once, we could not use the tissue harvested from one of those cadavers, as it contained Calcium carbonate. It turned out that that boy had been drug addicted and the mineral was used to cut the drug. After the 3-year project, now the standards are represented by the internal organs of foeti of induced abortions. Their tissues are "clean", since they were not exposed to environmental pollution
9- The study mentioned above involved the verification of malformed (animal and human) foeti. In a few instances, dust was detected in their tissues, and that showed that nano-contamination can be shared between mother and child through the umbilical circulation.

The new diagnostic tool developed allows to detect inorganic particulate matter present in pathological tissues. Its identification is a fundamental step to identify the possible source of pollution and the kind of exposure the patient suffered [15-24].

Discussion

The results obtained through our research should induce us to revise our way of understanding the impact of environmental pollution on our health. We should start by considering a simple natural law, stating that nothing can be created and nothing destroyed, but everything can just be transformed. It is a matter of fact that all forms of combustion change matter and produce micro- and nanoparticulate whose size depends mainly on the temperature reached in the crucible, and that most of that particulate, which, in many instances, is neither biodegradable nor biocompatible, gets dispersed in the environment. So, the air, the soil, water, vegetables are polluted and both humans and animals are the victims of that condition through the inhalation of the air and the ingestion of water and vegetables. The situation grows worse to humans who eat animals whose flesh is polluted, and nowadays that pollution may be imported from places which are very far from where those humans live. In our century, it is particularly hard to set boundaries to everything and pollution is no exception.

What the majority of the systems aimed at getting rid of wastes do is reduce them to a very small size, sometimes incomparably smaller than the original, and disperse them. The problem is that the smaller the size of that dust, the more penetrating and aggressive to the organism it is, and making it smaller and smaller does not seem particularly wise.

One of the most promising results of our research is the possibility it offers to trace back the source of pollution and the kind of exposure the subject underwent, by comparing what we detect in his

pathological specimens with the environment where that subject lives or work, or the food he eats. So, it will not be too difficult to understand whether the strategy of having wastes “disappear” by making them small enough to become hard to detect or we had better resort to different solutions.

In conclusion, what is necessary to do is:

1- study in a meaningful, statistical way homogenous cohorts of patients, living in the same environment and having developed the same disease. The environment to investigate may include risky working places such as incinerators, power plants, burning oil wells, cement works, firing grounds, etc. The information gathered will be used to set up prevention programmes;

2- study food “from the fork to the farm” and detect what the sources polluting it are. The study should also include animal feed. As our method allows to work on archived biological samples, “mad cow” disease could and should be part of the study, in order to verify if cattle were actually fed with exhausted lubricants mixed with animal flour. A focused study could be carried out on the poltry food in order to verify if there is a nanocontamination. If so, the infected animals must be verified for this contamination and the interaction nanoparticle-virus should be evaluated ;

3- a) analyze as basic research ice carrots in the Antarctica to check if environmental nanopollution was already present in the past and to what extent. Hiroshima and Nagasaki destruction and Chernobyl accident with its Cesium dust could be particularly interesting. b) Then, again as basic research, how bacteria and parasites behave and develop after having interacted with nanodust should be studied. c) Viruses are of the greatest interest to study how they adhere to nanoparticles and how (and if) they change, for example in pathogenicity, after having interacted. d) Nanodust seems to be teratogenic and this is an issue that certainly deserves to be deeply investigated;

4- develop traps and filters to capture nanodust and sensors to detect and measure it. Without any doubt, this will prove very useful to nanotechnology industries to protect workers;

5- develop a network of infrastructures to monitor nanodust in the environment, capable of alerting people in case of danger (see, for example, what was not available at Chernobyl);

6- set up courses and schools to educate scientists and technicians in this new, particular field of environmental science. This is an urgent necessity to nanotechnology companies producing or working with engineered nanoparticles.

7- Special attention must be paid to security, since nanoparticles can be silent and not expensive bullets to the cell nucleus and could be used as such.

Further directions of research can be the study of the so-called industrial sterility, malformed foeti in specific industrial areas, rare diseases and new pathologies investigated, but not yet understood. Epidemiological studies with homogenous cohorts of patients should be carried out, where, starting from the analysis of the embedded particulate matter, exposure is verified and the source of pollution is identified.

The results of our research have been readily applied by the Italian senatorial commission dealing with the problem of pathologies contracted by peacekeeping troops. A not negligible number of them were in the vicinity of the explosion of high-temperature rounds (e.g. depleted-uranium or tungsten weaponry) or were involved in the destruction of weapons which was carried out by heap them up in large holes dug in the ground and have them burn. Some of those soldiers were also close to burning oil rigs. In all those cases, they inhaled particulates produced be the combustion of large quantities of matter and, inevitably, inhaled and, sometimes, ingested them. Part of those soldiers developed diseases that can be classified as nanopathologies.

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