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# BIOLOGICAL ASSESSMENT OF MEDICAL DEVICES CONTAINING NANOMATERIALS

## Scientific Report

## FOREWORD

Knowledge and practices in healthcare are constantly changing thanks to new emerging technologies that improve the quality of diagnostics and patient care. However, this profusion of innovations is not always easy to follow for everyone.

The mission carried out by the French Health Products Safety Agency (Afssaps) is to monitor and ensure the safety of healthcare products. Especially, it is responsible for the evaluation of the benefits and risks associated with the use of medical devices. It may thus propose recommendations in particular.

The main aim of such health safety recommendations is to summarise scientific knowledge for manufacturers, healthcare professionals and patients/users, and to provide expert opinions on a given topic. They thus assist in decision-making by defining what is appropriate, what is inappropriate or no longer appropriate, and what remains uncertain or controversial.

Recommendations on the biological assessment of medical devices containing nanomaterials, which are presented in this document, were formulated by a group of multidisciplinary, scientific experts appointed and named in the *Bulletin officiel Santé-Protection sociale – Solidarité* n°2010/11 (Official Bulletin - Health and Social Protection - Solidarity No. 2010/11) dated 15 December 2010 based on decisions taken by the General Director of Afssaps on 13 October 2010 (see Appendix I).

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## SUMMARY

The fostering of technologies on a nanometric scale, namely nanotechnologies, allows innovative applications to be developed. Many medical devices containing nanomaterials are currently under development and some have already been launched on the market. However, as few studies on nanoscale materials are available and since their properties sometimes differ from those of their bulk counterparts (*i.e.* without a nanometric dimension), the related health risk remains unclear.

Based on these facts, the French Health Products Safety Agency wants to establish whether conventional evaluation methods are suitable to assess properly the risks associated with the use of medical devices containing nanomaterials. Therefore, in 2010, it set up a work group consisting of multidisciplinary experts whose mission was to evaluate current knowledge of medical devices containing nanomaterials and their biological assessment. Then they were asked to put forward recommendations for manufacturers involved in the development of this type of medical device. The findings of this group are documented in the report entitled "Biological assessment of medical devices containing nanomaterials".

The first part of this report gives an overview of the advances brought about by nanomaterials and their use in medical devices already on the market and those under development. The second part outlines current knowledge of the biological effects of nanomaterials. The third part focuses on the current regulatory and standardisation framework to examine its suitability for medical devices containing nanomaterials. Finally, based on these analyses, the report gives recommendations for the manufacturers of medical devices containing nanomaterials to improve the biological risk assessment during the life cycle of the medical device: from design via application all through to recycling after use.

In conclusion, current guidance documents are appropriate for the biological assessment of medical devices containing nanomaterials. However, case-by-case adaptations are required in order to take into account the specific features of the nanomaterials. Firstly, as in any medical device evaluation, the favourable benefit/risk ratio must be highlighted. More specifically, the benefits of adding nanomaterials to the medical device must be discussed (benefits should clearly outweigh potential risks). Secondly, the recommendations proposed by the French Health Products Safety Agency provide support for the manufacturers of medical devices containing nanomaterials. These recommendations mainly concern information disclosure and transparency, the identification and characterization of the materials used and the precautions to be taken when evaluating biological risks.

The constantly evolving world of nanotechnologies presents a real challenge for scientists and regulatory authorities alike. To promote knowledge and innovation whilst ensuring better risk control, international, co-ordinated, multidisciplinary actions must continue in order to improve the quality and safety of products used by healthcare professionals and patients. The French Health Products Safety Agency pays specific attention to new medical devices containing nanomaterials launched on the French market.

# INTRODUCTION

The fostering of technologies on a nanometric scale, namely nanotechnologies, allows innovative applications to be developed. In fact, the specific physico-chemical and biological properties of nanomaterials are used in various domains such as electronics, energy, cosmetology, medicine and pharmacy. These extremely varied promising nanomaterials could have a considerable impact in the field of medical devices with regard to the diagnosis, treatment and prevention of diseases.

Many medical devices are currently under development and some have already been launched on the market. However, as nanoscale materials have not been sufficiently studied and since their properties sometimes differ from those of their bulk counterparts (*i.e.* without a nanometric dimension), their potential effects on health remain unclear. Consequently, the French Health Products Safety Agency is currently concerned with establishing whether conventional evaluation methods are appropriate for properly addressing the risks associated with the use of medical devices containing nanomaterials.

The aim of this work is to establish recommendations for manufacturers for the analysis and assessment of the biological risks associated with medical devices containing nanomaterials. This applies to all medical devices (including active implantable medical devices and *in vitro* diagnostic medical devices) containing nanomaterials and likely to come into contact with the patient's or user's body. This report thus aims to provide material for reflection in evaluating the benefit/risk ratio in a health safety framework. It is based on a summary of current scientific knowledge relating to the biological properties and behaviour of nanomaterials.

A bibliography and market analysis of medical devices containing nanomaterials was performed up to November 2010. The first part of the report provides an overview of the innovations brought about by nanomaterials and their use in medical devices already on the market and those under development. The second part outlines current knowledge of the biological effects of nanomaterials. The third part focuses on the current regulatory and standardisation framework to examine its suitability for medical devices containing nanomaterials. Finally, based on these analyses, the report gives recommendations intended for the manufacturers of medical devices containing nanomaterials to improve the analysis and biological risk assessment during the life cycle of the medical device: from design via application all through to recycling after use.

Only issues associated with medical devices containing nanomaterials have been investigated in this study. The health-related consequences of exposure to nanomaterials of a professional, industrial, food, cosmetic or environmental origin are not studied. The risks are inherent to a defined application and cannot be transposed or extrapolated from one context to another: each case must be considered individually.

# I. State of the art relating to nanomaterials used in medical devices

The prefix **nano**, taken from the Greek word *νανος* meaning *dwarf*, refers to a billionth of the base unit: a nanometre is therefore a billionth of a metre ( $10^{-9}$  m or nm). As far back as 1959, physicist Richard Feynman introduced the concept of nanotechnology in his presentation entitled, "There's plenty of room at the bottom", which considered handling molecular and atomic materials as macroscopic objects. Nanotechnologies took off in the 1980s mainly thanks to the invention of two optical microscopy instruments allowing observation of and interaction with material on an atomic or sub-atomic scale: the scanning tunnelling microscope (STM) in 1981 and the atomic force microscope (AFM) two years later. A few years earlier, the researchers started to use changes in physico-chemical properties when shifting from a macroscopic scale to a nanometric scale, to create new multifunctional materials with better performance profiles. Some nanomaterials have been used for their properties since Antiquity but it was with the dawning of the XXI<sup>st</sup> century that industrial productions diversified in terms of the chemical nature of the nanomaterials manufactured, with fullerenes and the miniaturisation of electronic components.

Among the sectors involved in nanotechnologies such as energy, the automobile and construction industries, clothing, cosmetics and food, the Health sector is pinning its hopes on nanomaterials and nanometric devices to revolutionise this particular field. Nanomedicine promises fundamental changes in terms of prevention, diagnosis and treatment of diseases using nanosystems with targeted complex systems to repair or treat cells. Applications have been developed in surgery, diagnosis, cancer treatment, molecular imaging, medical devices and tissue engineering, to name but a few. Many devices - the fruit of nanotechnological research - are currently under development and some have already been launched on the market.

## I.A. Terminology

The exact definition of the terms used to describe the technology and materials used on a nanometric scale are still under discussion world-wide at scientific, industrial and legal level. Nevertheless, the universally accepted definition refers to materials on a nanometric scale as those with at least one dimension between 1 and 100 nm and with the concept of new physical, chemical and biological properties specific to this small scale. When this report was written, given that there was still no clear-cut international consensus with regard to terminology, **we used the definitions provided by the International Organization for Standardization (ISO)**,<sup>2</sup> Consequently, the meaning of the various terms used is detailed as following, in order to ensure a better understanding of this document.

- **Nanoscale**

According to the ISO definitions, **nanoscale** refers to the range in dimensions between approximately 1 and 100 nm.<sup>2</sup> It should be noted that the borders are approximate, indicating a range in size that is typically but not exclusively between 1 and 100 nm. The lower limit size was introduced to avoid single atom or small groups of atoms from being assimilated with nano-objects or elements of nanostructures. Similarly, although exceeding the 100-nm scale, some objects are considered as nano-objects if their characteristics are different from those extrapolated from the same object on a bigger scale.

- **Nanotechnology**

**Nanotechnology** refers to the manipulation and control matter on a nanoscale to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms, molecules or bulk materials. The terms "manipulation and control" include material synthesis.

Nanotechnologies therefore involve the production of structures, devices and systems using processes that allow the material to be structured on a nanoscale.

- **Nanomaterial**

**Nanomaterials** play a crucial role in nanotechnologies. Nanomaterials are materials, with one, two or three external dimensions in the nanoscale (**nano-objects**), or having internal structure or surface structured on a nanoscale (**nanostuctured materials**) and present with one or more new physical, chemical and biological properties specific to this small scale. This generic term includes nano-objects and nanostructured materials.

It is useful to distinguish natural and incidental nanomaterials from their manufactured counterparts. **Manufactured nanomaterials** are produced intentionally by man within an industrial or research setting to have specific properties or specific composition. Conversely, **natural nanomaterials** can be nanoparticles or nanometric aggregates originating from space, volcanoes, forest fires, minerals such as clay or the decomposition of animal skeletons. **Incidental nanomaterials** of anthropic origin refer to atmospheric nanoparticles or ultrafine particles emitted by traffic, incinerators or in factory smoke, etc. They can also form in the atmosphere as by-products of volatile organic pollutants.

**Free nanomaterials** refer to nanomaterials that are not encapsulated or connected in some way to prevent them from being released in the organs, tissues or cells of the user.

**Nano-reinforced materials** include nano-objects in their matrices to introduce a new function or to alter physical and mechanical properties. Nanocomposites are a typical case, for instance used to heighten resistance to wear and tear in mechanically reinforcing applications.

- **Nanostructured material**

A **nanostructured material** refers to a material with a surface or internal structure on a nanoscale and possessing one or more new physical, chemical and biological properties specific to this small scale. A material with a surface containing nanometric pores is a typical example of a nanostructured material.

The surfaces of materials can be nano-structured by incision, lithography, the inclusion of nanoparticles or with a nanoscale coating. These coatings are generally obtained by physical or chemical deposits (plasma, electrochemistry and laser ablation, etc.).

- **Nano-object**

A **nano-object** is a material with one, two or three external dimensions on a nanoscale. The morphology of nano-objects varies.

A **nanoparticle** is a nano-object with three external dimensions on a nanoscale. Nanoparticles are not all spheres but may have the shape of needles, extended rods, spring structures, etc.

Nano-objects with two external dimensions on the nanoscale and a larger third dimension include **nanofibres, nanotubes, nanofilaments** or **nanorods**.

Nano-objects with one external dimension on the nanoscale and two other substantially larger dimensions typically include **nanofilms, nanolayers** or **nanocoatings**.

## **I.B. Specific features of the nanoscale**

The properties of a material depend on its structure and chemical composition. However, switching to the nanoscale can trigger changes in key physico-chemical characteristics. Below a certain dimension, it is possible to change one or more of the physico-chemical properties: melting point, magnetic, electrical, optical properties, etc. The variation can sometimes be sudden such as super paramagnetism.

### **I.B.1. Dominant surface effects**

An increase in the surface/volume ratio is a direct consequence of size reduction. The smaller the object, the greater its external surface in relation to its volume.<sup>3</sup> A solid silver sphere weighing 10 g has an external surface area of approximately 5 cm<sup>2</sup>, whereas, for the same mass, nanoparticles 10 nm in diameter have a total external surface area of approximately 600 m<sup>2</sup>, *i.e.* a 10<sup>6</sup>-fold increase in

the exchange surface area. A microcrystal (1  $\mu\text{m}$ ) of iron presents less than 1% of its atoms on the surface whereas a nanocrystal (1 nm) has more than 90% on its surface.<sup>4</sup> Consequently, the specific surface area (external surface area per unit of mass), which is the real exchange surface area, is greater for nano-objects.

Nanomaterials are often characterized by a crystallised, orderly centre with a locally disordered, even amorphous surface. Given the large proportion of atoms on the surface of a nano-object, the crystalline network is subject to constraints, which lead to deformities and re-arrangement of the atoms. This configuration therefore alters the phenomena appearing on the external surface, essentially adsorption, absorption and the binding of external chemical species. This specific chemical reactivity of nanoparticles is therefore widely used in chemical catalysis and also in biological applications.

## **I.B.2. Agglomeration and aggregation**

The thermodynamic instability at the surface of nano-objects makes the latter highly reactive. Increased surface energy and the high dispersion rate specific to nanoparticles contribute to the agglomeration and aggregation phenomena often observed with these materials.<sup>5a, 5b-d</sup> Such behaviour has been documented and investigated in the colloidal studies.

An agglomerate is an association of particles bound by weak forces (Van der Waals, electrostatic or surface tension) with the particles being adjacent to each other. Agglomerate morphology is not a property of the nanomaterial but the result of a temporary state of dynamic balance between the effects of dispersion and agglomeration in a suspension or aerosol.

The aggregate is a heterogeneous nanoparticle in which the various components are linked by strong bonds (covalent type). It may be due to the fusion of several primary nanoparticles; the aggregation properties determine the final form of the aggregate (compactness, dimensions, etc.) and its interaction with the surrounding environment (e.g. penetration of the respiratory system and cells). All of this information may prove crucial in the biological assessment of medical devices containing nanomaterials.<sup>6</sup>

## **I.B.3. Effects of size on thermodynamic properties**

If the decrease in size falls below the critical nucleus, the crystal becomes unstable. This triggers either a change in the crystallographic structure or an increase in volume.<sup>7</sup> An increase in the surface/volume ratio causes pressure towards the interior of the sphere and triggers changes in the thermodynamic properties of the material such as a decrease in the melting point, a reduction in latent heat and an extension of the phase coexistence region. A drop in the solid-liquid phase transition temperature can be observed with size decrease.<sup>8</sup> For example, the melting temperature of gold exceeds approximately 1000°C for nanoparticles of 10 nm, and around 500°C for nanoparticles of 2

nm. This melting point depression is particularly drastic for nanoparticles reaching critical diameter, smaller than 5 nm. However, for nanoparticles incorporated in a matrix, the melting point can be higher or lower depending on the force of the interaction between the nanoparticles and the matrix.

#### **I.B.4. Effects of size on mechanical properties**

In some cases, a reduction in the grain size can lead to lighter products whilst maintaining the same physical and mechanical properties compared to conventional bulky materials, or even improving them.<sup>9</sup> For instance, copper formed from nanocrystals of 6 nm is five times more resistant to mechanical deformation than microcrystals of 50  $\mu\text{m}$  because the small size limits internal structural imperfections (dislocations, impurities, etc).<sup>10</sup>

Historically, nanostructured materials have been used for centuries but their development witnessed a turning point from the 1980s onwards mainly thanks to microscopes that could detect fine microstructures. The industrial and medical development of nanostructured metals focuses essentially on copper, nickel, zinc, aluminium, iron, silver, gold and titanium.

Although these metals display increased resistance to mechanical wear and tear, and are harder and stronger thanks to nanostructures, they often have lower ductility (ability to deform under tensile stress without fracture).<sup>9</sup> According to the Hall-Petch law applied to conventional materials, there is an inverse relationship between grain size and the amount of applied force necessary to deform a crystalline material: the smaller the grains, the more the material can be deformed thanks to dislocation phenomena (propagation of minute defects within the crystalline structure), thus the stronger the material. It has, however, been noted that some nanocrystalline materials possess mechanical properties as a result of this relationship or even present a negative Hall-Petch effect. In fact, below about 30 nanometres, the volume of the grain joints is no longer negligible and other physical phenomena occurring in the intergranular space become predominant over dislocations.

#### **I.B.5. Quantum effects**

When the dimensions of the nanomaterials become comparable to typical physical values of the system (*e.g.* Bohr exciton radius-distance between the electron and its hole-, Fermi wavelength level or the magnetic exchange length), quantum phenomena appear. The physico-chemical properties (electrical, optical and magnetic) are radically different from those of the bulk material. Quantum phenomena are predominant essentially for sizes below one-tenth of a nanometre.<sup>8, 11</sup>

For systems with delocalised electrons such as metals or semi-conductors in particular, the movement of the excitons can be so confined that the energy bands disappear in favour of discrete energy levels generating quantum properties.<sup>12</sup> This is typically the case of quantum dots in which the movement of electrons in three dimensions is restricted by quantum confinement.

Quantum properties depend on the size of the material within nanoscale. Under ultraviolet (UV) source, the quantum dots of selenide and cadmium change colour depending on their size: green grains for 3 nm change to yellow for 4 nm, and then to red at 5 nm.<sup>8</sup> The optical properties of these nanocrystals can be used in medical imaging applications. They offer numerous advantages compared to fluorescent molecules: brighter and more stable for dynamic imaging, a broad spectrum allowing excitation via a large variety of sources, fine tuning fluorescence according to the size of the nanocrystals, etc.

When the magnetic elements are sufficiently small (less than approximately 15 nm), ferromagnetic materials become superparamagnetic.<sup>13</sup> The multi-domain structure ("Weiss" domains), which characterizes magnets and which minimises global magnetic energy cannot be formed. The nanoparticle therefore becomes single domain, *i.e.* the spins carried by the atoms forming this nanoparticle all have the same direction. A permanent magnetic moment appears and the nanoparticle can be considered as a single magnetic domain. An external magnetic field can align the moments carried by a distribution of nanoparticles, thus considerably increase the global magnetic field and viscosity of the medium within a few milliseconds. When this field is cancelled, heat agitation randomly redistributes all these magnetic moments without retaining any magnetism.<sup>14</sup> The pattern is thus similar to the paramagnetism of atoms but the moment carried by the nanoparticles is far greater than the atomic spin, hence the term super paramagnetism. This unique magnetic property has, for instance, led to the development of SPIO (*Super Paramagnetic Iron Oxide*) for medical applications such as treatment by hyperthermia,<sup>15</sup> imaging or diagnostics.

### **I.B.6. Effects of the crystalline structure**

Although nanoparticles or nanocomposites are incorporated in a material to strengthen it and serve only as accessories, other materials display interesting intrinsic mechanical and chemical properties. Belonging to the fullerene family, **carbon nanotubes** possess properties that do not exist in nature: 100 times more resistant and 6 times lighter than steel, harder than diamond. Equipped with excellent thermal and chemical stability, some nanotubes are even conductors or semi-conductors. These tubular nanomaterials are formed from carbon atoms arranged in a regular, graphene type structure. The simplest form is SWCNT (*Single-walled carbon nanotubes*), consisting of a single rolled-up sheet with a diameter of approximately 0.4 nm and up to one micrometre in length. In a more complex form, these nanotubes consist of multi-concentric cylinders of carbon and have a diameter of 2 to 100 nm (*Multi-walled carbon nanotubes*, or MWCNT).

### **I.B.7. Nano-scale and biological scale**

In order to improve the interaction of materials with biological media for medical applications, attempts are increasingly made to mimic the biological systems by nanostructuring the material. The

nanoscale of natural mineral particles would confer optimal robustness to the material and would adapt more readily to natural defects.<sup>16</sup>

In fact, nature seems to benefit from nanoscale structures to produce high-performance biological systems. A good example is the composition of natural bone, which is often described schematically as a composite of biological origin with fibrillar reinforcements in which solid inclusions of 10 to 50 nm are incorporated in a soft protein matrix. With a mineral composition similar to natural bone (calcium and phosphate), synthetic hydroxyapatite, often used as a bone substitute, possesses excellent osteoconduction (passive property of the material to receive bone regrowth) on a macroscopic scale. However, it cannot be completely resorbed, especially if it is highly crystallised and pure and therefore remains long-term in the body as an implant. A size reduction of hydroxyapatite particles on a nanoscale could enable these synthetic nanoparticles to be bioresorbable.<sup>17</sup>

Furthermore, as we saw earlier, the more atoms on the exposed surface, the greater the exchange surface with the external elements. This specific feature of nanomaterials is extremely useful for biological applications. In fact, when a material is introduced into a biological medium, the first stage is the rapid adsorption of serum proteins or of any other biological element on the surface of the material. These proteins will then adopt different conformations depending on the physico-chemical properties of the surface (e.g. hydrophobia, roughness, topography, chemical reactivity). The type, quantity and conformation adopted by the plasma proteins subsequently determine their interactions with cell membrane receptors, thus allowing or preventing the adhesion and proliferation of cells on the surface of the material.<sup>18a, 18b</sup>

The interaction between proteins and nanomaterials should only be influenced by the unique surface properties intrinsic to nano-objects such as a larger specific surface, finely structured roughness or topography, or modified electronic distributions, etc. A surface of this kind could, for example, provide more sites for the adsorption of proteins and promote cell adhesion and proliferation. The nanostructured titanium surfaces of orthopaedic implants have thus been shown to significantly increase the adhesion of osteoblasts compared to untreated surfaces.<sup>18b, 19, 20, 21</sup>

Some authors advocate the hypothesis that an increase in the proliferation of osteoblasts on nanostructured surfaces was due to the similarity between the nanoscales of these nanomaterials and the size of the proteins.<sup>20, 21</sup> Vitronectin - a specific serum protein - which was adsorbed in greater quantities on nanostructured ceramics than conventional ceramics, promoted osteoblast adhesion.<sup>18a</sup> The same pattern of behaviour was observed with fibronectin - another protein of blood plasma and the extracellular matrix, which also promoted osteoblast proliferation.<sup>20</sup> Nanoporous ceramics thus presented better biocompatibility than conventional ceramics. A similar observation was also made with metals, polymers and nanostructured composites. Webster *et al.* went even further by postulating that the nanoscale topography of a surface was sufficient to promote the adhesion of osteoblasts regardless of surface chemistry.<sup>21</sup>

Nanomaterials exhibit unique properties and can be put to good use in the health sector. Synthetic materials, which mimic biological systems with increasing efficacy up to the nanoscale details, should enhance their biocompatibility. Moreover, thanks to their small size, nanoparticles should be able to circulate and penetrate numerous cells, possibly reaching targeted areas of the body even if they are considered difficult to access with current technologies. Nevertheless, nanoparticles are still foreign bodies likely to be treated as such and eliminated by the body's protective mechanisms. Nanotechnologies foster the hope of optimum, personalised medicine: intelligent, multifunctional nanoprobes would be capable of detecting at a very early stage abnormal cells that trigger disease, of destroying these cells or dispensing the exact dose of appropriate medication for the patient at the desired time and of conveying medical data in real time to doctors thus allowing closer monitoring of the disease.

## **I.C. Medical devices containing nanomaterials**

Nanomedicine is one of the most promising nanotechnological applications. It uses new physical, chemical and biological properties related to the nanoscale structures of nanomaterials. This report focuses specifically on medical devices. The consolidated version of directive 93/42/CEE(Directive 2007/47/EC)<sup>22</sup> defines a "medical device as any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, together with any accessories, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes, and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease,
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap,
- investigation, replacement or modification of the anatomy or of a physiological process,
- control of conception,

- and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means."

These include, for instance, suture threads, syringes, hip prostheses, artificial hearts, haemodialyzers, scanners, pacemakers and laboratory reagents, etc.

In this section, detailed examples of medical applications are given in order to provide some ideas of the use of nanomaterials in medical devices already on the market or under development. They are also listed in Appendix II of this report. This list is not intended to be an exhaustive inventory but is only designed to show that nanotechnologies in the case of medical devices affect a broad

spectrum of medical applications, ranging from traditional medical equipment to sophisticated electronic biomimetic devices via orthopaedic, dental or cardiovascular implants.<sup>23</sup> This report is limited to medical devices and will not discuss applications relating to drug delivery (e.g. polymer nanoparticles) or contrast agents in medical imaging, which are not classified as medical devices.

### **I.C.1. Medical equipment**

The improved resistance and robustness of materials thanks to nanostructures appeal to many manufacturers who see the latter as interesting components for the aerospace industry, armaments, sports equipment or in our case, medical devices.

- ***Needles for sub-cutaneous intradermal injection***

Manufacturers have been extremely innovative in recent decades to improve medical devices used to administer drugs. Benefiting from miniaturisation techniques developed in electronics, the size of hypodermic needles has considerably reduced, now amounting to a micrometre. Micro-needles are designed to be as small and sharp as possible. The aim is to minimise the depth to which they penetrate the skin, consequently reducing tissue lesions and the sensation of pain.

Often made from metal (nickel, titanium, gold, etc.), silicone or biodegradable polymers, this new generation of injection systems is presented in the form of a matrix of micro-needles, just a few hundredths of a micrometre in length and with a submicronic tip diameter. Given their dimensions which are slightly above the nanometric scale, these injection systems are not nanotechnological systems *per se*. These devices are nevertheless useful for administering micro- or nano-doses of drugs, insulin or vaccines transdermally or even into the cornea. Potential applications could extend to systems used for diagnostic purposes or to monitor a patient's biological parameters.

Devices already launched on the market or at an advanced development stage include MicroPyramid™ from NANOPASS TECHNOLOGIES (Israel) injecting vaccines and insulin, MicroTrans™ from VALERITAS (USA), Macroflux™ from ZOSANO PHARMA (USA), NanoJect™ from DEBIOTECH (Switzerland) and Pyraderm™ from APOGEE TECHNOLOGY (USA). The manufacturers interested in devices for monitoring and diagnostic purposes include, amongst others, KUMETRIX (USA) for measuring glucose levels in diabetics, and MICRONIT MICROFLUIDICS (The Netherlands).

- ***Wound care***

During the era of the Greek physician Hippocrates, silver was used for its germicidal properties. Silver nitrate was used in the composition of numerous treatments including those for infectious diseases, before being replaced by colloidal metallic silver particles in the late XIX<sup>th</sup> century. The discovery of antibiotics including penicillin sent silver into oblivion before interest in this metal was subsequently rekindled over the last twenty years due to its broad spectrum of antimicrobial, antifungal and antiviral activity, providing an alternative to antibiotic therapy. Silver can be found in gels, creams,

powders or emulsions and, especially in dressings. Applied to serious wounds such as burns, these dressings differ in terms of composition, rate of release and mode of action of the active metabolite.<sup>24</sup>

The exact mechanism of action of silver has not been elucidated to date; both silver ions and metallic silver nanoparticles display antimicrobial activity in an aqueous medium.<sup>25a, 25b</sup> More detailed information on the mechanism of action and toxicity is given in part II.B.2. of this report.

The first silver-based dressings contained silver salts (e.g. Actisorb™ by SYSTAGENIX - USA, Arglaes™ and SilvaSorb® by MEDLINE INDUSTRIES - USA, Urgotul SSD® by LABORATOIRES URGO - France), but few dressings with metallic silver nanocrystals are marketed. The most well-known dressings containing metallic silver nanoparticles are Silverlon™ from ARGENTUM MEDICAL (USA) and Acticoat® from SMITH & NEPHEW (United Kingdom). Contact between the dressing and sterile water or exudate would trigger the solubilisation and/or ionisation of metallic silver. Indicated for serious wounds, Acticoat® uses Silcrist™ technology. It is contended that the advantage of the metallic form compared to silver salt would be a faster and better release of the active substance.<sup>26,27,28</sup> By way of comparison, it has been proposed that silver nitrate would be immediately released in a biological medium and its efficacy would persist for only 2 hours whereas Acticoat™ would remain effective for up to 7 days.<sup>29</sup> In the presence of an aqueous medium (e.g. wound), for Silverlon™, all of the metallic silver nanoparticles would be hydrolysed in the form of Ag<sup>+</sup> ions whereas the Acticoat® dressing would release silver in the form of Ag<sup>+</sup> and Ag<sup>0</sup>. In order to explain the prolonged duration of efficacy observed with Acticoat®, Dunn *et al.* suggested that, in the Ag<sup>0</sup> form, silver would be deactivated less rapidly than Ag<sup>+</sup> ions by the chloride counter-ions or by organic particles in the biological medium.<sup>29</sup>

### • **Medical textiles**

The use of silver as an antimicrobial agent also extends to textiles and other essential medical equipments in controlled environments such as operating rooms: masks, gowns, bandages, etc. Silver nanoparticles can be incorporated in textile fibres by immersion in a colloidal solution of silver or by melt-spinning the polymer with silver nanoparticles or even by spraying nanoparticles on the tissue. However, in order to be efficient, the silver particles must be present on the fibre surface and efficacy may diminish with washing. NanoFense™ surgical masks containing silver nanoparticles and marketed by APPLIED NANOSCIENCE (USA) is intended to filter influenza viruses. Other masks manufactured by SCOUTBURG (Taiwan) are also available. The Korean manufacturer HYOSUNG offers textiles possessing permanent antimicrobial properties, branded under the name of Mipan® Magic Silver Nano. As a general rule, it is difficult to identify the type of silver in these products (metallic or ionic? nanoparticle or nano- or micro-metric coating?).

The properties of other metal oxides are also used. Magnesium oxide nanoparticles also possess antibacterial properties.<sup>30</sup> This is why they are incorporated in the NanoMask® filtration system produced by manufacturer EMERGENCY FILTRATION PRODUCTS (USA) intended to halt viral and bacterial contaminants.

### • **Nanoprobes and nanorobots under development**

Undergoing projects involving nanoscale active implantable medical devices use ultra-sensitive sensors,<sup>31</sup> autonomous engine and power source,<sup>32</sup> arms for precision handling and molecular computers. The components used in these devices are supposedly biocompatible and even bioresorbable.<sup>33</sup> These nanorobots should be able to scan the body and cells to test for any diseases, eliminate or directly repair damaged cells whilst preserving healthy cells at the same time (theranostics).<sup>34</sup> This would transport medicine from the macroscopic scale to the micro/nanoscale of biological systems.<sup>35</sup> These devices are, however, extremely complex to develop and are still at the design stage.

## **I.C.2. Dental and orthopaedic products**

The orthopaedic implant and dental market is soaring, especially due to the ageing of the population. The materials used to manufacture implants can be divided into four categories: metals, ceramics, polymers and composites.

### **• Composites for dental restoration**

A dental restoration composite generally contains a photopolymerisable resin matrix, an inorganic filler, additives and colour pigments. Resins are traditionally *bis*-GMA, TEDMA and TEGDMA type polymers with variable viscosity. The filler consists of very fine particles of different sizes ranging from 0.01 to 50  $\mu\text{m}$ , to enhance the mechanical properties of the composite. Indeed, good particle size distribution optimizing the use of space would prevent composite from shrinking, which can amount to 20% of the volume.<sup>36</sup> The larger particles come from ground quartz crystals whilst the finer particles are nanoparticles of 10-100 nm, often made from silicon or zirconium oxides.

The use of nanoparticles as fillers began in 1950 with the marketing of a range of Aerosil<sup>®</sup> powders by DEGUSSA (Germany). However these nanoparticles tend to agglomerate. This is why, since 2000, manufacturers have each developed a methodology to trap these nanoparticles in a polymer matrix in order to isolate them from each other and to obtain a better size distribution. According to these manufacturers, the advantage of these nanoparticles over Aerosil<sup>®</sup> powder aggregates is a better rate of loading and a slight improvement in mechanical and rheological properties.

Some composites containing nanoparticles are used for dental restoration. The 3M ESPE manufacturer (USA) has several ranges of dental products ranging from Filtek<sup>™</sup> Supreme photopolymerisable restoration nanocomposites to the Adper<sup>™</sup> Scotchbond<sup>™</sup> SE self-etch adhesive containing nanofillers of silanised zircon via Ketac<sup>™</sup> N100 nano-ionomer material. Other composites are marketed in France such as the Kappalux Nano from PRODUITS DENTAIRE PIERRE ROLLAND (PIERRE ROLLAND DENTAL PRODUCTS) (France). German dental restoration products are also available such as Grandio<sup>®</sup> from VOCO (Germany), as well as Japanese products including Optiglaze from GC CORPORATION (Japan).

### • **Bone filling products**

Bone replacement products are often indicated in both orthopaedic and dental surgery. Since the natural mineral phase of bone and teeth are composed of calcium and phosphate, synthetic materials with a similar chemical composition are proposed such as hydroxyapatite ( $[\text{Ca}_5(\text{PO}_4)_6(\text{OH})_2]$ ) and calcium phosphate ( $[\text{Ca}_3(\text{PO}_4)_2]$ ).

Studies have shown that these nanoparticles formed a mineral layer, which is said to promote the repair of dental enamel but which could also have an antibacterial effect on the surface of the tooth.<sup>37</sup> Hence dental products such as toothpastes and mouth-washes, which mostly are not medical devices, also contain nanoparticles.

In orthopaedics, when bone tissue is required, an autologous bone graft is the reference technique. When this is inadequate in terms of quality or quantity, synthetic bone replacements are used as an alternative to or in combination with the autograft. Their use can reduce or prevent morbidity risks associated with the harvest procedure of autologous bone often from iliac crest. Slightly crystallised hydroxyapatite and calcium phosphate ( $\beta$ -TCP) promote bone regrowth by providing *in situ* the raw material to reconstruct the natural bone.  $\beta$ -TCP and slightly crystallised hydroxyapatite nanoparticles are easily absorbed unlike strongly crystallised, pure hydroxyapatite.

Hydroxyapatite gel is a slightly crystallised blend of hydroxyapatite nanocrystals and water. The specific surface area of the material is supposed to be augmented thanks to the nanocrystalline form: 100 m<sup>2</sup>/g versus 1 m<sup>2</sup>/g for a conventional synthetic hydroxyapatite powder. Therefore, clinically speaking, there should be a greater contact with recipient tissue, thus increasing the reactivity and rate of absorption.

Marketed products containing hydroxyapatite nanoparticles include injectable bone filling gels such as Nanogel<sup>®</sup> from TEKNIMED (France) and Nanostim<sup>™</sup>, also branded under the name of Ostim<sup>®</sup> by AAP BIOMATERIALS (Germany) and distributed by MEDTRONIC in France. These gels do not possess mechanical properties because they do not harden. If they are used in an area of bone structure stability, the traditional processes of osteosynthesis are also required. Similarly, the bone substitute, PerOssal<sup>®</sup> from AAP BIOMATERIALS (Germany), is manufactured from hydroxyapatite nanocrystals such as those used in Nanostim<sup>®</sup>. However, the bone graft product, FortrOss<sup>®</sup> from PIONEER SURGICAL TECHNOLOGY (USA), combines the hydroxyapatite nanoparticles of NanOss<sup>®</sup> technology with the E-Matrix osteoconductive matrix. Other products could be calcium phosphate-based such as the Vitoss<sup>®</sup> Scaffold cancellous bone void filler from ORTHOVITA (USA). The nanoscale porosity of the latter is supposed to provide rapid bone remodelling. All of these products are indicated in both orthopaedic and dental surgery.

The use of silver nanoparticles with antimicrobial properties in bone cements is also under investigation.<sup>38</sup>

### • **Nanostructured metallic implants**

Implants are inserted in a patient body for a potentially long but nevertheless limited period of time. Hip prostheses, for example, have a lifespan of approximately 10-15 years.<sup>20</sup> Metallic alloys are selected for their excellent mechanical properties and resistance to corrosion. However, the gradual loosening of implants with natural bone has been observed, particularly when they are cemented. These complications requiring repeat surgeries are often attributed to defects on the implant/natural bone interface.

To improve the integration of the implant in bone, the materials can be subjected to chemical or topographical surface treatment. The aim is to trigger rapid, guided and controlled tissue formation around the orthopaedic prosthesis or dental implant. The extracellular matrix formed should ideally resemble native bone as closely as possible in terms of composition, structure and biomechanical properties in order to ensure the stable anchorage of the prosthetic materials.

Previously we saw that nanostructured surfaces mimicking the nanoscale structure of the natural bone could promote the adhesion and differentiation of bone cells *in vitro*. SYBRON IMPLANT SOLUTIONS (USA) thus proposes pure titanium implants, devoid of chemical contamination, and which would have good bone integration thanks to the nanoscale topography of the Puretex<sup>®</sup> nanoporous surface. Similarly, the NanoImplant<sup>®</sup> dental implant from TIMPLANT (Czech Republic), made from nanostructured titanium, supposedly offers mechanical advantages and faster colonisation by fibroblasts.

Although some researchers consider the nanoscale topography of the surface of an implant to be a sufficient criterion for promoting osteoblast proliferation and therefore bone remineralisation,<sup>21</sup> others have attempted to develop implants with bioactive nanoscale surfaces to promote cell adhesion and bone growth. Hydroxyapatite or calcium phosphate coatings contribute to the recalcification of natural bone around the implant thanks to nanoscale topography and the reservoir of raw material. This was the approach selected by the DOT manufacturer (Germany) who markets the microporous Bonit<sup>®</sup> coating containing hydroxyapatite nanocrystals. For example, this coating is used in Nanos<sup>™</sup> orthopaedic prostheses marketed by SMITH&NEPHEW (United Kingdom) and in Symax<sup>™</sup> joint prostheses from STRYKER (France). NanoTite<sup>™</sup> implants from BIOMET 3i (USA) contain calcium phosphate nanocrystals.

Many studies are focused on the potential medical benefits of different coatings, either made from nanoporous ceramics (e.g. Debiostent<sup>™</sup> currently being developed by DEBIOTECH, Switzerland), or incorporating silver nanoparticles for their antiseptic properties or even coatings with biomimetic polymers in their structure and/or incorporating proteins.

- **External knee prosthesis**

Advances in prosthetic technology also concern bionics with significant improvements being made in external knee prostheses in terms of mobility, energy efficacy and natural gait. According to the manufacturer, the Rheo Knee monoaxial knee prosthesis from OSSUR (Iceland) should give amputees smooth, natural joint movements thanks to finely tuned control via the magneto-rheological fluid. Outside the patient's body, this fluid contains iron particles of 100 nm to 1 µm in an oil, which could immediately react to the magnetic field applied depending on the desired movement. The particles were then arranged in a chain, opposing minimal resistance to bring fluidity to the walking movements.

### **I.C.3. Cardiovascular devices**

- **Stents**

Coronary heart disease associated with narrowing of the vascular network (stenosis), can be treated by carrying out coronary angioplasty with the implantation of a device which keeps the artery open. This device is known as an endoprosthesis or stent. Coronary stents are often metallic or in polymer. There are bare stents and stents containing bioactive molecules (known as "active stents"). Despite excellent results, one of the main complications of this technique is intra-stent restenosis. It is mainly promoted by neo-intimal hyperplasia of smooth muscle cells in the blood vessel wall and extracellular matrix deposit.<sup>39</sup> Bare-metal stents are also associated with the risk of thrombosis. Current research therefore try to find more effective, longer-lasting solutions.

Initial attempts should focus on reducing the thrombotic risk by modifying the material used.<sup>40</sup> Manufacturers have therefore attempted to correct the contact defect between the stent and the cells by using polymer or ceramic nanoporous nanoscale coatings for bare stents (e.g. ALCOVE SURFACE, Germany). CELONOVA BIOSCIENCES (Canada) tried to optimise its coronary stent, Catania™, made from a cobalt and chrome alloy, by coating it with about 50 nm thick of Polyzene®-F polymer. It is contended that the surface of the Polyzene®-F-treated stent would be haemocompatible and could promote total, rapid healing of the vessels. It thus would reduce platelet activation during and after surgery, and help to prevent inflammatory tissue reactions responsible for restenosis.

Among the active stents for which attempts have been made to reduce the risk of restenosis, the VestaSync™ stent from MIV THERAPEUTICS (Canada) has an ultrafine hydroxyapatite coating with a porosity of 100-500 nm, compared to 14-15 µm in conventional porous coatings.<sup>41</sup> The manufacturer is currently involved in clinical trials (end of phase II to date) in order to assess the efficacy of the device with a view to obtain CE labelling.

- **Catheters**

Obviously, the idea of incorporating active substances does not apply solely to stents but is also used for other medical devices. In particular, the manufacturer ACRYMED (USA) offers coatings incorporating silver nanoparticles (SilvaGard™ technology). It has been incorporated in the ON-Q® SilverSoaker™ anaesthesia catheter, which is already marketed in the United States. The use of silver nanoparticles possessing antimicrobial properties for other invasive medical devices such as central venous catheters are currently under development (clinical trial).<sup>38</sup>

Increasingly sophisticated medical procedures often require appropriate devices and tools such as, for example, catheters or balloons with smooth, ultrafine walls. Conventional material reinforcements are too voluminous for use in such fine objects. Smaller reinforcements are therefore required: e.g. clay, ceramic, silica nanoparticles or carbon nanotubes. These nano reinforcements incorporated in a polymer matrix would bestow polymer nanocomposites with improved mechanical properties<sup>42</sup> including greater rigidity without friability, which is essential for catheters.

- **Synthetic vascular grafts**

*Electrospinning* is a process used for creating polymer nano or microfibres using electrostatic forces to obtain innovative "textiles". When the electrical tension applied between the spinning nozzle and counter electrode is sufficiently high, the polymer solution is drawn and forms a very fine liquid jet towards the counter-electrode. Applications in this field of research include implants or regenerative medicine.<sup>43</sup> There are numerous options because pore size and quantity can be varied and bioactive molecules or nanoparticles can be incorporated in the final material by experimenting with the polymer solution or counter electrode, which can also be liquid. The density of the final "textile" can be modulated to mimic human tissue structure with implantable applications for bone, cartilage and the vascular system.

The NICAST manufacturer (Israel) obtained CE labelling in 2008 for the AVflo™ self-sealing vascular access graft made from electrospun fabric. The Company is planning to launch an ameliorated version of the graft and the NovaMesh™ intra-abdominal hernia mesh, also composed of electrospun polymer nanotissue, this year on the European market.

Other research scientists are also developing artificial arteries using nanoscale polymer materials to mimic natural blood vessels. An English team is about to carry out clinical studies with a polymer vascular graft containing UCL-NanoBio™ nanocages on its surface, forming a patented polymer nanocomposite.<sup>44</sup> These nanocages would stimulate the proliferation of circulating endothelial cells, which would help to repair damaged blood vessels. A Russian company (ROSKARDIOINVEST) is attempting to develop nanostructured artificial heart valves, which would be less resistant to blood flow and would reduce the risk of thrombosis.

## **I.C.4. Surgery**

### **• *Surgical instruments***

The use of nanotechnologies in the field of surgery is essentially aimed at making procedures less invasive. To do this, improvements can initially be made to existing instruments and material. For instance, incisions made with diamond-coated scalpels are finer and more precise thanks to a lower friction coefficient. The advantage of the nanoscale diamond layer is its weak physical adhesion to materials and tissues, thus facilitating penetration. Its chemical and biological inertness is supposed to boost the material lifespan. The roughness of the surface of the blade marketed by GFD GESELLSCHAFT FÜR DIAMANTPRODUKTE MBH (Germany) is only approximately 20-40 nm. This type of surgical instrument could improve ophthalmological surgery or neurosurgery.

Other manufacturers such as AB SANDVIK MATERIALS TECHNOLOGY (Sweden) attempt to improve material. They have developed stainless steel containing nanocrystals of this metal measuring about ten nanometres. This new metal is used to make suture needles, for instance (Sandvik Bioline 1RK91™ needles). They would combine good resistance with good ductility (reduced risk of needle rupture). Finally, research is also geared to develop nanoscale knives made from a hard silicon compound (20 nm curvature radius) or carbon nanotubes, capable of cell ablation.<sup>45</sup>

### **• *Neurosurgery***

Internal or external catheters for draining cerebrospinal fluid can cause bacterial infections which sometimes quickly spread to the brain and around the meninges. The use of catheters impregnated with silver nanoparticles could prevent these infectious complications.<sup>46</sup> A randomised clinical study is expected to confirm the results of a pilot study.

### **• *Nanometric precision surgery***

Thanks to nanotechnologies, surgery is reaching an additional level of precision.<sup>47</sup> Femtosecond lasers can be used to perform cell (e.g. neuronal cells) or chromosome surgery. Laser beams can also be used to trap, move and manipulate cells, organelles, biological molecules, proteins or DNA. Under the action of laser light, chemical groups of these nanotweezers react by closing or opening the tweezers thus formed by absorbing photons of different wavelengths.

### **• *Surgical nano devices***

The progress of electronic miniaturisation allows improvements in the medical sector thanks primarily to the development of increasingly sophisticated MEMS/NEMS (micro/nano-electromechanical systems). Nano tools could assist surgeons, facilitating handling procedures at nanoscale or by providing biological information thanks to the probes. These nano tools would be controlled by humans using computers to recommend and/or carry out minimally invasive surgery. For example, the MAKO surgical device from ORTHOSENSOR (USA) would provide surgeons at the Holy Cross Hospital in the United States with accurate information on personalised orthopaedic knee implantation thanks to its nanosensors.

## I.C.5. Nanotechnologies in the treatment of cancers

Traditional cancer treatments include surgery, chemotherapy, immunotherapy and radiotherapy. Thermotherapy is an alternative to these treatments in the event of treatment failure or side effects. This technique involves heating tumour cells, which are more sensitive to heat than healthy cells.<sup>15</sup> Heat is obtained thanks to an external energy source based on microwaves, ultrasound, optical devices or a magnetic, electric field created between the antennae. The main defect associated with conventional hyperthermia techniques is the heterogeneity of heat distribution in the tissues, which can cause numerous side effects.

The use of nanoparticles in cancer heat therapy seems to be really beneficial when used in synergy with some conventional treatments. This technique is supposed to be able to destroy tumours whilst limiting the effects on organs and healthy tissues. The nanoparticles could be used to treat tumours, location or severity of which would make medicinal treatment ineffective and surgery tricky. In the short term, survival time can be increased and adverse events limited thanks to the localised action of nanoparticles. The three devices of this kind, which are currently most advanced in terms of development, are:

- Nano-cancer therapy from MAGFORCE NANOTECHNOLOGIES (Germany), which obtained CE marking in 2010,
- NanoXray from NANOBOTIX (France), which is now ready to enter the clinical study phase,
- AuroShell from NANOSPECTRA BIOSCIENCES (USA), which is also at the clinical study phase.

MAGFORCE NANOTECHNOLOGIES nanoparticles are superparamagnetic iron oxides of approximately 15 nm, covered with aminosilanes. They can be activated by an external magnetic field. They are indicated in the treatment of multiple glioblastoma,<sup>48</sup> but the German manufacturer is attempting to extend the indication to other types of cancer. NANOBOTIX nanoparticles are made from hafnium oxide and are activated by X-rays for the treatment of soft tissue sarcoma at the extremities. As for gold-covered silica nanoparticles from NANOSPECTRA BIOSCIENCES, they are 150 nm in diameter. They are activated by laser light and could be used against solid brain and neck tumours.<sup>49</sup>

Brachysil™ from PSIVIDA (Australia) is a medical device under clinical evaluation for the treatment of prostate cancer. To be precise, it contains 30 µm-size microparticles of silicon with BioSilicon nanoscale pores incorporating radioactive phosphorus <sup>32</sup>P. This treatment has been formulated to deliver local targeted doses of β-radiation following biodegradation of the silicon shell.

The NanoKnife™ system from ANGIODYNAMICS (USA) is a new tumour ablation technique based on the irreversible electroporation\* of cancerous cells (CE labelling obtained in 2009). Electrical frequencies are created between the probes positioned on the lesion. They consequently trigger nanoscale pores in the cell membranes and cause cell death.

Other hyperthermia devices made from different materials and/or using other energy sources are currently under development, albeit at research stage, or are classified rather as drugs than medical devices since they are associated with active substances or specific proteins of some tumour cell receptors.

## **I.C.6. Implantable active medical devices**

### **• *Artificial organs***

Technological progress such as electronics applied to medicine facilitates the concept of replacing complex organs in the human body, such as the heart, by artificial organs. A multidisciplinary consortium of American research scientists recently managed to create an artificial kidney containing a filter composed of thousands of nanoporous silicon membranes that could selectively filter toxins.<sup>50</sup> The nanopores would be sufficiently dense and of an adequate shape so as not to alter the blood flow.

New cardiac stimulators or new audio prostheses also benefit from nano-electronic advances, with sensorial nano probes capable of storing and rapidly transmitting data for even greater reactivity. The nano-electronic components used in these devices are encapsulated and therefore do not come into contact with the body.

### **• *Retinal prostheses***

Research scientists are developing retinal implants from nano-electronic elements to treat blindness due to pigmentary retinopathies. The implant is composed of an electrode chip attached to the retina in the epi-retinal or sub-retinal position. The electrodes transmit the information recorded by an external camera to provide the patient with rudimentary vision.

Several projects are in the pipeline, in Germany, Japan, the United States, Australia and France. Research scientists at RETINA IMPLANT (Germany) are currently launching a second clinical study on their improved retinal prosthesis. The most advanced device is Argus™ in the United States, developed by SECOND SIGHT MEDICAL PRODUCTS (USA). It is currently at the clinical phase and in the process of receiving CE labelling. The Argus™ medical device consists of a series of platinum electrodes on a silicon plate. The difficulties to be overcome in perfecting these prosthetic systems are the interferences between electrodes and the resolution of the reconstituted image. As the latter

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\* Technique for creating pores in cell membranes by electric pulses.

largely depends on the number of electrodes, some researchers advocate the use of 3D electrodes to reduce the interference.

Regarding the system biocompatibility, the non-biocompatible or electronic parts of the device are often protected by a shell. Other manufacturers, however, attempt to replace this cumbersome form of protection by a biocompatible, ultrafine film of diamond nanoparticles (approximately 5 nm in diameter), which was inert in corrosive media.<sup>51</sup> For example, the Institute for Vision and the AEC (Atomic Energy Commission) have opted for a nanostructured diamond layer to enhance the biocompatibility and performance of retinal stimulation electrodes.

### **I.C.7. Diagnostic and continuous monitoring devices**

Research and development teams are striving to perfect applications for monitoring the physiopathological condition of the patient or tools allowing the disease to be followed up.<sup>52</sup>

Some manufacturers propose diagnostic devices for type I diabetes that analyse the air exhaled by the patient. An American team at the MIT (*Massachusetts Institute of Technology*) created a sub-cutaneous device for monitoring blood sugar levels *in vivo*.<sup>53</sup> The device was based on carbon nanotubes that become fluorescent depending on the quantity of glucose in the blood. Research scientists must now find an "ink" in which these nanotubes can be mixed to create a sort of "tattoo", which would facilitate the continuous monitoring of blood glucose levels.

NANOSPHERE (USA) proposes the Verigene device containing gold nanoparticles to carry out genetic and pharmacogenetic tests. Sensors could also be incorporated in the implants to follow in real time the interaction between the material and the biological medium, by providing data on whether or not the cells proliferate on the surface of the implant and the types of cell involved. Doctors could thus monitor implant biocompatibility *in situ* (at cell level).

IVDMDs (*in vitro* diagnostic medical devices) are becoming more sophisticated with genetic and protein biochips and *lab-on-chips* for detection with molecular precision.

## **I.D. Overview**

This section highlights the wide-ranging use of nanotechnologies in the world of medical devices and opens up interesting avenues. Some devices - the fruit of nanotechnological research - have already been launched on the market whilst numerous others are currently at the development stage, in fields such as orthopaedics, surgery, oncology and cardiovascular or dental products. Progress can also be made in more common applications such as medical textiles and medical instruments for a global improvement in healthcare. Future nano-devices or nano-sensors could take the world of active medical devices and *in vitro* diagnostic devices by storm. This brief account is not

intended to be exhaustive but aims to provide an overview of the wealth of options available with nanotechnologies in the medical device sector alone.

Moreover, thanks to technological advances, we are gradually witnessing a sliding of boundaries between drugs and medical devices. The boundary is becoming more and more tenuous with nanoparticles being used to treat cancers or implantable medical devices used to monitor blood glucose levels for instance. Classification is no longer straightforward and calls for an in-depth investigation of the specific mode of action (principal or accessory).

Finally, amongst the perspectives outlined, some are still at the early research stage or often at the feasibility demonstration stage. Prototypes are still pending finalisation in the laboratory and in preclinical trials in order to be transformed into realistic, marketable medical devices. Nevertheless, nanotechnologies are genuinely important tools for the future of medicine, improving existing instruments and creating new medical devices that are more intelligent, more effective and more biocompatible. They provide opportunities that could off-set current limitations in terms of disease management, culminating in a better quality of life for patients and even longer life expectancy.

## II. Biological assessment of nanomaterials

The fostering of nanotechnologies arouses enthusiasm in terms of the numerous possibilities for application but also generates concern regarding their potential biological impacts. In fact, the unique properties of nanomaterials, which have led to innovative applications, could allow them to reach inaccessible places in living organisms with a different reactivity compared to conventional materials. It is precisely their small size, large specific surface area and increased reactivity that enable them to bind to biological elements with greater efficiency and which raise concern about substantial consequences in terms of toxicity.

The toxicity examples considered in this report are taken from studies on nanomaterials used in various applications and environments (drugs, cosmetology and pollution, etc.). They do not necessarily reflect the reality of medical devices since studies of the nanomaterial adverse effects are more detailed for nanoparticles or nanotubes than for other types of nanomaterials (such as nanostructured materials). Similarly, the data presented in this report are to be used with caution and cannot necessarily be extrapolated.

### II.A. Current knowledge relating to the toxicity of nanomaterials

The risk to man of any given product is the combination of the intrinsic hazard of the product and human exposure to this product. According to current studies, though still scarce and sometimes contradictory, it is generally admitted that the toxicity of a nanoscale particle would differ from its conventional counterpart; which does not necessarily mean that the nanomaterial is more toxic. For example, some published studies indicated that CuO nanoparticles were indeed more genotoxic than microparticles. Conversely, other studies showed that, in some cases, TiO<sub>2</sub> nanoparticles were less genotoxic than their microscale counterparts.<sup>54</sup> The toxicity of TiO<sub>2</sub> nanoparticles will be discussed in detail in section II.B.3.

To better understand the potential hazard related to nanomaterials, we are going to review their potential biological effects. However, this report is not aimed at collecting or assessing extensively all nanomaterial toxicology studies conducted to date. It will only refer to key points recorded so far on this topic. For further information, the reader is invited to consult scientific reports and reviews available on the subject.<sup>3, 55</sup>

## II.A.1. Interactions between nanomaterials and biological systems

Firstly, when a nanomaterial is exposed to a biological medium, its behavior is determined by various factors and does not depend solely on its intrinsic characteristics. In fact, on coming into contact with a biological environment, nano-objects are immediately covered with a dynamic protein “corona” of variable composition.<sup>56</sup> Most of the studies focusing on protein adsorption on the surface of nanoparticles used plasma or serum (containing approximately 3700 proteins),<sup>57</sup> as models of biological fluid. They were interested in the effects of plasma protein adsorption on cell recognition, especially by macrophages. Some studies also focused on interactions with the mucus and pulmonary surfactant. The bioavailability of nano-objects and ensuing cell responses depend on the interaction between the proteins in a biological medium and nano-objects.

However, these two *in vitro* biological fluid models poorly reflect *in vivo* situations. Indeed, in general, the chelation of divalent ions (such as calcium) by citrate ions added to plasma in order to block the onset of the coagulation cascade, also blocks the amplified activation of the complement system (non-specific immune defence system). The addition of controlled doses of calcium ions allows the amplified coagulation phenomena to be evaluated when coming into contact with a material surface. Similarly, in the serum, the important absence of fibrinogen suppresses any effect related to that protein, whereas the addition of controlled doses of calcium and magnesium ions allows the amplified activation of the complement to be evaluated. Consequently, the rapid, simultaneous *in vivo* activation of these two amplified systems when in contact with a material surface cannot be observed *in vitro*.

### • Kinetics of protein binding to nano-objects

The composition of the protein corona adsorbed on nano-objects at a given time depends on concentration and the adsorption kinetics of the proteins present in the biological fluids. It is important to determine which proteins are adsorbed on the surface of nanomaterials, but it is also crucial to understand the binding affinities and stoichiometries involved. The affinity of a protein for the surface of a nano-object differs from its affinity for this same bulk material (dependent on size, available surface area and curvature of the nanoparticle).<sup>58</sup> Furthermore, the couple nano-object/adsorbed proteins is likely to change over time: the proteins present in high concentrations will thus be adsorbed very rapidly and will cover the surface of the nano-objects. But these proteins can subsequently dissociate from the material and be replaced by other proteins present in smaller concentrations but with greater affinities (Vroman effect).

For instance, albumin is rapidly adsorbed by various types of nano-objects (lipids, polystyrene, PEG-PHDCA, etc.). The quantity of albumin adsorbed is then stabilized, even reduced to be replaced by other proteins such as fibrinogen, apolipoproteins, C3 proteins of the complement system and IgGs, etc.<sup>58b, 59</sup>

- ***Protein adsorption depends on the properties of nanomaterials***

Some intrinsic properties of nano-objects affect the adsorption of plasma proteins. The latter has been seen to increase with the zeta potential of the nano-object, which reflects its surface charge,<sup>58b, 60</sup> without any variation in the profile of the proteins adsorbed.

The hydrophobia of the nano-object surface not only influences the quantity of proteins adsorbed but also the type of proteins. Generally speaking, hydrophobic nano-objects, which adsorb more plasma proteins than hydrophilic nano-objects were opsonized more rapidly (captured by the immune system to be subjected to phagocytosis) .<sup>58b, 61</sup>

For some authors, the surface properties (charge and hydrophobia) are also probably more important for protein adsorption than the actual composition of the nanomaterial (type of material, shape and size). However, the latter cannot be overlooked because numerous chemically different compounds diffuse on the surface of nanomaterials, thus modifying the physico-chemistry of their surface. Protein structure, stability, activity and functionality can also be altered following their adsorption on a nanomaterial.<sup>58a, 62</sup>

The presence of nanostructures on surfaces also determines protein adsorption on these materials.<sup>63</sup> For example, the concentration and conformation of fibronectin adsorbed on nanostructured tantalum surfaces differed depending on the topography of the nanostructures.<sup>64</sup> In particular, the RGD sequences contained in fibronectin, which were essential for cell recognition, did not present the same accessibility to cells depending on nanostructured surfaces. The organization of nanostructures on materials was also crucial for protein adsorption and cell reactions. Grafting densities and the lengths of polymer "nanobrushes" (PDMA, PNIPAM) affected protein adsorption: the stronger the brush grafting densities on the surfaces and the longer the brushes, the fewer plasma proteins were adsorbed.<sup>65</sup> These nanobrush effects have been observed in a theoretical model of poly(oxyethylene) (PEO) brushes grafted on a surface and demonstrated under experimental conditions via nanoparticles with a poly(lactide-glycolide) (PLGA) core and PEO (poly(oxyethylene) coating).<sup>66, 67, 68</sup>

The surface properties of nanomaterials are therefore of paramount importance for protein adsorption.<sup>69</sup>

- ***Protein adsorption affects the biodistribution of nano-objects and cell response***

The adsorption of proteins on nano-objects, which is influenced by the properties of the latter, can alter their overall size and surface charge. These factors are therefore likely to impact upon the internalization of nano-objects in cells, cell response and their distribution in the body.<sup>70</sup>

The competitive adsorption phenomenon of proteins on a nanomaterial plays a key role in the amplified activation of the complement. Indeed, nanomaterials - foreign bodies potentially activating this system - are taken up within minutes by the macrophages located in the organs of the Mononuclear Phagocyte System (MPS) such as liver and spleen.

Therefore the adsorption of opsonins such as IgGs, complement proteins and fibrinogen promoted phagocytosis and eliminated nano-objects from circulation. These elements were then sequestered in the organs of the reticulo-endothelial system and concentrated in the liver and spleen.<sup>58b, 71</sup> Inversely, the albumin adsorbed on the surface of the nanoparticles seemed to prolong their presence in the blood<sup>58b</sup> and could reduce the inflammatory response.<sup>72</sup> However, the presence of albumin adsorbed on the surface of PLA nanoparticles did not always prolong their presence in the blood.<sup>73</sup>

Regarding the surface nanostructure, the brush grafting density was shown to lead to variable repercussions on blood coagulation and platelet activation by influencing the type of proteins adsorbed: the surfaces grafted at high density by PDMA brushes did not activate the platelets whereas these surfaces grafted by the same brushes but with lower density triggered coagulation and platelet activation.<sup>65a</sup>

Other effects of surface nanostructures have been shown on nanoparticles with a poly(alkyl cyanoacrylate) (PACA) core and polysaccharide coating (dextran, chitosan, etc.), either in brushes or in loops. Whereas the increase in brush length reduced activation of the human serum complement in the presence of nanoparticles, an increase in loop length increased this activation.<sup>74</sup> In this case, plasma protein adsorption was not related to complement activation.

Similarly, for nanostructured surface materials, the quantity and type of proteins adsorbed are essential for cell adhesion, translocation and differentiation.<sup>18b</sup> For example, Yang *et al.* commented that titanium surfaces with nanoscale roughness adsorbed fibronectin more effectively and in larger quantities than albumin. Osteoblast adhesion was promoted to greater extent on the surfaces of titanium covered with fibronectin than albumin.<sup>75</sup>

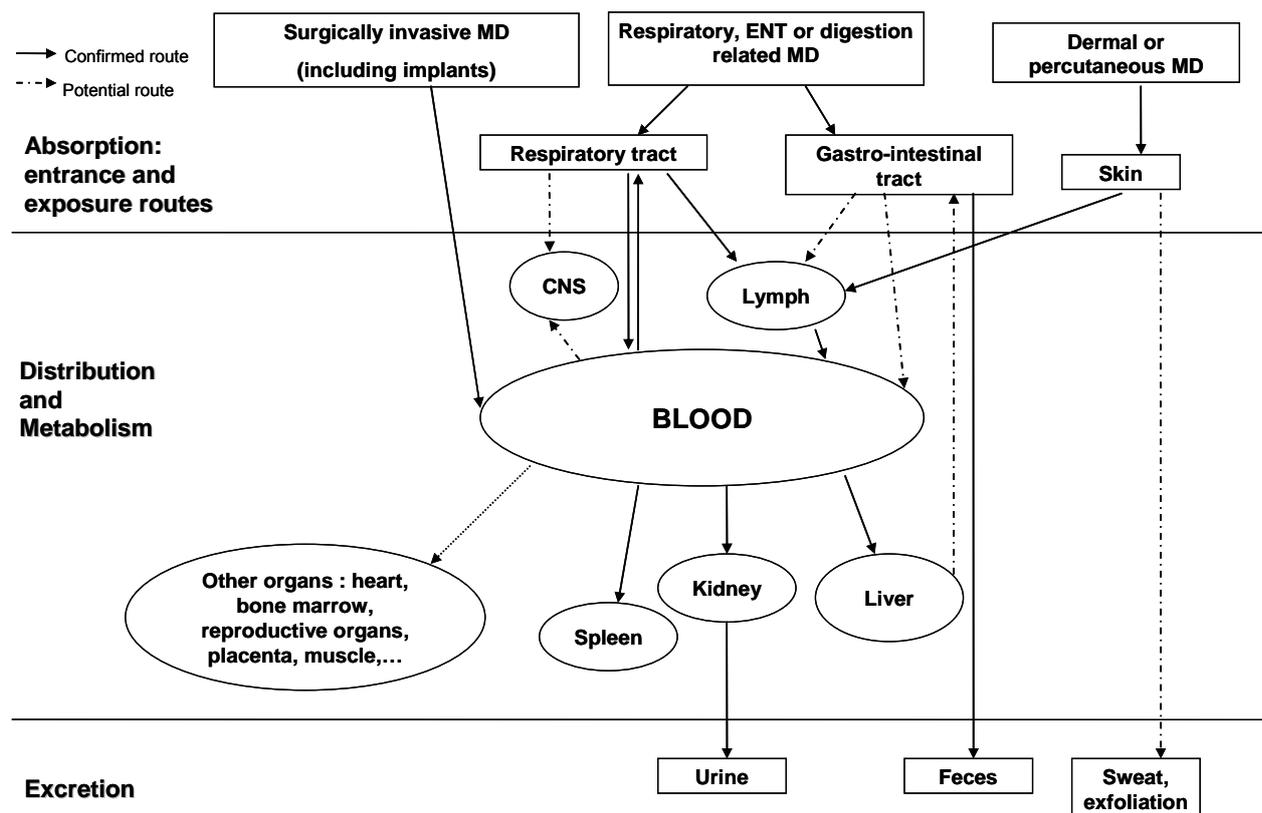
## II.A.2. Toxicokinetics and biodistribution

Knowledge in "macro/micro" cannot necessarily be transposed to the "nano" scale, particularly regarding the fate of nanomaterials in the body. The absorption, distribution, metabolism and elimination (ADME) phenomena seems to differ compared to conventional materials. Consequently, toxico-kinetic studies are essential for evaluating the toxicological risks that may rise when using a medical device containing nanomaterials. Four main parameters influence this toxico-kinetic study: the route of administration, object size, surface reactivity (charge, chemistry) and animal species.

When using a medical device, nanomaterials may penetrate the respiratory system in the case of respiratory or ENT related MDs, the digestive system with digestion related MDs, pass through the skin for cutaneous or percutaneous MDs, eventually reaching the blood. Nanomaterials can also be directly found in the blood when invasive surgical MDs are used, including implants in particular. Then,

carried by the blood flow, they can be found in the liver and "target" organs, which store nano-objects. They can be detected in the urine either by elimination or when using a genito-urinary MD. Finally, they can be eliminated in the faeces depending on their physico-chemical characteristics (see Figure 1).

As we can see in the following diagram (Figure 1), some transport routes have been highlighted in publications whilst others remain hypothetical. The quantification of nanomaterial translocation, their accumulation and retention in the organs and target tissues remain largely unknown.<sup>55c</sup>



**Figure 1. Bio-kinetics and possible distribution of nanomaterials contained in medical devices.** The plain arrows represent routes confirmed by studies whilst the dashed arrows indicate hypothetical routes. MD: medical device, CNS: Central Nervous System - Figure adapted from Oberdörster *et al.*<sup>1</sup>

### • Absorption by skin

Healthy skin is generally an effective physical barrier against environmental aggression. It consists of three main layers: the epidermis, dermis and sub-cutaneous layer. Most chemical products are halted by the outer layer of the epidermis, namely the stratum corneum.

This is precisely the case for TiO<sub>2</sub> nanoparticles according to numerous *in-vitro* and *in-vivo* studies, which showed limited penetration through the stratum corneum of healthy skin.<sup>55c, 55i, 76</sup> A recent *in-vivo* study in particular showed that the penetration of TiO<sub>2</sub> nanoparticles was not significant

for healthy pig skin.<sup>77</sup> These nanoparticles used in commercial products are generally in rutile form, which is less reactive than the anatase form.<sup>55c</sup> They are often coated with a layer of silica or alumina, which is functionalised for greater stability. These primary nanoparticles of 10-30 nm tend to agglomerate to form aggregates of several microns.

Let's look at the example of silver nanoparticles: *in-vitro* studies on human skin have shown slight absorption by intact skin whereas, in the case of abraded skin the systemic penetration of silver nanoparticles was possible,<sup>78</sup> or even considerable.

Other studies showed that very small nanometric particles (< 10 nm) could penetrate more deeply,<sup>79</sup> the risk of absorption once again being greater in case of damaged skin (e.g.: burned, abraded or psoriatic skin).

Furthermore, mechanical flexion of the skin was also shown to greatly facilitate the penetration of fullerene nanoparticles<sup>79a</sup> and beryllium microparticles.<sup>80</sup> Conversely, although nanoparticles have been detected in hair follicles, their passage through the skin has not been confirmed.<sup>81</sup>

The duration of exposure also seems to affect cutaneous absorption. In fact, Wu *et al.* recently showed that TiO<sub>2</sub> nanoparticles (4 and 60 nm) did not penetrate beyond the deep layers of the epidermis in pigs following cutaneous exposure of up to 30 days. However, in hairless mice, longer exposure could trigger significant chronic toxicity with the generation of free radicals and collagen depletion.<sup>79c</sup> After 60 days, the nanoparticles had crossed the dermal barrier, reached different tissues and triggered pathological lesions in major organs such as the liver.

### • **Absorption by the lungs**

Deposit of nano-objects in the respiratory tract depends on numerous factors including size, the force of inhalation and the structure of the airways. The toxico-kinetics of this route of entry was studied extensively within the scope of environmental (atmospheric pollution) and occupational health safety. According to mathematical models and those of the International Commission for Radiological Protection, almost 90% of nanoparticles of less than 100 nm inhaled were deposited in the respiratory tract, part of it was stopped in the upper airways leaving approximately 50% to diffuse into the alveolar region, whereas fewer particles of 100 nm to 1 µm penetrated the upper airways.<sup>82</sup> Nanoparticles of less than 10 nm were stopped in the upper airways.

The nano-objects present in the airways were mainly managed by muco-ciliary clearance to be expectorated or transferred to the gastro-intestinal tract to be eliminated in the faeces.<sup>55f</sup> Other substances, being soluble, could dissolve in the mucus that bathes the epithelium and pass into the blood and lymphatic circulation to be subsequently distributed to other organs, such as the kidneys for elimination. In the alveolar region, clearance was achieved by resident alveolar macrophages. If they were not cleaned, insoluble nanomaterials would accumulate and overload the pulmonary defence mechanisms or even partly translocate through the pulmonary barrier. Their bio-persistence could trigger harmful consequences such as inflammation, chronic toxicity and carcinogenic risks as has already been observed with asbestos fibres. Carbon nanotubes with a similar fibrillar structure have

been seen to be eliminated only very slowly from the lungs of rodents in animal studies: 81% could still be detected 60 days post-exposure.<sup>83</sup> Recent studies on various radiolabelled nanoparticles and carbon nanotubes following inhalation by rats showed that approximately 1% of the nano-objects inhaled could migrate to the systemic system and accumulate in various organs including the liver and spleen.<sup>84, 85, 86</sup>

### ● **Oral absorption**

Nanomaterials may reach the gastro-intestinal tract due to medical devices for digestive applications but also as a result of the translocation of inhaled nano-objects or even dental products. Several studies have shown that micro- and nanoparticles were absorbed by the digestive tract mainly via the Peyer plates in the small intestine and via intestinal enterocytes.<sup>87</sup> The size and charge of the nanomaterials appeared to influence the rate of absorption. In the case of gold nanoparticles, the smaller they were the better their gastro-intestinal absorption and distribution in more organs.<sup>88</sup> Similarly, positively charged polystyrene nanoparticles were absorbed more efficiently than neutral nanoparticles whereas negatively charged ones would diffuse through the layer of mucus and interact with the epithelial cells.<sup>89</sup>

Ingested nanoparticles seemed to be eliminated rapidly: 98% in the faeces within 48h and the rest in the urine.<sup>55j</sup> Some studies also indicated partial translocation in the blood circulation and lymph glands with systemic distribution in organs such as the liver and kidneys. Indeed, the degradation products originating from dental prostheses or composites<sup>90</sup> that were absorbed via the oral route could accumulate in these organs, thus leading to effects on health such as fever, swelling of the spleen and liver, suppression of the biliary circulation and acute renal failure. These symptoms have been observed one year after the insertion of ceramic dental bridges and have disappeared on removal of the prostheses.<sup>91</sup>

### ● **Systemic distribution and metabolism**

Detected either directly in the blood or indirectly, nanomaterials can be opsonized, recognised by the reticulo-endothelial system and subjected to phagocytosis by macrophages in order to eliminate them from circulation. They were then distributed in various organs such as spleen, liver and kidney.<sup>92</sup> They could also be detected in the heart, lungs or bone marrow. Translocation to the Central Nervous System (CNS) was also possible. Enzymes such as proteases or metallothioneins in the organs could be involved in the metabolism of metallic nano-objects.<sup>87</sup>

Surface functionalisation of nanomaterials is particularly important here because it affects bio-distribution and kinetics. For instance, a PEG (polyethylene glycol) polymer envelope would partly prevent them from being taken up by the liver and spleen<sup>93</sup> and would prolong their presence in the circulation.<sup>61, 94</sup> Just like surface properties, the size and shape determine the fate of nanomaterials in the body. Some research scientists have shown in particular that the widest biodistribution was observed with the smallest gold nanoparticles (10 nm).<sup>92</sup> Others have found biodistribution to be similar but biopersistence and the transfer rate differed depending on the size of the polystyrene nanoparticles.<sup>95</sup>

### • **Elimination**

The elimination of nanomaterials has not yet been fully elucidated and depends essentially on the route of exposure and the physico-chemical parameters of the material studied (size, surface properties, etc.). Ingested or inhaled nanomaterials were mainly eliminated in the faeces like all foreign particle entering via these routes.

Regarding nanomaterials present in the systemic circulation, the conventional route of elimination via the kidneys is often mentioned, as was the case for fullerenes and carbon nanotubes.<sup>96</sup> Another route has been suggested for polystyrene nanoparticles, namely the liver with excretion in the bile.<sup>97</sup> This is an excretory route known in pharmacology but which has yet to be confirmed for nano-objects.

The elimination of quantum dots is, on the other hand, extremely difficult to determine despite clearly identified biodistribution thanks to their fluorescence. In fact, elimination studies are contradictory since the results largely depend on coatings and the size of the nanoparticles.<sup>98</sup> Nevertheless, according to a study, they could still be detected in the lymph glands and bone marrow of mice 133 days after intravenous injection.<sup>87</sup>

Some researchers fear that the biopersistence of nanomaterials raise the risk of chronic toxicity or even trigger the onset of cancers. However, data relating to the consequences of nanomaterial accumulation in the body are seldom reported at the present time.

## **II.A.3. Cytotoxicity**

When the cells are exposed to substances that they can subject to phagocytosis but do not eliminate, they initiate a response that can be characterized by the secretion of inflammation factors such as pro-inflammatory cytokines. This triggers an inflammatory reaction activating the immune system, which can involve B lymphocytes or macrophages within the scope of a delayed hypersensitivity response. These responses are associated with changes in the expression of different genes including genes for inflammation, apoptosis or control of the cell cycle. They can also be responsible for DNA lesions indicating risks of genotoxicity and carcinogenicity.

Numerous cytotoxicity studies have been carried out to evaluate the toxic potential of nanomaterials but no consensus has so far been reached due to variations in experimental methods, the cell lines selected and, quite simply, the nanomaterials used.<sup>99</sup> In fact, cytotoxic effects differ depending on the size, form, chemical composition and surface properties of the nanomaterial. Moreover, the results of cytotoxicity tests should be interpreted with caution depending on the exposure time since long exposure can obviously trigger a more cytotoxic effect. The cytotoxicity of nanomaterials is, however, conditioned by two factors: the ability to be internalized by cells and the ability to trigger cytotoxicity (by oxidative stress, apoptosis, etc.).

Just like nano-organisms (such as viruses), nano-objects are able to penetrate inside cells and interact with intracellular biological species.<sup>55j</sup> The conventional internalization route is endocytosis,

especially for agglomerates of nanoparticles located in the cytoplasmic vesicles.<sup>100</sup> Some scientists have also suggested that nanoparticles of a few nanometres could penetrate cells through ion channels or membrane pores whilst others believe that the passage of nanoparticles could also take place passively by diffusion and interaction between the surface of the nanomaterials and the cell membrane, without vesicle formation.<sup>101</sup> Van der Waals, electrostatic, steric or surface tension interactions may occur. This type of internalization presents a risk because the nanomaterials can shift freely inside the cell where they will come into direct contact with cytoplasmic proteins and organelles. Cell internalization by diffusion was observed for small nanoparticles, especially if they were lipophilic (e.g. 25-50 nm<sup>102</sup>).

The location of nano-objects in the cell depends on their size. Microparticles were found in large cytoplasmic vacuoles whereas smaller nano-objects were found in small vesicles or roamed free in the cytoplasm.<sup>55f</sup> However, the latter tended to accumulate in cells and also finally clustered together in cytoplasmic vacuoles. The presence of nanoparticles in the mitochondria, albeit rare, has also been described.<sup>103</sup> Within the cells, the nanomaterials could interact with biological components and disrupt cell function. Dose can also influence the intracellular distribution of nanoparticles. Indeed, Yang et al. (2010) showed that, although lysosomes were target organites, single-walled carbon nanotubes (SWCNTs) were also internalized within the mitochondria at high doses (80% lysosomes/20 % mitochondria as from 400 mg/kg po in male mice).<sup>104</sup>

Carbon nanotubes, quantum dots, fullerenes, gold or TiO<sub>2</sub> nanoparticles might be able to trigger apoptosis.<sup>98, 105</sup> Other nanomaterials could interact with the nucleus and cause DNA lesions or mutations.

Nevertheless, with regard to cytotoxicity mechanisms, the main mechanism of nanomaterials concerns their ability to trigger oxidative stress by forming free radicals such as reactive oxygen species (ROS), leading to the activation of signalling pathways sensitive to redox potentials.<sup>55j</sup> The process triggered would culminate in the production of cytokines and chemokines involved in pro-inflammatory responses. Organs of the reticulo-endothelial system would eventually be damaged via accumulation.

There are many sources of free radicals. As the surface of nanomaterials can be highly reactive, it witnesses numerous chemical reactions which occur between the nanomaterial and the surrounding species. Some materials such as transition metals can act as reaction catalysts, producing free radicals or ROS (Fenton or Haber-Weiss reactions). Free radicals were also formed by the conventional phagocytose response of cells when confronted with a foreign body. An increase in oxidative lesions was also observed due to the disruption in mitochondrial activity (electron transport chain).<sup>106</sup>

Nanomaterials seem to generate more free radicals and ROS than their larger counterparts, probably because of their larger specific surface area.<sup>107</sup> Although for some insoluble nanomaterials the role of size has been highlighted in cytotoxicity mechanisms, it is still too early to establish whether

the cytotoxicity observed in nanomaterials is also due to toxicity based on chemical composition (such as the release of ions that are active metabolites or reactions catalysed with surface elements). There is still not enough comparable, reliable data and the analytical and experimental methods employed are not adequate to draw a definite conclusion. In fact, research scientists have shown that a few nanomaterials interfered with the reading of some cytotoxicity tests.<sup>99, 108</sup>

#### **II.A.4. Immunotoxicology, delayed hypersensitivity and irritation**

When an organism is stressed, two types of defence mechanism are triggered: specific or non-specific. The non-specific response is immediate and appears swiftly after the aggression. This occurs in irritation reactions following the initial contact. On the other hand, a specific reaction is triggered only after several exposures. Delayed-type hypersensitivity is primarily a specific allergic reaction that calls for the patient to be pre-sensitized to the allergen: several contacts with the allergen are needed in order to elicit a response and involve the immune system.

The immune system is, by definition, the organism's shield against aggression, which could prevent it from functioning properly. This system is both highly complex and very finely regulated: it involves many components, both humoral (cytokines, chemokines, complement, antibodies, etc.) and cellular (dendritic cells, T and B cells, etc.), and any disruption could trigger stimulation or repression depending on the elements involved.

Research in nanomaterial immunotoxicity are rather focused on nanoparticles and the main results are detailed below. Manufactured nanoparticles can be classified into two main groups - those produced to be "furtive" and therefore to pass unnoticed by the immune system, and those targeting cell components and may interfere with this system.

- ***The "stealthiness" of nano-objects***

Nanoparticles are "stealth" when they are not identified by the defence mechanisms; this lack of recognition is a major issue in the design of nanoparticles. The physical characteristics of these nano-objects (size, shape, surface charge and hydrophilic/hydrophobic properties) will condition compatibility with the immune system. Polymers such as poly-ethylene-glycol (PEG) have often been used to coat nanoparticles in order to make them hydrophilic, which enable them to circulate unnoticed by the immune system.<sup>109</sup> Stealthiness is conferred by the effect of steric repulsion via a hydrophilic polymer corona, as explained earlier. This was the case for PEG (or PEO) in brush or loop format<sup>110</sup> and for polysaccharides in long brush format only.<sup>111</sup> These designs have, however, sometimes led to anti-PEG antibody production,<sup>112</sup> which eliminated PEG-nanoparticle complexes more rapidly from the body<sup>113</sup> and raised the risk of an inflammatory or anaphylactic response.

Moreover, many serum proteins can bind to nanoparticles and alter their treatment by immune cells (interaction with specific receptors), which can completely detract nanoparticles from their initial target.

- ***Nanoparticles and immune response***

A nanoparticle can impact upon the immune system in several ways. It can manifest in the form of inflammation and trigger allergic or even auto-immune reactions.<sup>114</sup> This will depend on the specific antigen properties of the nano-object, its adjuvant or inflammatory potential or its ability to activate the complement system. According to the situation, the outcome of these interactions will be an increase or inhibition of the immune response. The nanoparticles will then be considered as immunostimulant or immunosuppressant.

#### ● ***Nanoparticles and antigenicity***

The creation of a specific immune response is manifested by the production of antibodies and/or cells that will recognize the foreign body. Very few studies to date have shown that the particles could trigger an immune response specific to them. Anti-C<sub>70</sub> or C<sub>60</sub> fullerene antibodies have been described but it has not been possible to reproduce these results, even in the presence of strong adjuvants.<sup>115</sup>

#### ● ***Adjuvant effect***

An adjuvant is an element capable of enhancing the immune response triggered by a given antigen. Numerous publications highlighted the adjuvant ability of nano-objects:<sup>116</sup> for example, small molecules attached to gold nanoparticles triggered a stronger response (specific antibody synthesis) than in the presence of a conventional adjuvant and with less antigen.<sup>117</sup> The origin of this phenomenon was not clearly defined and may undoubtedly be due to the nanoparticle and the antigen considered. The nature and extent of the immune response varied depending on several parameters: duration time during which the antigen was present in the body, endocytosis by immune cells and activation of these same cells. Nano-objects can affect any of these variables: they can have a longer retention time in the tissues than proteins, they can be internalized in large numbers by the immune cells and remain in these cells, and they can also trigger an inflammatory response, which activates cells and in turn prompts an immune response. However, the drawback of this enhancing effect is that nano-objects can cause significant disruption such as allergic reactions: carbon nanotubes have been seen to increase allergy to ovalbumin by triggering acute inflammatory responses.<sup>118</sup>

#### ● ***Inflammatory effect***

The initiation of an immune response and, above all, the orientation towards pathways Th1, Th2, Th17 or Treg involve many parameters including cytokines, which play a key role. The secretion of these molecules is partly controlled by the inflammatory condition of the cells: several studies have shown that the various physical parameters of nano-objects could be implicated:

- the charge (cationic liposomes triggered cell activation, but not anionic liposomes)<sup>119</sup>;
- the size (polystyrene particles less than 100 nm had a greater stimulating effect on antibody production than particles greater than 500 nm). Th1 or Th2 responses appeared to have been triggered by PLGA nanoparticles of 80 nm and above or by dendrimers (macromolecules containing monomers which group together by branching out around a multifunctional centre) of 5 nm.<sup>120</sup> However, in these different cases, the fact that the authors used different reagents could also impact

upon the response triggered because even the contaminants of nanoparticle preparations can affect the responses obtained. The chemical modification of the particle surface allowed particles to interact with membrane receptors (TLRs, complement receptors, MARCO receptor, etc.) like pathogens, and targeted the particles towards the cells involved in the immune response such as dendritic cells.<sup>120</sup> The inflammatory reaction can also be due to oxidative stress caused by nanoparticles formed from metallic oxides;<sup>121</sup> depending on their oxidizing potential, these materials have a potentially significant impact on inflammation through to cytotoxicity or genotoxicity.<sup>122</sup>

#### ● **Internalisation by immune cells**

The cells involved in triggering immune responses (dendritic cells and, to a lesser extent, macrophages) are highly capable of internalization. Not only do these cells express several surface receptors internalizable after binding their ligand, but they are also capable of phagocytosis, *i.e.* they can internalise ligands of over 500 nm. Although the presence of nanoparticles within cells is largely documented by several publications, the internalization process has not been clearly established in most cases and probably differs depending on the particles. The binding of serum proteins to nanoparticles can assist internalisation as shown for fetuin;<sup>71</sup> several cell receptors were involved: receptors for the Fc fragments for immunoglobulins (fullerenes),<sup>123</sup> complement receptors (lipid nano-capsules of 10 to 100 nm),<sup>124</sup> mannose receptors for chitosan particles.<sup>125</sup> On one hand, internalisation increased with the zeta potential and, on the other hand, the large size of some particles or agglomerates promoted phagocytosis.<sup>126</sup> The internalization of nano-objects by immune cells could potentially disrupt the normal functioning of these cells and, therefore, the immune system.

#### ● **Immunosuppression**

Although most of the studies carried out with nanoparticles emphasised the inflammatory aspects, some showed that nano-objects could also trigger immunosuppression: carbon nanotubes could catalyse the TGF- $\beta$  synthesis by macrophages resulting in B-cell blockage.<sup>127</sup> Nano-objects are sometimes used to deliver steroidal immunosuppressants (treatment of arthritis) or for auto-immune treatments (transport of collagen for the treatment of rheumatoid arthritis),<sup>115</sup> but they can also display immunotolerant properties: peptides in the form of dendrimers could block allergic encephalomyelitis.<sup>128</sup>

#### ● **Nanomaterials and hypersensitivity**

The ability of nanomaterials to trigger a hypersensitivity reaction post-exposure depends first and foremost on its ability to cross the barrier, such as skin, and interact with proteins. The nano-object / protein complex is generally recognized by the immune system and is therefore essential for sensitization. We have already referred to experiments showing that nano-objects could act as vectors to induce or reduce allergic reactions directed towards proteins. Conversely, there is no clear-cut proof of hypersensitivity resulting from the presence of nano objects alone and which is directed against the latter. Nickel nanoparticles are an exception, however, because in this particular case it seems that nickel ions obtained from solubilisation of the particle were responsible for the inflammatory reaction<sup>129</sup> by reacting with (TLR4) receptors.<sup>130</sup> On a more general note, some scientists even think that the

toxicity observed in the presence of nanoparticles could be due to their solubilization in biological media or in cells, with the resulting ions being in fact more toxic than the nanoparticles from which they were derived.<sup>131</sup>

- **Potential for irritation**

The topical application of nanomaterials to the skin *in vivo* has generally led to mild to moderate irritation.<sup>76b</sup> Studies focusing on metallic oxide nanoparticles used in cosmetic products and sun creams have shown a slight irritant potential.<sup>55l</sup> Similarly, some carbon nanotube studies suggested that they did not trigger dermal<sup>132</sup> or ocular irritation.<sup>133</sup> However, the absence of any irritation contrasts with well-known contact dermatitis reactions observed with macroscopic carbon fibres of a similar structure.<sup>134</sup>

## II.A.5. Haemocompatibility

According to current standards for the biological assessment of medical devices (standards within the ISO 10993 series), haemocompatibility must be assessed for implanted medical devices or MD communicating externally, in direct or indirect contact with blood. Moreover, the propensity of nano-objects to access the blood and interact with biological elements emphasises the importance of this evaluation to even greater extent.<sup>1</sup>

Any artificial surface is normally recognized by the body as a foreign element which triggers non-specific defense reactions in the blood affecting cell elements (polynuclear cells, red blood cells and platelets, *etc.*), plasma proteins (e.g. the complement system) and chemical mediators. These reactions lead amongst other things to coagulation effects and fibrinolysis. Finally, platelet activation and thrombin production are two synergistic phenomena that could trigger thrombosis - a sign of material non-haemocompatibility in the cardiovascular system.

Radomski *et al.* have shown that carbon nanotubes and mixed carbon nanoparticles could trigger platelet aggregation *in vitro* and accelerated vascular thrombosis in a rat model with thrombosis, as seen with fine and ultrafine particles of urban pollution.<sup>135</sup> However, the lack of information on the characteristics of the nanomaterials studied limits the extent of these data. By studying the effect of various polystyrene nanoparticle parameters on haemocompatibility, Mayer *et al.* noted that size seemed to play an important role.<sup>136</sup> In fact, the smaller nanoparticles (20-30 nm) triggered haemolysis and inflammation. Conversely, these authors could not draw any conclusions regarding the effect of the surface charge - an observation shared by other research scientists regarding the internalization of polystyrene, gold and TiO<sub>2</sub> nanoparticles by red blood cells.<sup>101, 137</sup>

Although research in the haemocompatibility of nanomaterials is relatively scarce, two mechanisms can nevertheless be distinguished:<sup>5b, 55g, 136</sup>

- Direct mechanism: by thickening the blood, blocking the vessels, damaging the vascular walls or even creating a localized inflammatory reaction such as an atheroma plaque, which would reduce blood flow;

- Indirect mechanism: by triggering the release of inflammatory chemical mediators that diffuse into the blood and activate localised inflammatory reactions in the vessels of the affected organs.

In contrast, other experiments carried out with drug delivery nanoparticles such as nanoparticles containing alcohol/polysorbate or gadolinium, displayed their haemocompatibility with minor effects on platelet function.<sup>138</sup> This is why, based on current knowledge, contradictory and limited information still prevent any conclusions from being drawn with regard to the potential toxic effect of nanomaterials on the vascular system unlike ultrafine pollution particles, whose relationship with cardiovascular diseases has been clearly established.<sup>139</sup>

## II.A.6. Systemic toxicity

In this section, we shall examine the systemic toxicity of nanomaterials over time, i.e. acute toxicity (24 h), subacute (24 h to 28 days), subchronic (e.g. 90 days) and chronic (generally 6-12 months) when considering rodents. In the published studies, identification of the target organs was often overlooked and the characteristics of the nanomaterials tested were relatively limited.<sup>76b</sup> These preliminary studies nevertheless indicated slight to moderate systemic toxicity at the doses administered and via the routes of administration employed.<sup>140</sup>

The organs mostly affected belong to the reticulo-endothelial system including the liver and the spleen. This is consistent with the frequently documented uptake of nanomaterials by this system. The kidney is another organ usually affected since it has been identified in some toxico-kinetic studies as the primary route of elimination for numerous nanomaterials including carbon nanotubes and fullerenes.<sup>141</sup> The accumulation of some nanomaterials in lysosomes raised the risk of dysfunction.<sup>76b</sup> The biopersistence of insoluble nanomaterials could lead, amongst other things, to changes in lysosomal permeability and enzymatic activity, and to macrophage apoptosis. *In vitro* quantum dots have been observed to be involved in activating autophagia mechanisms.<sup>142</sup> Autophagia is a process of degradation of part of the cell cytoplasm by its own lysosomes, sometimes contributing to cell death, but also acting as a cell defence mechanism.

Thanks to their unique optical and electrical properties, fluorescent quantum dots have shown their usefulness primarily as imaging agents and their applications should increase in the future. However, the enthusiasm for this new nanomaterial is obscured somewhat by potential toxicity, especially regarding quantum dots which contain cadmium. In fact, quantum dots often contain a metallic core of cadmium-selenium, surrounded by an initial envelope to make them biocompatible, and possibly a secondary functionalized envelope for screening or modifying their bio-kinetic properties. Concerns have been raised regarding the stability of this nucleus-envelope structure. If, *per chance*, the metallic nucleus was exposed, cadmium ions are known to trigger signs of acute and chronic toxicity in vertebrates and raises real environmental and health problems.<sup>98</sup> Quantum dots are an excellent example of the influence of size and surface properties on the systemic toxicity of nanomaterials because their biodistribution can be monitored. In a study of acute toxicity *in vivo*, Geys

*et al.* showed that, at a high dose, the quantum dots of CdSe/ZnS with a carboxylic function caused more pulmonary thromboses than those containing amines.<sup>143</sup>

Initial toxicity studies have focused on local effects, mainly pulmonary and dermal, with different test conditions and sometimes insufficient characterization of the nanomaterials studied. This is why it is not easy to generalize and predict the toxicity profile. The problem is all the more worrying for nanomaterials because of their ability to translocate in the body and reach hitherto fairly inaccessible organs (such as the brain). There is thus a real need to boost our knowledge of the systemic toxicity of nanomaterials, especially in the long-term, with validated analytical methods and protocols.

## **II.A.7. Genotoxicity**

According to current standards for the biological assessment of medical devices (standards within the ISO 10993 series), analysis of the genotoxicity of a medical device is a key stage in the biological risk assessment because damage to DNA may lead to the development of cancers but can also impact upon the reproductive system and foetal development. Some nanomaterials have been seen to cross cell membranes<sup>55j, 100-102</sup> and subsequently also penetrate cell nuclei. The passage of nanomaterials is due to diffusion or active transport across the nuclear membrane, which possesses nanoscale pores.<sup>144</sup> Another possibility for direct contact between nano-objects accumulated in the cells and DNA occurs at the moment of cell division when the nuclear envelope disappears. The primary genotoxic effects of conventional materials follow a direct or indirect mechanism but secondary genotoxic effects can also occur, and even indirect effects (the last point is discussed in greater detail on section II.B.3).

### **• Direct primary effects**

Nanomaterials have similar dimensions to cell and nuclear components. The diameter of a nucleosome is 10 nm and that of microtubules is 25 nm. By way of comparison, strands of DNA are a micrometer long and a few nanometres in diameter, similar to carbon nanotubes. Nanoparticles may well penetrate the nucleus and interact directly with DNA and nuclear proteins. Nano-objects can produce free radicals by reacting with cell components on the same scale and induce DNA lesions or disrupt chromosome separation during mitosis (aneugenic potential). The process of cell division may be disrupted as a result and cell traffic disorganized.<sup>55c</sup> Above all, the presence of silica nanoparticles in the nucleus could lead to the formation of intranuclear protein aggregates, which subsequently triggered the inhibition of replication, transcription and cell proliferation.<sup>146</sup>

### **• Indirect primary effects**

The genotoxic effect can also be caused by an indirect mechanism involving either a pro-oxidizing effect or inhibition of DNA repair. When the fragile redox equilibrium between anti-oxidizing agents and reactive oxygen species (ROS, *Reactive Oxygene Species*) is disrupted, the phenomenon of oxidative stress is triggered. Endogenous oxidative lesions of DNA or those involved in the

mitochondrial respiratory chain increase, leading to the production of ROS and the discontinuation of ATP synthesis. Metallic oxide nanoparticles are particularly involved in these phenomena because the released metallic ions are likely to catalyse the conversion of metabolic molecules into free radicals.

The second consequence of the indirect primary genotoxic effect concerns DNA repair. Failure of the repair process could trigger mutagenic and carcinogenic effects. When the DNA is damaged, a key effector protein, p53, is activated. If the lesions are too extensive, protein p53 triggers cell apoptosis. The activity of this protein is therefore a good indicator of the genotoxic effect.

Ahamed *et al.* determined the genotoxic effect of silver nanoparticles triggering an increase in the expression of p53 in fibroblasts and embryonal stem cells.<sup>147</sup> These results indicated that exposure to silver nanoparticles could lead to DNA damage. In fact, silver nanoparticles have been observed to penetrate the nucleus.<sup>148</sup> AshaRani *et al.* have suggested the involvement of an indirect primary mechanism. The DNA lesions observed were essentially linked to dysfunction of the mitochondrial respiratory chain and an increase in the quantity of ROS. However, currently the ability of silver nanoparticles to promote oxidative stress is still under discussion and the mechanism of their genotoxicity remains unclear.<sup>149</sup> Overall, it is still difficult to determine whether or not the nanoparticles studied (all types) interact directly with DNA.<sup>150</sup>

#### • **Secondary effects**

The endocytosis and/or phagocytosis of nano-objects can also lead to additional, secondary DNA lesions. These secondary effects, which are mainly associated with inflammation, are due to the oxidative stress and inflammatory response triggered by nano-objects. The radical species formed, such as hydroxyl radicals, can react with polyunsaturated fatty acids thus triggering lipid peroxidation, which, in turn, can lead to the formation of DNA adducts. DNA lesions also take the form of chromosomal fragmentation and mutation points.

Although the size and surface chemistry affect the biological response, the chemical composition also appears to play an important role in the magnitude of the ensuing response.<sup>149</sup> In an *in-vitro* study published by Papageorgiou *et al.*, cobalt-chrome nanoparticles followed a different genotoxic mechanism from their microparticle counterparts on fibroblasts.<sup>151</sup> The more extensive DNA lesions observed were probably due to the side effects associated with inflammatory phenomena.

Inflammation can trigger the production of hypochlorous acid via the myeloperoxidase of polynuclear neutrophils. Hypochlorous ions inhibit DNA repair via the excision-resynthesis mechanism by blocking the expression of several genes involved in this mechanism in particular.<sup>152</sup>

#### • **Evaluation of nanoparticle genotoxicity**

Discrepancies are currently observed in published conclusions, which make them difficult to interpret.<sup>55b, 55g, 149-150, 153</sup> There are several reasons for this. Generally, in numerous toxicity studies published to date, a lack of precise physico-chemical characterization of the nanomaterials studied and the differences between the experimental conditions (e.g. dose units, protocols) make comparisons difficult. Apparently contradictory results have been recorded with the same

nanomaterials especially in terms of genotoxicity as compared to other parameters.<sup>149-150</sup> Genotoxicity is difficult to evaluate because of the diversity of experimental methods used.

This is why it is highly advisable to identify the characteristics of the nanomaterials studied and to choose cell lines and protocols most suitable for the study since inappropriate models can generate irrelevant results that cannot be extrapolated to man. Without rigorous control of the test parameters, it would be difficult to identify the factors affecting the toxicity observed. It would, however, be useful to establish whether the nanomaterial genotoxicity observed is specific to the chemical element or to the nanometric size. For instance, concerning cobalt, a study allocated the genotoxic effect observed to ions arising from metallic nanoparticles.<sup>154</sup>

## II.A.8. Carcinogenicity

Although several studies highlighted the ability of nanomaterials to trigger DNA lesions, few data are actually available regarding their carcinogenic potential. A study comparing nanomaterials with their conventional counterparts (bulk materials) showed that nanoscale metals triggered a carcinogenic effect whereas bulk materials caused only a chronic inflammatory reaction such as a granulomatous reaction, usually against a foreign body.<sup>155</sup>

A genotoxic process and chronic inflammation can lead to a carcinogenic effect. The spectrum of persistent carcinogenic effects with fibrillar materials such as asbestos raises concerns regarding carbon nanotubes with a similar structure.<sup>55g, 156</sup> In fact, some research scientists have shown that long, multi-walled carbon nanotubes could cause mesothelioma in the abdominal cavity of mice (peritoneum, tissue comparable to pleura).<sup>157</sup> All these authors have emphasized above all the importance of bio-persistence and the size of the carbon nanotubes tested with regard to the carcinogenic phenomenon, as with asbestos. This was for instance one of the possible causes provided by Muller *et al.*, who did not succeed in proving a carcinogenic effect *in vivo* after 2 years of study, as the multi-walled carbon nanotubes used in the experiment were not long enough to trigger tumours.<sup>158</sup> The potential toxic and carcinogenic effects of carbon nanotubes will be discussed in more detail later on (part II.B.1).

The same phenomenon could also account for the carcinogenicity of slightly toxic but sparingly soluble inhaled pollution nanoparticles (e.g. TiO<sub>2</sub>).<sup>55b, 159</sup> Pulmonary overload and chronic inflammation due to biopersistence could lead to tumour formation. The possible mechanisms include DNA lesions and the production of ROS accompanying inflammation.

## II.A.9. Reproductive and developmental toxicity

Current knowledge relating to the toxicity of nanomaterials towards reproduction and foetal development are very limited.<sup>160</sup> DNA damage can trigger mutations, which can also disrupt

reproduction and the development of subsequent generations. Moreover, the high mobility of nanoparticles may allow them to diffuse into the reproductive organs and cross the placental barrier.

Research scientists have monitored radiolabelled gold nanoparticles injected into female gestating rats. Some scientists have detected a very small quantity of nanoparticles with a diameter of 5 to 30 nm in the foetus (0.005-0.018% of the dose injected).<sup>161</sup> Others, however, have not detected the passage of radiolabelled nanoparticles<sup>162</sup> or non-radiolabelled nanoparticles of 4 to 40 nm.<sup>163</sup>

An *in-vitro* study of polystyrene nanoparticles has, however, shown that nanoparticles might cross the placenta depending on their size.<sup>164</sup> Similarly, quantum dots could also cross this barrier even with a silicon or PEG coating.<sup>165</sup> Nanoparticles of TiO<sub>2</sub> injected subcutaneously into female mice disrupted spermatogenesis in the offspring along with histological changes in testicles and alterations in cerebral gene expression.<sup>166</sup> Carbon nanotubes injected intravenously into the tail of male adult mice caused reversible damage to the testicles and generated ROS without changing hormone concentrations or the fertility of the rodents.<sup>167</sup>

Thus the effects vary considerably from one type of nanomaterial to another.<sup>160</sup> Consequently, it is impossible to generally apply the results of studies conducted with one type of nanomaterial to all nanomaterials already used in medical devices (or elsewhere).<sup>160</sup> Very few studies have shown a teratogenic or reprotoxic effect despite an increasing number of studies highlighting the potential passage of nanoparticles through the placental barrier. The few reprotoxic effects observed to date seem relatively minor or inconclusive, but the risk is nevertheless present.

## **II.A.10. Neurotoxicity**

The Central Nervous System (CNS) is impermeable to a large number of molecules thanks to the barrier that separates the blood vessels from the extracellular space of the nervous tissue (blood-brain barrier). This protective barrier is essentially composed of endothelial cells, which are interlinked by tight junctions that prevent the diffusion of molecules, even small ones such as ions. Furthermore, access to cerebral tissue is governed by several active systems of selective transport, mainly ion channels. Therefore, the blood-brain barrier is not only a physical obstacle, passively preventing the passage of undesirable substances, but it is also an extremely selective filter, restricting access to highly lipophilic molecules.<sup>87</sup>

### **• Risk of exposing the CNS to nano-objects**

The neurotoxicity of metals has drawn attention to the risk presented by nano-objects in particular. Initially, after implanting a MD containing nanomaterials in the brain, there is a risk of accumulation of substances with a potentially hazardous outcome for the patient, the brain being a closed environment allowing only very few substances to enter and exit. On the other hand, the degradation products including nanoparticles released due to the wear and tear of implanted medical devices (outside the brain) can be detected in the blood and allowing a potential translocation to the brain, leading to a possible risk of neurotoxicity.

### • **Ability of nanoparticles to cross the blood-brain barrier**

Under normal conditions, few nano-objects cross the blood-brain barrier.<sup>55a, 104, 168</sup> However, a disruption to this barrier or to the selective transport systems (e.g. in the event of disease or the genetic deformation of this barrier) could facilitate penetration. In particular, its permeability to nano-objects is increased *in vivo* in the case of some physiopathological situations such as hyperthermia, even aggravating the pathological process.<sup>169</sup>

Apart from their high mobility, gold nanoparticles measuring a few tens of nanometres, would be readily transported through the barrier by passive diffusion or by transport-mediated endocytosis including active systems of selective transport.<sup>92, 170</sup>

Nanomaterials can also force their way through by altering the barrier. In fact, the specific surface properties of nanoparticles and especially metallic nanoparticles, could allow them to interact with epithelial cell membranes in the blood-brain barrier, thus causing alterations and neuronal degeneration.<sup>171</sup> It is interesting to note that in this *in vivo* study, copper and silver nanoparticles were more toxic than aluminium nanoparticles, though of the same size (50-60 nm). In a mechanistic point of view, according to *in-vitro* studies, the damage done to the barrier by nanoparticles could be due to their ability to trigger oxidative stress, generate reactive oxygen species and trigger inflammation and some neurodegenerative diseases.<sup>75</sup>

### • **Direct access of inhaled nanoparticles**

Conversely, there is another, more direct way of accessing the CNS for inhaled nanoparticles. *In-vivo* studies have shown that nanoparticles deposited on the olfactory and nasal epithelium can translocate to the CNS *via* the olfactory nerve and directly access the brain, but this mechanism has yet to be confirmed in man.<sup>172</sup> Despite the physiological and anatomical difference between rats and humans, as the olfactory system in the rat is more developed, the possibility of nanoparticles inhaled by the olfactory route penetrating the human brain cannot be overlooked.<sup>76b</sup>

### • **Neurotoxic risk of nanoparticles**

Although the pathological consequences of the translocation and accumulation of nano-objects in the brain are still unclear, the possible formation of cerebral oedema, potentially leading to cerebral lesions and neurological diseases, can nevertheless be considered. In addition, if the ability of nanoparticles to enter the CNS is worrying, it is also promising since it is an exploratory route for transporting treatments to this seemingly inaccessible organ to treat neurological diseases.<sup>173</sup>

## II.B. Current knowledge of the toxicological assessment of some nanomaterials

Several nanomaterials can be used in medical devices (gold nanoparticles, nanodiamonds, nanometric silica, iron oxides, nanometric hydroxyapatite, nanometric titanium, etc.). However, we have voluntarily chosen to develop the toxicological evaluation for just three of them:

- carbon nanotubes (still under development for MD).
- silver nanoparticles (used in MDs already on the market),
- nanoparticles arising from degradation products due to the wear and tear of medical devices.

### II.B.1. Carbon nanotubes

Particular attention is paid to the potential toxicity of carbon nanotubes because of their unique biological properties, which are responsible for their high potential in medicine. The use of carbon nanotubes in medical devices is currently at the development stage. As far as we know, no medical device with carbon nanotube has been marketed to date.

A carbon nanotube (CNT) consists of one or more sheets of graphene rolled one over another to form a cylindrical structure. Carbon nanotubes have proved interesting in medicine because of their relatively important length-to-diameter ratio. In fact, possessing similar dimensions to DNA strands, carbon nanotubes can interact more efficiently with biological materials because they are on the same scale. The surface of these CNTs can easily be functionalised to modulate their biological behaviour, enhancing their biocompatibility and showing potential applications in anti-infective treatments (under development).<sup>174</sup> Thanks to their specific physico-chemical properties, carbon nanotubes can be considered for use in the recognition and detection of highly sensitive molecules. Their strong optical absorbance in particular makes them good candidates for cancer treatment. However, as for any health product, the risk of toxicity must be weighed against potential benefits.

However, because of their close fibrillar resemblance to asbestos, the toxicological risks of carbon nanotubes give cause for concern.<sup>175</sup> Although initial results have shown that some CNTs currently used could trigger toxicity via the respiratory route under test conditions, it is difficult to obtain a clear consensus in terms of toxicity due to the differences in materials and the operating conditions of the published studies.<sup>175a, 175c</sup>

Some studies have shown that CNTs can trigger a granulomatous reaction and pulmonary inflammation, even pulmonary fibrosis.<sup>83, 157a, 176b</sup> This pulmonary toxicity could also induce cardiovascular effects.<sup>177</sup> At cell level, Porter *et al.* observed the internalization of single-walled carbon nanotubes (SWCNT) by transmission electron and confocal microscopy.<sup>178</sup> The cytotoxicity of CNTs

was due to oxidative stress, which could trigger genotoxicity.<sup>179</sup> Finally, the biopersistence of these nanomaterials in the lungs<sup>83, 180</sup> is especially of concern since, like asbestos, it could trigger mesothelioma observed by some research scientists.<sup>157a, b</sup> In direct contrast to these toxicity data, other studies have shown that the CNTs were tolerated locally following intra-tracheal instillation in rats<sup>181</sup> and were non-carcinogenic after intraperitoneal injection.<sup>158</sup>

It would appear that toxicity depends on the intrinsic characteristics of the CNTs, such as length, shape<sup>182</sup> and state of aggregation.<sup>183</sup> Short CNTs could be subjected to phagocytosis by macrophages and eliminated more easily than long fibres - hence they could be less toxic.<sup>157a</sup> For instance, Müller *et al.* showed that over 80% of multi-walled carbon nanotubes (MWCNT), 5.9 µm in length, were still present in the lung tissue of rats after two months compared to 36% for MWCNTs of 700 nm.<sup>83</sup> Regarding the state of aggregation, dispersed SWCNTs, administered via the intratracheal route, do not trigger granulomatous reactions, unlike aggregated SWCNTs.<sup>183</sup> Toxicity results also depends on the physico-chemical difference between the carbon nanotubes studies (whether or not functionalised).<sup>86</sup>

The contradiction in the results obtained in various studies can also be due to the disparity in terms of test conditions.<sup>86</sup> Some doses administered were criticised as being too high and hardly realistic clinically.<sup>184</sup> Moreover, it is important to remember that some experiments were carried out on carbon nanotubes in the form of aggregates administered by intra-tracheal inhalation or unnatural pharyngeal instillation. These aggregates could not have been inhaled because of their large size. Thus the toxicity test results do not necessarily reflect respiratory exposure in real situations.<sup>76b</sup>

Furthermore, some toxicity results appear to be misinterpreted whilst others depend on CNT manufacturing processes.<sup>86</sup> On one hand, some carbon nanotubes interacting with some colorimetric agents in the toxicity tests used such as MTT, generated false positive results.<sup>108</sup> On the other hand, even if research scientists test the cytotoxicity of carbon nanotubes using other non-colorimetric methods, their toxicity results would be tempered by the possibility of metallic contamination, which would intensify the cytotoxicity observed.<sup>185</sup> Kagan *et al.* postulated that the iron residues used as a catalyst in CNT production would increase oxidative stress by comparing the inflammatory response of macrophages between carbon nanotubes made from contaminated and purified SWCNT.<sup>186</sup> It should be noted that the induction of mesothelioma was observed on raw, non-purified carbon nanotubes.<sup>157a, b</sup> It seems that the surface defects could also be responsible for the pulmonary toxic effects observed.<sup>187</sup>

In view of the initial, albeit contradictory, toxicity results,<sup>55h</sup> the risk of a "fibre" type toxic pulmonary effect, similar to asbestos, seems to be associated with carbon nanotubes, with the potential incidence of genotoxicity and carcinogenicity being due to lung inflammation. The experimental studies indicated danger but the carcinogenic risk to man cannot be considered evident given the limitations of the experiments carried out.<sup>188</sup> Not all of the carbon nanotubes inhaled necessarily trigger symptoms similar to asbestos. The size and form are important parameters for toxicity in addition to impurities and surface defects. No definitive conclusion can currently be drawn in

terms of the identification of toxic potential, given the wide physico-chemical variety of the carbon nanotubes studied.<sup>188</sup>

Few carbon nanotube toxicity studies have been carried out using other routes of exposure. The published papers tend to show the harmless nature of nanomaterial, particularly upon contact with the skin<sup>132</sup> and when administered intravenously.<sup>189</sup> Reversible damage has been reported in rat testicles without, however, affecting their fertility.<sup>167</sup> *In-vitro* studies essentially confirmed the lack of toxic effects with highly purified carbon nanotubes on cardiac cells<sup>190</sup>, osteoblasts and fibroblasts.<sup>191</sup> Although the liver appeared to be a preferred accumulation site,<sup>175a</sup> most of the single-walled carbon nanotubes administered by intravenous injection and monitored by radiolabelling<sup>96</sup> or fluorescence<sup>189</sup>, were rapidly eliminated via the kidneys.

Consequently, caution should be exercised when interpreting the results, accurately identifying the factors responsible for the toxicity observed whilst using nanomaterials for the intended purpose and remain wary of false positive results. There are still many gaps in our knowledge of the toxicity of carbon nanotubes, especially following exposure to other routes used more frequently for medical devices than the pulmonary route.

Nevertheless, CNTs are an interesting alternative for medical applications. The benefits of using these nanomaterials should in this case be weighed against the potential risks.

## II.B.2. Silver nanoparticles

Silver has been used since Antiquity, especially in health products, and generally presents few risks to man regardless of route of exposure - respiratory, oral, cutaneous or intravenous.<sup>55c, 192</sup>

Silver ions, obtained from dissolving metallic silver in a biological medium, is responsible for the bactericidal activity<sup>46</sup> and seems to be biologically more active than metallic silver.<sup>55c, 193</sup> The exact mechanism of action is unclear to date but ions appear to bind readily to proteins and amino acid residues, interacting with cell membranes, and can irreversibly denature bacteria and viruses, hence its usefulness as an anti-infective, anti-viral agent. The bactericidal activity of silver nanoparticles depended on the size and shape since the interaction of these nanoparticles with bacterial membranes was influenced by their specific surface area.<sup>194</sup> Nanoparticles less than 10 nm were the most reactive,<sup>194a</sup> like triangular, truncated shapes.

Some studies showed that metallic silver nanoparticles have an anti-inflammatory action, especially by selectively inducing the apoptosis of inflamed cells and thus promoting wound healing<sup>195</sup>, whereas silver ions *per se* were devoid of anti-inflammatory activity. The presence or absence of a

surface coating on the silver nanoparticle also alters the rate of solubilization and their biological properties. More basic research on the mechanisms of action of silver nanoparticles has highlighted the potential of this nanomaterial to promote oxidative stress, possibly culminating in apoptosis. By interacting with proteins and enzymes possessing thiol groups, silver nanoparticles could disrupt mitochondrial function.<sup>196</sup> They also triggered DNA lesions indicative of genotoxicity but the data gathered to date were not sufficient to corroborate this finding.<sup>149</sup> A comparison of the activity of silver nanoparticles (13 nm) with silver microparticles (2-3 µm) in an *in vivo* study suggested that the shift from a microscale to a nanoscale played a key role in triggering apoptosis.<sup>197</sup>

Adverse effects such as allergy or silver intoxication (argyria) are known and controlled. Argyria, a rare phenomenon, is due to prolonged exposure through ingestion and inhalation, leading to deposits of silver in the skin (argyria), giving a blue colouration to the skin and secondary mucosa or the eye (argyrosis). Generally, silver ions produced from the ionisation of metallic silver in media such as body fluids or tissue exudates, are readily absorbed by the body, and are quickly found in the systemic circulation before being eliminated via the liver and kidneys.

Although the toxicity of metallic silver particles via the pulmonary and oral routes has been documented, few information is available regarding the risks of cutaneous exposure, which is nevertheless of specific interest given the medical devices already on the market or under development (dressings, medical textiles).

Regarding skin absorption, *in vitro* studies on human skin have shown slight absorption through intact skin whereas potentially high quantities of silver nanoparticles can penetrate damaged skin systemically<sup>78</sup>. The case of a young patient with severe burns was reported. This patient developed hepatotoxicity and argyria after one week's topical treatment with Acticoat® dressings.<sup>198</sup> However, these effects proved reversible on treatment withdrawal.

Although clinical studies indicated dermal biocompatibility with dressings containing silver nanoparticles,<sup>29, 199</sup> some *in vitro* studies showed that silver nanoparticles had cytotoxic effects on keratinocytes and fibroblasts.<sup>200</sup> It nevertheless seems difficult to compare the data obtained *in vivo* on the skin (which is covered with stratum corneum) and the information documented for keratinocytes or fibroblasts. Moreover, Poon *et al.* recognised that the sensitivity of cells to the toxic activity of silver (metallic and ionic) decreased when the biological environment became more complex to mimic clinical conditions.<sup>200b</sup>

Another route of exposure to nanoscale silver is its incorporation in the coating applied to invasive surgical medical devices.<sup>22</sup> An *in vitro* study of the impact of a silver nanoparticle coating on a venous catheter showed that the antimicrobial activity of the catheter was accompanied by an acceleration in blood coagulation and highlighted a risk of thrombosis.<sup>201</sup> Similarly, the AVERT clinical study on heart valves coated with Silzone metallic silver to reduce the risks of endocarditis had to be

stopped after 2 years due to the risk of thrombosis and leaking valves observed in several patients. The polymer coating impregnated with metallic silver was 400 nm thick. The results seemed to link the adverse effects observed to this fine silver layer of Silzone, although the data were not statistically significant.<sup>202</sup>

Finally, the use of silver nanoparticles for their bactericidal activity raises increasing health safety concerns because their use is becoming more and more widespread. This is also the case in mass products, thus considerably increasing exposure to Man and more generally to ecosystems. However, these questions of a general nature are beyond the scope of this report, which is limited to the potential risks to patients exposed to medical devices containing nanoscale metallic silver.

### **II.B.3. Nano-objects arising from degradation products due to the wear and tear**

Nanomaterials can be detected in patients using a medical device, as they could come from impurities and manufacturing residues. As the case of dental implant debris in the gastro-intestinal tract was detailed in chapter II.A.2, we will now examine the biological effects of nanoparticles released by joint prostheses (hip, knee, shoulder, ankle, discal, trapezo-metacarpal, etc.). Particles of micro- and nanoscale were detected nearby implants with friction coupling, cements or composites. Coupling is mainly of metal-polyethylene, metal-metal, polyethylene-ceramic or ceramic-ceramic type.

Implanted joint prostheses are subject to many constraints which trigger wear and tear and degradation: friction and abrasion between joint surfaces, micro movements on the interface with the bone, corrosion and/or erosion. The rate of wear and tear of polyethylene is generally in the range of 100  $\mu\text{m}/\text{year}$ , generating billions of particles of a few micrometres.<sup>203</sup> Conversely, the metallic debris is small, ranging from 10 to 90 nm (averaging around 50 nm) and is released at the rate of  $10^{12}$ - $10^{14}$  particles per annum in a metal-metal coupling.<sup>204</sup> The quantity of nanoparticles smaller than 50 nm may, however, be underestimated given the detection limits for current analytical tools. By way of illustration, a study on the mechanisms of wear and tear particle formation in metal-metal joints showed that globular, needle-shaped nanoparticles were released *in vivo*.<sup>205</sup> These authors noticed that the origin of each type of nanoparticle was different. The globular nanoparticles stemmed from nanocrystals detached from the nanocrystalline layer on the surface whereas the needle-shaped nanoparticles were due to the fracture of martensite - a component of hardened steel.

#### **• *Toxico-kinetics of implant debris***

Wear and tear debris are traditionally subjected to phagocytosis by macrophages, which transport them as far as the lymph glands for excretion.<sup>206</sup> When this excretory route is overloaded, the particles and macrophages are retained locally and a granulomatous inflammatory response appears around the prosthesis. Phagocytosis is a dynamic process depending on the size of the foreign particles, which is generally greater than 500 nm. The very large particles of over 10  $\mu\text{m}$

stimulate the formation of giant multi-nucleated cells. Regarding nanoparticles, they can undergo pinocytosis, which is non-specific endocytosis.

Nanoparticle mobility was also illustrated in the dissemination of implant degradation particles, especially if the implants were inadequately fixed.<sup>207</sup> In fact, they could be found distributed systemically through the body, typically in the synovial tissues (bone, cartilage and joint cavities) and the lymph glands as well as in the liver, spleen and bone marrow.<sup>207</sup> However, neither the mechanism of their transportation to the latter organs nor the consequences of the presence of these nanoparticles was known.

The onset of inflammation is a frequently observed response, but the accumulation of nanoparticles from implants could also disrupt the functioning of the immune system,<sup>208</sup> generate osteolytic lesions around the prosthesis and even lead to the detachment of the said prosthesis. Their involvement in immune dysfunction highlighted the risk of a hypersensitivity reaction. Lastly, these metallic nanoparticles were responsible for the necrosis of lymphatic glands.<sup>207</sup> However, the exact mechanism of their toxicity was not fully known and could involve their ionic form as an active metabolite.<sup>209</sup> A case of granulomatous hepatitis occurring 8 years after the implantation of a polyethylene-titanium hip prosthesis was recorded.<sup>209</sup>

#### ● **Cobalt-chrome nanoparticles**

One of the materials most widely used in prostheses is the cobalt-chrome alloy. Consequently, nanoparticles of cobalt and chrome arising from the wear, tear and degradation of these implants could be present in the body. The genotoxic and carcinogenic potential of cobalt has been documented in particular for the ionic forms, such as the  $\text{Co}^{2+}$  ion.<sup>210</sup> In contrast, the genotoxic and carcinogenic characteristics of metallic cobalt nanoparticles has scarcely been investigated.

The carcinogenicity of chrome and cobalt have been thoroughly examined in recent years and were the subject of an evaluation of the IARC (*International Agency for Research on Cancer*) in 1990 for chrome and 1991 for cobalt.<sup>211</sup> Metallic chrome and its trivalent salts have been classified as group 3 substances (agent that cannot be classified in terms of carcinogenicity), whereas hexavalent chrome salts were classified in group 1 (carcinogenic agent). Regarding cobalt, this element was classified in group 2B as a potentially carcinogenic substance for man. This was based on tangible evidence in laboratory animals but proof in man was still uncertain.

The *in vitro* and *in vivo* genotoxic effect of ions  $\text{Co(II)}$  would be due to the production of reactive oxygen species and inhibition of DNA repair. Studies showed that the genotoxicity of cobalt nanoparticles was probably due to their dissolution and ionisation.<sup>151, 154</sup>

One group of research scientists showed DNA damage, without significant cell death, caused by nanoscale and microscale products resulting from the wear and tear of joint prostheses in Co-Cr, through a barrier consisting of several layers of BeWo cells, modelling roughly the placenta barrier.<sup>145</sup>

The cells communicated with each other via junctions, thus allowing either the passage of cobalt and chrome ions into the cells of the lower layers not exposed to particles, or enabled the transmission of intracellular signals, which would diffuse to the fibroblasts. The genotoxic effect of chrome and cobalt nanoparticles therefore would follow a new indirect mechanism, transmitted by BeWo cells. This effect was also observed with Co-Cr microparticles and may not therefore be specific to the nanoscale. Moreover, doses used to trigger a genotoxic effect were higher than those found in real clinical situations, as willingly acknowledged by these research scientists. These results must be confirmed by other concordant studies before any conclusion is drawn in haste or any generalisation is made.

### ● **Titanium dioxide nanoparticles**

The surface of the implants can be covered with a layer of titanium dioxide to prevent corrosion and enhance biocompatibility. The thickness of the dioxide layer generally varies between 1 and 10  $\mu\text{m}$  and its composition includes crystalline anatase and rutile structures. On one hand, it is highly resistant to corrosion by chloride ions. On the other hand, this intermediate layer isolates the material from the surrounding medium. The thickness and composition of the passive layer develops over time under the influence of the environment: initially, it is titanium dioxide  $\text{TiO}_2$ , then by dissolution/precipitation, blends of  $\text{TiO}_2$ ,  $\text{TiO}$ ,  $\text{Ti}_2\text{O}_3$ ,  $\text{Ti}_3\text{O}_2$  titanium oxides are detected. Partly destroyed over time by chemical or mechanical attacks, the layer is dynamically reformed. Finally, since titanium dioxide is in direct contact with the biological medium, an evaluation of the biocompatibility of nanoparticles of titanium dioxide arising from the degradation of the coating is necessary.

Until recently, titanium dioxide was considered as slightly toxic. Nevertheless, since February 2006, the CIRC reclassified pigmentary, ultrafine  $\text{TiO}_2$  as a "possible carcinogenic agent for man" (group 2B).<sup>212</sup> Nevertheless, the intrinsic toxicity of titanium oxide nanoparticles is still subject to debate currently. Ingestion and contact with the skin do not appear to trigger major side effects (see II.A.2).<sup>55c, 76a, 77, 140</sup> However, pulmonary toxic effects, inflammation and DNA lesions were observed in animals with nanoparticles of  $\text{TiO}_2$  introduced into the respiratory tract. *In-vivo* studies have shown signs of systemic toxicity such as apathy, lethargy, loss of appetite and tremor.

Regarding nanoparticles of  $\text{TiO}_2$ , toxicity seems to depend not only on the crystalline structure but also on the size and surface properties.<sup>213</sup> Inactive catalytically, nanoparticles of rutile were twice less cytotoxic than those of anatase.<sup>214</sup> They induced necrosis of the test cells whereas their anatase counterparts seemed to initiate cell apoptosis by triggering the formation of reactive oxygen species.<sup>215, 105</sup>

## II.C. Relevant characterization parameters for the toxicological evaluation of nanomaterials

Since the impact of nanomaterials on biological systems is still only partly known, precise characterization is essential in order to understand the factors that determine biological behaviour. Chemical composition or even size is no longer the only adequate factor for determining the toxicity of nanomaterials, which depends on numerous other physico-chemical factors (form, state of aggregation, surface properties, electronic properties and solubility, etc.). The major diversity of nanomaterials further complicates the task of evaluating and classifying the risk associated with these nanomaterials. For instance, there are currently around 50 000 different types of carbon nanotubes depending on the raw material, manufacturing processes and catalysts used.<sup>55g</sup>

A complete characterization of the nanomaterials used is desirable but calls for considerable time, money and implementation procedures, which can become complex. Of all the possible parameters for defining a nanomaterial, a general consensus has been reached for 8 basic parameters considered to be the most relevant for its biological assessment<sup>1, 216</sup> :

- Size and size distribution
- Morphology
- Aggregation / agglomeration state
- Solubility and dispersability
- Specific surface area
- Composition (chemical composition and crystallographic structure, amongst others)
- Surface charge
- Surface chemistry

This approach would provide a minimum, standardised characterization of nanomaterials before carrying out any toxicological test. Without an adequate description, the results of the toxicity tests will be of limited value and will be difficult to compare to those obtained with other nanomaterials.

### II.C.1. Physical parameters

- ***Size, size distribution and morphology***

Size is the criterion on which everyone agrees in the definition of nanomaterial and nano-object. This is why it is important to clearly characterize this parameter especially since the concepts of dimension and morphology are crucial for assessing the toxicity of nanoparticles. We have seen that the toxicity or safety of carbon nanotubes changed according to size.<sup>83, 157a, 182</sup>

In addition to size, the morphology of the nanomaterial is also important. As seen previously, the bactericidal activity of silver nanoparticles depended not only on size but also on shape.<sup>194a</sup> According to Chen *et al.*, the cytotoxic potential of TiO<sub>2</sub> nanomaterials depended on the shape of the nanomaterial: anatase titanium oxide was found not cytotoxic in the form of rods (2D), unlike the spherical shape (3D).<sup>217</sup>

#### - Microscopic methods

Several methods can be used to determine the size and morphology of nanomaterials.<sup>5b, 216d, 218</sup> Amongst the microscopic methods, which are the most powerful tools for these identifications, measuring protocols are developed based on two main, complementary instruments: the *Atomic Force Microscope* (AFM) and the *Scanning Electron Microscope* (SEM) or *Transmission Electron Microscope* (TEM).

For many years, TEM has been used in research laboratories to study nanoparticles because it has a sub-nanometric resolution. However, the image obtained is a projection of a nano-object on a plane, which can introduce a bias when measuring size or morphology. Furthermore, in the case of nanostructured materials, the sample to be analysed must be carefully prepared: cutting a fine layer to allow electrons to pass through the sample.

In the case of SEMs, progress made in the field of sources and the control of electron beams allows lateral resolutions slightly less than one nanometre to be reached nowadays with the latest generation of SEMs (e.g. Magellan from FEI), which makes them competitive for measuring nanoparticles. Furthermore, the SEM is a multipurpose identification tool with a fast scanning mechanism and a very wide measuring range. It is used on industrial production lines, especially in the microelectronic sector. This resolution is however limited in height by the deflection of secondary electrons collected by lateral detectors and it is strongly influenced by numerous parameters: electron energy, the electric properties of the nano-object or its surface charge, etc.

Finally, AFM holds a key role in the National Metrology Institutes because it is the reference instrument that facilitates the recording of measurements which can be directly related to International System (I.S.) units on the nanoscale. By scanning a sample with a fine probe, the AFM is capable of reconstructing a 3-dimension topographical image of a surface in ambient air, under vacuum or in a liquid environment. The resolutions thus obtained are vertically below one nanometre but depend horizontally (XY) on the size and geometry of the tip (image artefacts induced by the tip, approximately 10 nm in size).

Within the European standardisation framework, the methods used to measure nano-objects or nanostructured materials are being developed mainly using both SEM (good uncertainty of measurement in XY) and AFM (good uncertainty of measurement in Z).<sup>219</sup> This synergy could enable 3-dimensional metrological measurements of nano-objects.

Finally, regardless of the type of instrument used, the validity of the measurement must be established by checking that the measurements and sampling are significant.<sup>220</sup> The choice of reference materials is also crucial.

### **- Size distribution**

Most manufactured nanoparticles are not perfect homogeneous spheres but are generally a combination of several sizes and various morphologies. In some current methodologies, the size of a nanoparticle is deduced from the diameter of an equivalent sphere, which would give the same intrinsic properties as the complex particle. The size ranges and relative amounts of the particles present in the mixture studied must be clearly identified in order to assess the biological risks associated with nanomaterials. This is why the size of a nanomaterial cannot be described without also specifying size distribution.

### **- Measurements depending on the technique and the batch**

The size measurements of a nanoparticle are based on different identification methods, which are not necessarily comparable because the instruments do not measure the same mesurand. The various techniques do not measure the same thing and depend on the type of sample: in powder, dispersed in a liquid, incorporated in a solid matrix or with a coating. For example, size measurements by TEM cannot detect organic coatings whereas the latter are taken into account with DLS (*Dynamic Light Scattering*). More precisely, DLS measurements are measurements of the hydrodynamic diameter of the nanoparticles, i.e. their core diameter with the first hydration sphere, whereas TEM gives only the core diameter. Domingos *et al.* reported that the size measured could considerably differ from the manufacturer's indications depending on the technique used.<sup>218</sup> It is therefore important for all nanomaterial characterization to include the measured size, measurement uncertainty, size distribution observed and the methods used for measuring.

### **- Variability of the nanomaterials produced**

Information provided by the raw material manufacturers must be taken with caution. Indeed, Park and Grassi showed that a commercial batch of 30 nm nanoparticles actually contained nanoparticles ranging from 5 nm to 300 nm.<sup>221</sup> Likewise, in a sample of spherical nanoparticles, these authors found spheres as well as rods. Furthermore, the physico-chemical characteristics of the nanomaterials can change over time and depending on the environment.

### **• Aggregation/Agglomeration, solubility and dispersability**

Nano-objects are likely to change in size and shape once in the biological environment. Yet it's precisely the state of the nano-object when it interacts with biological species that determines the resulting biological effects. Nanoparticles have a tendency to aggregate and agglomerate in an aqueous medium, which disrupts measurements of size.<sup>216b, c, 218</sup> When the biological effects of a nanomaterial depends on its size, then the state of dispersion, aggregation or agglomeration is of paramount importance.

In fact, studies have shown that carbon nanotubes did not have the same pulmonary toxicity as dispersed or agglomerated fibres.<sup>222</sup> Mercer *et al.* noted, for instance, that dispersed SWCNTs (0.69 µm in diameter), were incorporated more rapidly into the alveolar interstitial spaces of mice following pharyngeal aspiration because of their smaller size. Less well recognised by macrophages,

dispersed SWCNTs triggered generalised fibrosis contrary to non-dispersed SWCNTs (balls, 1.52 µm in diameter).<sup>223</sup>

In the polydispersed systems, it is often difficult to establish whether the various size groups are those of primary nano-objects or the size of aggregates and agglomerates. In an aqueous medium, the aggregation and agglomeration of nano-objects are controlled by a subtle balance between superficial and intermolecular forces involving interparticles and interactions between particles and their environment. Slight disruptions in the medium such as the pH, ionic force or concentrations can substantially alter the dispersion state of the nanoparticles.<sup>6</sup> The agglomeration pattern of the nanoparticles thus changes depending on the medium studied. Murdock *et al.* noted that the addition of serum to the culture medium could lead to an improved dispersion of metallic nanoparticles in some cases.<sup>216c</sup>

The dispersion state is a dynamic state that must be investigated at different times by recording size distribution measures in comparison to the initial "ideal" state of dispersion in order to qualitatively establish the level of aggregation and agglomeration.<sup>216d</sup> The term dispersability is used to qualify the ability of a material (dispersed phase) to be evenly distributed in another medium (dispersive medium or continuous phase, e.g. a carrier liquid). Depending on the surface of the nano-objects (especially if they are functionalised), there may be evidence of dispersion in the carrier liquid. In this case, the final suspension would be more stable over time.

A dynamic study of the dispersion state calls for a reliable method to measure the initial state in which the nano-objects are ideally dispersed in solution. Various methods such as sonication, dispersing agents or surfactants are routinely used for dispersion. However, the use of a chemical surfactant may damage the cells and interfere with the toxicological evaluation. For example, several studies have indicated that the cationic surfactant CTAB (cetyl trimethyl ammonium bromide), normally used to stabilise gold nanorods, might be the main cause of toxicity with this nanomaterial.<sup>94, 224</sup>

Expressed via the same unit as dispersibility but involving different concepts, solubility is an essential parameter for evaluating the biological risks of any type of material. A material is said to have been dissolved when a molecular suspension is obtained in a single homogeneous, temporary stable phase in another material (solvent). Internal forces of interaction within the nanoparticle are considerable and can prevent a nanoparticle from dissolving in its medium. Conversely, the dispersibility of nano-objects calls on external forces between the nano-objects (collective effects). Although these two parameters shed light on the behaviour of the test material in a liquid medium, they provide different information.

Biodegradation of a compound is often associated with notions of solubility and persistence. An insoluble, non-degradable nanomaterial demands specific attention because it could imply persistence and an accumulation of the nanomaterial in the body, ultimately triggering chronic toxic effects.<sup>225</sup> Similarly, a soluble nanomaterial will produce ions which can also be toxic. By comparing different nanoparticles *in vitro*, Brunner *et al.* showed that solubility affected toxicity.<sup>226</sup> The toxicological profile of a nanomaterial, once dissolved in a biological medium in its molecular or ionic form, is similar to the solubilised bulk material and *a priori* the risks encountered should be known

thanks to the clinical data history of the bulk material with the same chemical nature. Naturally, a better understanding of the risks would facilitate risk analysis and management, but does not preclude them from being taken into account.

Thus the state of aggregation and agglomeration of nano-objects coupled with dispersibility and solubility must be known, especially in cases where properties are modified in biological media, because the toxicological consequences may be affected accordingly.<sup>216c, 222a</sup>

- **Specific surface area**

Specific surface area refers to the exposed surface of a material characterized by the ratio between the surface area on the mass of the material studied [ $S = 3/(\text{density} \times \text{radius})$ ] and is therefore independent of the quantity of material used. This parameter is therefore particularly useful for describing nanoparticles with a strong tendency to aggregate and agglomerate. It is also a relevant characterization for porous surfaces, for which a distinction is also made between the external and internal surface.

Since interactions between nanomaterials and biological components take place on the surface of the nanomaterial, determination of the specific surface area is vital for the study of potential adverse effects.<sup>227</sup> The specific surface area measured definitely depends on the dimensions and morphology of the nanomaterials. For example, Grabinski *et al.* clearly noted more interaction between cell membranes with single-walled carbon nanotubes than with multi-walled carbon nanotubes probably because of the larger specific surface area of single-walled carbon nanotubes according to these authors.<sup>182</sup> However, more interactions do not necessarily mean more toxicity because the authors have not noticed any difference in cytotoxicity between these two types of carbon nanotubes although other comparative studies have highlighted greater cytotoxicity in single-walled carbon nanotubes.<sup>228</sup> In fact, the toxicity of carbon nanotubes certainly depends on other factors, as we saw earlier (part II.B.1).

The specific surface area of nanoparticles and nanoporous nanomaterials is often measured by a gaseous adsorption method using the BET (Brunauer-Emmett-Teller) theoretical analysis technique.<sup>229</sup> Given the small size of the gaseous molecules adsorbed, the specific surface area measured is only slightly affected by nanomaterial agglomeration. However, one of the limits of this method is that it is only valid for powders and/or solid, dry materials.<sup>216a, 216d</sup> Other methods can also be used such as gas/surface titrations.<sup>230</sup>

Some researchers suggest that the toxicological evaluation of nanomaterials as a function of specific surface area should be recommended in addition to the mass, which is a traditional unit of measurement.<sup>1, 55k, 227</sup> This point will be discussed further in the report (part III.B.2).

## II.C.2. Chemical parameters

### • **Composition and contaminations**

Composition refers to all of the relevant parameters used to describe the composition of a material such as chemical composition, crystallographic structure, crystalline state, molecular conformation/configuration, etc.

The characterization of the chemical composition must include both expected and undesired substances such as **impurities**. Since the exposed surface area of the nanoparticles is large, there is an increased risk of adsorbed contaminants. The impurities can stem from the preparation, production, sterilisation and storage. The impurities encountered with polymer nanomaterials include residual monomers, oligomers of polymerisation products (known as low molecular weight impurities), etc.

Endotoxins - biological contaminants - can completely alter toxicological results. Inoue *et al.* in particular noted that endotoxins could considerably enhance the pulmonary inflammation of carbon black nanoparticles, which only trigger minor effects on their own.<sup>231</sup>

Similarly, the contaminants can also be chemical. Studies have shown that some adverse effects of carbon nanotubes could be attributed to iron<sup>185-186</sup> and yttrium impurities.<sup>232</sup>

Moreover, some impurities inherent in toxicity test protocols can also lead to confounding conclusions. For example, the results of a study, which attributed the ability to induce oxidative stress in fish<sup>233</sup> to fullerenes were criticised because of the possible presence of residual THF (tetrahydrofuran) solvent.<sup>234</sup> Subsequent research has indeed confirmed that the adverse effects observed were associated to residual THF.<sup>235</sup>

### • **Surface chemistry and surface charge**

Surface chemistry refers to many superficial properties that govern the direct interaction between nanomaterials and the environment, especially the biological environment. Surface chemistry includes, amongst other things, elements that balance solubility, catalytic properties, surface properties and superficial adsorption and desorption. This surface chemistry depends in particular on the molecules present on the surface of the nanomaterial. Its description should at least include details of the chemical composition and, in the case of coating: thickness, homogeneity, solidity of anchorage with the core, etc. Regarding polymer nanomaterials, low-molecular-weight compounds can diffuse to the surface, and alter the chemistry of the latter.

### • **Coating**

The choice of coating is often directed by the intended application. The external surface, aqueous solubility and functionalisation affect stability as well as the aggregation and agglomeration state but also impact upon the way in which the nanomaterial interacts with biological molecules<sup>56</sup> and

therefore its biological behaviour such as toxicokinetics or biological activity.<sup>236</sup> Often the surface of nanomaterials used in medical devices is modified or functionalised to improve their biocompatibility.

For instance, functionalisation with the PEG (polyethylene glycol) polymer is often used to avoid recognition by the macrophages and phagocytosis in order to prolong the circulation time in the body, as has been seen with gold nanorods.<sup>237</sup> The half-life *in vivo* of quantum dots covered with short methoxy-PEG chains (750 Da) was 12 minutes versus 70 min for those with longer chains (5000 Da).<sup>238</sup> Although coating with a polysaccharide increased the genotoxic potential of silver nanoparticles towards fibroblasts and embryonal stem cells,<sup>147</sup> the functionalisation of CdSe quantum dots which were deemed toxic, with a ZnS layer can reduce this cytotoxic effect.<sup>239</sup> Once again, this coating must not degrade once in the body and must not display toxic properties.

Surface molecules can be very dynamic, interacting with molecules in the surrounding medium. Several possibilities exist for modifying the surfaces. These will have a major impact on the interaction of the nanomaterial with biological systems. It is the core / coating system which controls the overall behaviour of the nanomaterial. This is why the biological risks inherent in nanomaterials should ideally be assessed on the nanomaterials together with its coating whilst paying particular attention to the possible biodegradation of the latter. If that is the case, a toxicological study for the resulting degradation products should also be carried out.

#### ● Surface charge

It is useful to establish the surface charge of a nanomaterial because it provides information on its dispersion properties in the medium and on its ability to adsorb ions and surrounding biological particles.<sup>58b, 60</sup> The toxico-kinetics of nanomaterials depends greatly on the surface charge, which may change over time and depending on the environment. In their study, Goodman *et al.* showed that the surfaces of gold nanoparticles functionalised with positive chemical charge (cationic) molecules displayed better affinity with negatively charged cell membranes, and therefore contributed to the cytotoxic action.<sup>240</sup>

When the nanoparticles are in suspension in a liquid, they can be charged and are surrounded by an ionic cloud. The resulting electric charge known as zeta potential, is traditionally used to indirectly characterize the surface charge. The apparent zeta potential of a nanomaterial depends on the surface chemistry, adsorbed species, pH and the ionic composition of the medium studied.

When evaluating the biological risks of a nanomaterial, zeta potential is a good indicator of its biological activity.<sup>241</sup> Generally speaking, a high zeta potential (in absolute value) indicates better dispersion in solution, better stability and therefore less likelihood of aggregation and agglomeration and, potentially, less toxicity. In fact, according to a study carried out by Hu *et al.*, the higher the zeta potential (in absolute value), the less cytotoxic the metallic nanoparticles (ZnO, CuO, Al<sub>2</sub>O<sub>3</sub>, La<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, SnO<sub>2</sub> and TiO<sub>2</sub>, ZnO) to *E. Coli* bacteria.<sup>242</sup>

Generally, the surface charge must be evaluated under similar conditions to clinical conditions (vehicle, culture medium, presence of proteins, etc.).

### **II.C.3. Conclusion**

The eight parameters presented in this report provide a minimal standardised approach for the evaluation of physico-chemical properties, which is strongly recommended for identification purposes before carrying out any toxicological study involving nanomaterials. Generally, the parameters selected for the characterization of a nanomaterial must provide information on three criteria: appearance (size and size distribution, morphology, aggregation/agglomeration state, specific surface area), constitution (chemical composition, surface chemistry) and interaction influences with environmental elements (solubility/dispersability, surface charge). Other physico-chemical parameters are also relevant such as hydrophobia, redox potential, photocatalytic properties and the ability to trigger radical formation, etc.

Characterization should be performed on the most typical samples (representative of biological medium) with statistical validity, whilst specifying the technique used. ISO standardisation texts and those of the OECD should be consulted for more information on the methodologies to adopt in order to carry out these characterization procedures correctly. We wish to point out that reference to the supplier's indications will not suffice because the physico-chemical parameters of the nanomaterial (especially aggregation/agglomeration, surface chemistry) can change over time. Priority must be given to strict reproducibility between batches. Care must be taken to ensure that suitable methods are chosen (analytical or even biological) to guarantee batch uniformity and reproducibility. Otherwise, significant differences in terms of biocompatibility results and performance may be observed as a result of a minor, physico-chemical variation.

### **III. Are the current reference systems for medical devices suitable for medical devices containing nanomaterials?**

Regulation aims to create a framework to minimize the potential biological risks to patients when using medical devices, as compared to the expected medical benefits. Thus in order to launch a medical device on the market, the manufacturer must justify a favourable benefit/risk ratio. The development of nanotechnologies brings many hopes in terms of new diagnostic and treatment applications but also raises issues concerning potential harmful biological effects, which have not been fully elucidated. Regarding the specific properties and behaviour of nanomaterials, which often differ from their bulk counterparts, are the methods for evaluating the benefit/risk ratio and the current risk management procedures applicable to medical devices containing these nanomaterials?

#### **III.A. A review of current regulation relating to medical devices**

Current regulation for medical devices is based on several European directives, the main ones are listed below: Directive 90/385/EC relating to active implantable medical devices (AIMD) and Directive 93/42/EC relating to medical devices<sup>22a</sup> (recently revised and consolidated by Directive 2007/47/EC<sup>22b</sup>) and Directive 98/79/EC relating to *in-vitro* diagnostic medical devices. These directives are based on the premise that the medical device manufacturer is responsible for risk management.

Thus, to launch medical devices on the European market, manufacturers must ensure that the devices comply with the essential safety requirements listed in Appendix I of these European Directives by providing evidence in terms of efficacy and safety of the device in order to minimise the risks associated with the use of such devices by patients and users.

##### **III.A.1. Directive 93/42/EC and CE labelling**

In Directive 93/42/EC, medical devices are divided into four classes depending on the level of risk (duration of use, invasiveness, active medical device, *etc.*): I for a low risk; IIa, IIb and III for a high risk. For instance, invasive devices or implants that come into contact with the central nervous system, heart or central circulatory system but also devices incorporating a therapeutic substance such as antimicrobial silver nanoparticles are classified in group III.

For medical devices in classes IIa, IIb and III as well as AIMDs, compliance is assessed by an independent organization known as the notified body. In case of conformity, it issues a CE labelling certificate allowing manufacturers to launch their product on the European market. For medical devices with a high risk for human health (devices in IIa, IIb and III classes as well as AIMDs), the

manufacturer, authorised representative or distributor have to inform the French Health Products Safety Agency (Afssaps) of the use of such devices in France and to provide a copy of the labelling and instructions for use (decree No. 2002-1221 of 30 September 2002 and decree No. 2010-270 of 15 March 2010, corresponding to article L.5211-4 and R.5211-66 of the French Public Health Code). For Class I medical devices, a declaration to the French Health Products Safety Agency by the manufacturers or authorised representatives with a head office in France, is sufficient. As the competent authority for medical devices, the French Health Products Safety Agency will not issue a marketing authorisation unlike the case of drugs. Upstream from CE labelling, it nevertheless intervenes by authorising clinical trials to be held in France. Once the devices have been launched, the French Health Products Safety Agency coordinates vigilance activities and monitors the market. It takes appropriate health safety measures if required.

Directive 2007/47/EC, which was applied in March 2010, has increased the need for clinical data in the conformity assessment procedures. It also introduces the concept of Post Market Clinical Follow-up to monitor devices after CE labelling.

### **III.A.2. EN ISO 14971 Standard (Risk management)**

The essential health safety requirements stipulated in European Directives for medical devices define the results to achieve and the risks to be addressed but do not provide technical solutions. Compliance with harmonized European standards is on a voluntary basis and presumes conformity to these essential requirements. The EN ISO 14971 standard issued by the International Organization for Standardization is entitled “Application of risk management to medical devices”.<sup>243</sup>

This international standard helps the manufacturer to implement, document and maintain an ongoing process throughout the life-cycle of a medical device in order to identify hazardous phenomena associated with a given medical device, to estimate and evaluate the risks associated with these hazardous phenomena, to manage these risks and monitor the efficacy of the controls. The manufacturer should apply the following principles in the order stipulated:

- to eliminate or reduce risks as much as possible (inherent safe design and manufacturing) whilst taking into account existing technology and practices at the time of design together with any relevant technical and economic considerations in order to ensure a high level of protection in relation to health and safety;
- to take appropriate protective measures as required, including warnings in relation to risks that cannot be eliminated;
- to inform users of residual risks due to any failure in the protective measures taken.

Risk management applies right from the initial design of the medical device. A list of questions that can be used in order to identify medical device characteristics that could impact on safety factors is provided in Annex C of EN ISO 14971.

Annex I of this standard provides guidance regarding the application of risk analysis in relation to biological hazards. The effects can range from short-term effects such as cytotoxicity, irritation to the skin, eye and mucosal surfaces, acute systemic toxicity or haemolysis, to long-term effects such as sensitisation, genotoxicity, subacute, subchronic and chronic effects, carcinogenicity (tumorigenicity) or effects on reproduction including teratogenicity. The biological risk analysis should include the following:

- the physical and chemical characteristics of different material options,
- any history of clinical use or human exposure data,
- any existing toxicity and other biological safety data on the product and its component materials,
- test procedures within the EN ISO 10993 standards - part 1 framework.

### **III.A.3. EN ISO 10993 Standard (biological evaluation)**

There are currently 20 standards in the EN ISO 10993 series, grouped together under the general heading "Biological evaluation of medical devices" (see Figure 2)<sup>243-244</sup>; some of these are currently under revision. The first part, EN ISO 10993-1 entitled "Evaluation and testing within a risk management process", sets the general principles for the biological risk assessment pertaining to medical devices.<sup>244r</sup> The use of tests such as those described in EN ISO 10993 series brings scientific validity to the process used to evaluate the biological response. It also provides the general public with greater assurance regarding the biological safety of medical devices whilst taking the necessary measures to ensure the ethical use of animals.

The EN ISO 10993-1 standard explicitly outlines the process for evaluating the biological risks of a medical device. It determines the main issues to be addressed in order to evaluate the biological response based on the classification of devices according to the nature and duration of contact with the human body.

A table summarising the various biological risks to be taken into account based on this consideration is provided in Annex A to the EN ISO 10993-1 standard. The main biological effects to address are: cytotoxicity, sensitisation, irritation or intradermal reaction, systemic toxicity (acute, subacute/subchronic), genotoxicity, implantation (local toxicity) and haemocompatibility. This table does not provide an exhaustive list of tests to be carried out, but provides a guidance framework for compiling the most suitable biological risk evaluation programme for the medical device in question taking into account the nature and duration of exposure. Along these lines and depending on the case, additional biological risks should also be evaluated such as: chronic toxicity, carcinogenicity,

biodegradation, toxicokinetics, immunotoxicity, reproductive / developmental toxicity or any other specific toxicity (e.g. neurotoxicity). The biological risk evaluation programme should be well thought-out and justified according to the medical device in question and its specific application.

Part 1:	Evaluation and testing within a risk management process
Part 2:	Animal welfare requirements
Part 3:	Tests for genotoxicity, carcinogenicity and reproductive toxicity
Part 4:	Selection of tests for interactions with blood
Part 5:	Tests for <i>in vitro</i> cytotoxicity
Part 6:	Tests for local effects after implantation
Part 7:	Ethylene oxide sterilization residuals
Part 8:	Selection and qualification of reference materials used for biological tests
Part 9:	Framework for identification and quantification of potential degradation products
Part 10:	Tests for irritation and skin sensitization
Part 11:	Tests for systemic toxicity
Part 12:	Sample preparation and reference materials
Part 13:	Identification and quantification of degradation products of polymeric medical devices
Part 14:	Identification and quantification of degradation products from ceramics
Part 15:	Identification and quantification of degradation products from metals and alloys
Part 16:	Toxicokinetic study design for degradation products and leachables
Part 17:	Establishment of allowable limits for leachable substances
Part 18:	Chemical characterization of materials
Part 19:	Physico-chemical, morphological and topographical characterization of materials (technical specification)
Part 20:	Principles and methods for immunotoxicology testing of medical devices (technical specification)

**Figure 2. List of parts included in the EN ISO 10993 standard series**

### **III.B. Caveats regarding medical devices containing nanomaterials**

Based on the general information compiled for nanomaterials in section II of the report, this section highlights the relevant data to be extracted for the specific evaluation of medical devices containing nanomaterials.

It is also important to note that any *in vivo* or *in vitro* test must be carried out in accordance with the Good Laboratory Practice guidelines.

### **III.B.1. Physico-chemical characterization and batch reproducibility of nanomaterials used in medical devices**

Before carrying out any biological assessment to check the biocompatibility of a medical device containing nanomaterials, it is essential to characterize the nanomaterial using a systematic, precise approach. This should enable a more accurate toxicity evaluation of a given nanomaterial in relevant biological media and to ensure the reproducibility of the results obtained. Characterization is strongly recommended prior to evaluating the biological risks of the nanomaterial. Toxicological tests should be carried out on the nanomaterial under conditions that mimic as closely as possible the clinical situation.

According to Article 6 of the EN ISO 10993-1 standard relating to the process for the biological risk assessment, material characterization is an essential preliminary step in the biological risk evaluation process.<sup>244r</sup> As we saw in part II.C. of this report, this applies in particular to nanomaterials used in medical devices. Precise characterization is essential for two reasons:

- 1) to interpret the results of biological tests more effectively;
- 2) to gain a better understanding of the risks involved if there is any modification on the material (change of supplier, change in the manufacturing process, etc.) and to ensure the reproducibility of test batches in order to provide consistent results.

At the present time, the physico-chemical characterization of materials described in the EN ISO 10993-18 and 19 standards<sup>244c, 244q</sup> was written for chemical substance in bulk form. However, many physico-chemical parameters influence the potential toxicity of nanomaterials used in medical devices. This is why it is important to adapt to this particular case. As discussed in part II.C. of this report, the following eight physico-chemical parameters are required to characterize nanomaterials prior to any biological assessment :

- Size and size distribution
- Morphology
- Aggregation / agglomeration state
- Solubility and dispersability
- Specific surface area
- Composition (chemical composition and crystallographic structure, amongst others)
- Surface charge
- Surface chemistry

The parameters are not intended to provide an exhaustive list. The manufacturer must decide on the most suitable characterization parameters for assessing the biological risks of his medical device containing nanomaterials and should even supplement them if required. It is important to note the difficulty in characterizing some parameters (e.g. size or aggregation/agglomeration state), which change depending on the operating conditions (under vacuum or in solution, for instance) and the test medium (polar/apolar, pure aqueous/biological medium, etc.) over time and during the life cycle of the device.

Before evaluating the biological risks, it is obviously important to know the precise composition of the materials used in the medical device being studied, as stipulated in the regulatory guidance documents. Particular attention should be paid to the description of the impurities because these may be present in great amount and could have a substantial impact on the overall toxicity of the medical device, as shown for carbon nanotubes. Concerning pyrogenic impurities, the various types of tests carried out to detect such substances are discussed in Annex F of the EN ISO 10993-11 standard (rabbit pyrogen test, LAL, etc.).<sup>244j</sup> *In vivo* tests are an integral part of the biological risk evaluation process for any implantable medical device in direct or indirect contact with blood, for devices intended for the nasal or gynaecological sphere. It also concerns medical devices when the manufacturing process involves water. It is also strongly recommended for devices containing nanomaterials which tend to readily absorb impurities.

We wish to emphasize again that the parameters stipulated do not constitute an exhaustive list but are recommended to characterize more appropriately a nanomaterial used in a medical device. It is up to the manufacturer to decide and justify the most suitable parameters for the characterization in the biological risk assessment inherent to his medical device containing nanomaterials. The method used and the measurement uncertainties should be specified for each parameter measured. The measurements must be recorded in an appropriate number of samples.

The characterization information provided by the raw material supplier is certainly useful but does not exempt the manufacturer from checking the accuracy of these data in relation to the nanomaterial incorporated in the final medical device. Indeed, the characteristic parameters of the nanomaterial can change over time, especially during the manufacturing process.

Finally, good characterization also enables the manufacturer to check the conformity and reproducibility of batches. Production must be controlled and reproducible in order to ensure that the nanomaterials contained in the medical device are rigorously identical. It is the only way to ensure that the biological risk evaluation carried out on the medical device containing nanomaterials is valid for all of the batches produced.

This is why the manufacturer of the medical device containing nanomaterials must take care that the characterization of nanomaterials received from suppliers is valid, reproducible between batches and that the samples used for the biological risk evaluation are rigorously identical to the batches produced.

### **III.B.2. Sample preparation and dose metrics**

A biological risk assessment is carried out on the final product in accordance with current regulation. In fact, the combination of two non-toxic materials may create a product with toxic effects. This "cocktail effect" was observed by Salonen *et al* on combining fullerenes with gallic acid, resulting in cell membrane contraction.<sup>245</sup>

The preparation of samples and reference materials is the first stage in evaluating the biological risks of the medical device. It is detailed in the EN ISO 10993-12 standard.<sup>244k</sup> Samples coming into contact with reactive systems (e.g. biological medium) are called test samples. Test samples may be the medical device itself, or a typical sample or extract(s) of the medical device.

#### **• Preparation of test samples**

According to the EN ISO 10993-12 standard,<sup>244k</sup> biological tests can be carried out in direct contact with the medical device or using extracts. However, the method used to prepare the test samples should be adapted to the specific features of the nanoscale structure.

When nanomaterials are firmly anchored in a macroscopic matrix incorporated in the medical device, the current sample preparation protocols should be applicable, whether the direct contact method or extracts are used. Nevertheless, it is important to take into account the potential high reactivity on the surface of representative samples of nanomaterials.

However, the approach becomes trickier for free or slightly aggregated nanomaterials as well as for medical devices releasing nano-objects during extraction. Protocols thus have to be adapted to suit individual cases. Results should also be interpreted on a case-by-case basis. In particular, the behaviour of these free nanomaterials in the various extraction vehicles recommended in the EN ISO 10993-12 standard should be checked. In particular, solubility and the formation of aggregates/agglomerates can modify the biological response.

#### **• The importance of dispersion in test samples**

Generally speaking, validated methods for evaluating biological risks have been developed for soluble chemical substances. Free nanomaterials tend to form aggregates and agglomerates. This is why many research scientists try to obtain finely dispersed suspensions of the nanomaterials studied. This strong tendency to form aggregates or agglomerates can be reduced slightly with physical methods such as sonication or by adding surfactants or dispersive agents. Although these methods indeed improve the dispersion and, in some cases, the stability of free nanomaterials, they can also alter the evaluation of the biological risks because these conditions may no longer reflect clinical reality. Moreover, some surfactants could also be biologically active and modify the biological results.

It should be noted that the culture medium used to carry out some biological tests contain proteins that can act as "natural" dispersion agents. For example, cell culture medium with added serum essentially contain albumin. Since albumin is a zwitterion (an element that is both positively and negatively charged), it is a good dispersion agent. Some researchers would argue that the unknown part inherent to an *in vivo* experiment could be attributed to the serum, the exact composition of which cannot necessarily be controlled. This is one of the explanations put forward to justify the lack of correlation between *in vitro* and *in vivo* results for nanomaterials.<sup>246</sup> Therefore caution must be taken when extrapolating *in vitro* results to *in vivo* situations.<sup>55k, 87</sup> As we saw earlier, plasma proteins are very rapidly adsorbed by nano-objects and influence their biological fate. According to an *in vitro* study by Barret *et al.*, the addition of serum reduced the cytotoxicity of silica nanoparticles.<sup>247</sup>

As a general rule, biological risks should be evaluated under experimental conditions that best reflect clinical conditions, especially in terms of aggregation and agglomeration. Regardless of the method used to prepare the samples, a balance must be maintained between the need for dispersion and stability, while keeping in mind the influence that this may have on toxicity levels and the quality of the test protocols.

#### ● **Reference samples**

A certified reference material is a material accompanied by a certificate in which one or more value(s) of the property(ies) is(are) certified. A procedure establishes its traceability to an accurate realisation of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence. The use of reference materials allows the biological response of the material under specific test conditions to be determined qualitatively and quantitatively. In the case of nanomaterials, the production of reference materials is still in its infancy. Few reference nanomaterials actually exist, the main obstacle being the lack of standardized protocols and clearly defined physico-chemical parameters. The national metrology institutes are often unable to carry out reliable inter-comparisons in order to exchange or compare measuring data. In the field of dimensional nanometrology, calibration chains are being introduced at national and European level. However, the data released by the various laboratories involved in nanotoxicology studies are only comparable when traceability is established.

Hence positive and negative reference nanomaterials are still under development at the moment. There is a real need to collect enough data of well characterised nanomaterials in order to have reference materials available for comparison and benchmarking. Consequently, it is all the more essential to thoroughly characterize the test samples.

#### ● **Dose metrics**

The concept of dose metrics for test samples is crucial for evaluating biological risks because there is a need to specify what exactly is being studied. More and more people are questioning the relevance of the mass unit in the case of nanomaterials. By studying the inflammatory response induced in rats by the instillation of monodispersed nanoparticles of TiO<sub>2</sub> of different sizes, Oberdörster *et al.* was expecting a dose-dependent response according to the total mass instilled for each type of

nanoparticle. However, for the same given mass, nanoparticles of 25 nm triggered a stronger response than nanoparticles of 250 nm.<sup>248</sup> On grouping the results together, the authors noticed that a single dose-dependent response curve was obtained by tracing the inflammatory response according to the specific surface area. These results support the concept that the specific surface area is a totally relevant dose metric for evaluating the toxicity of nanomaterials. The biological response may also differ depending on the size of the surface area in question (nanoscale or microscale).<sup>1, 3</sup> Other researchers propose the number of particles as a dose metric for the *in vivo* evaluation of the pulmonary inflammatory response.<sup>249</sup> Porosity can also be considered for nanoporous materials.

The same conclusions were reached with *in vitro* tests carried out with other nanomaterials. Teeguarden *et al.* also commented that the response expressed as a mass unit did not take into account the effects induced by nanomaterials and was better correlated when the dose was adjusted according to the specific surface area or the number of particles.<sup>250</sup> Lison *et al.* ended with the same conclusion by investigating silica nanoparticles *in vitro*.<sup>251</sup> They showed that the degree of cytotoxicity and cell internalisation were proportional to a nominal unit including the specific surface area, mass and number. In fact, the internalization of nanomaterials by cells depended considerably on the size and the specific surface area. Furthermore, the advantage to think in terms of specific surface area is that this parameter is independent of the agglomeration state (but not of aggregation).

Consequently in the biological tests, mass which is the traditional unit used to calculate concentration ranges is perhaps no longer a relevant or appropriate dose metric for nanomaterials. If the biological risk assessment seems to better reflect reality when the test sample are prepared according to the specific surface area or the number of particles, these modifications are acceptable and must be documented in the risk analysis. Similarly, it might be wise to carry out extractions as a function of specific surface area as opposed to mass.

### **III.B.3. Toxicokinetics and the biodegradation study**

According to Annex A of the EN ISO 10993-16 standard<sup>2440</sup>, toxicokinetic studies are recommended:

- if the device is designed to be bioresorbable;
- if the device is a permanent contact implant and if biodegradation or significant corrosion is known or likely and/or if migration of leachable substances from the device occurs;
- substantial quantities of potentially toxic or reactive degradation products or leachables are likely or are known to be released from a medical device into the body during clinical use.

It is important to determine the type of degradation products in any medical device, especially if these products are on a nanoscale. The mechanism and kinetics of their elimination must be investigated since adverse effects may be triggered.

Designed for degradation products and extracts from medical devices in general, the toxicokinetic evaluation, as described in the EN ISO 10993-16 standard, is applicable to medical devices containing free nanomaterials or likely released nanomaterials. It is a prerequisite for any safety evaluation programme of medical devices containing nanomaterials or likely to release such materials.

We have seen that there is a risk of toxicity and translocation associated with free nanomaterials although this risk is only partly known. Given the recommendations presented in the standard, toxicokinetic studies should be considered for medical devices containing nanomaterials if they are in direct contact with the human body and, in particular, if they are implantable. These studies are all the more essential for medical devices containing free nanomaterials and for those with nanoscale degradation products. The latter case is particularly relevant for medical devices containing nanomaterials incorporated in a solid bulky matrix (but may become detached from this matrix in the biological medium). The risk management planning should examine this possibility, especially in case of malfunction. The toxicokinetic evaluation can identify the targeted organs and the state of the nanomaterials during their life cycle (solubility, aggregation, degradation products, etc.) and therefore contributes to a better understanding of their toxicity. The degradation products released by the nanomaterials and the impurities released during the manufacture of the devices should also be properly characterized.

The properties of nanomaterials may be different from those of conventional materials but they are nevertheless studied in a similar way. Evaluation of the absorption, distribution, metabolism and elimination (ADME) of free nanomaterials (including nanoscale degradation products) and their degradation or solubilisation products depend on 4 factors: the route of administration, animal species, size and the surface properties of the nanomaterial.<sup>252</sup> Bioavailability should be studied using relevant methods (e.g. fluorescent or radioactive labelling). Care must be taken above all to ensure that the marker selected does not modify the physico-chemical or biological properties of the free nanomaterials and therefore their biodistribution. It is also important to check that the marker remains firmly attached to the free nanomaterials throughout the study in order to avoid any confusion between monitoring the released marker and that of the free nanomaterials.

Imaging techniques, such as positron emission tomography (PET) or fluorescence imaging, do not give quantitative results but are suitable for the qualitative biodistribution study of free nanomaterials (invasive MDs, MDs via the ENT, pulmonary or gastrointestinal routes). These techniques are also valid for determining accumulation sites and translocation.

It is therefore highly advisable to carry out toxicokinetic studies on free nanomaterials and nanometric degradation products using a scientific approach suitable for each individual case, as stipulated in the EN ISO 10993-16 standard. The methodology could be adapted from ADME studies designed for drugs.<sup>252-253</sup> This toxicokinetic evaluation will provide a better picture of the risk in order to direct subsequent toxicity studies and adjust the risk management process for the medical device more effectively.

### III.B.4. Cytotoxicity tests

The EN ISO 10993-5 standard refers to *in vitro* studies to determine the cytotoxic potential of medical devices, materials and/or their extracts.<sup>244s</sup> The aim is to assess:

- cell lysis,
- the inhibition of cell growth,
- colony formation,
- other effects on cells (ex.: morphology, membrane lesions, metabolic dysfunction and inflammatory response, *etc.*).

The protocols for some standardised, validated methods such as "neutral red", "colony formation", "MTT" and "XTT" are described in the standard text.

#### • **Disturbances in cytotoxicity tests by nanomaterials**

Recently, the reliability of some methodologies used for nanomaterials has been questioned. In fact, the photometric absorbance and intrinsic fluorescence of some nanomaterials could alter colorimetric and fluorometric results. The highly reactive surface area of nanomaterials appeared to bind analytes or colouring agents, thus introducing artefacts and generating ambiguous or inconsistent spectrometric measurements.

For instance, carbon-based nanomaterials have been shown to absorb the molecules of the neutral red colouring agent and give false positive results.<sup>254</sup> The study conducted by Monteiro-Riviere *et al.* also stressed the difficulty in interpreting a cell count after labelling with trypan blue (another method used to evaluate cytotoxicity), with the aggregates/agglomerates of nanoparticles covering cells and thus complicating the analysis.

The MTT cytotoxicity test has also raised questions since inconsistencies have been observed between the results obtained with this method and those recorded using other techniques. The results of an MTT test, carried out on cells treated with single-walled carbon nanotubes, indicated a dose-dependent decrease in the number of living cells.<sup>108</sup> However, no significant cytotoxicity was detected in the WST test - a result corroborated by the LDH test and by labelling the cells with propidium iodide/annexin V followed by flow cytometry analysis. To explain this difference, Worle-Kirsch *et al.* suggested that the carbon nanotubes interact with MTT-formazan crystals but not with WST, XTT or INT crystals.<sup>108</sup> The binding of the carbon nanotube with the MTT soluble reagent would disrupt the colorimetric reaction, which reflects cell viability. Some researchers suggest using the colony-forming test to overcome the problems caused by these reagents.<sup>185</sup> The propidium iodide test is also another option.

Given the potential interference of nanomaterials on the test results, specific attention must be paid to the reproducibility, reliability and sensitivity of the cytotoxicity tests selected before making any rash interpretation. In particular, caution should be taken regarding the possible interference with nanomaterials during tests using colorimetric and fluorescent agents. In such cases, the corroboration of several results using different methodologies might be necessary in order to make a scientifically valid interpretation.

### **III.B.5. Genotoxicity tests**

The genotoxicity tests quoted in the EN ISO 10993-3 standard are a key part of any programme for evaluating the biological risks of an invasive medical device or one that is in prolonged/permanent contact with the body. This programme will vary depending on the context of the evaluation and the composition of the device.<sup>244f</sup> Various methods are currently available for assessing the different genotoxic risks associated with the devices.

#### **• *In vitro* genotoxicity tests**

The EN ISO 10993-3 standard recommends a series of *in vitro* genotoxicity tests. This series must include:

- either the three following different *in vitro* tests:
  1. a reverse mutation test on bacteria (Ames test),
  2. a gene mutation test on mammalian cells,
  3. a chromosome aberration test on mammalian cells;
- or two different tests: test 1. and a test on murine lymphoma.  
Indeed, tests 2. and 3. can be replaced by a test on murine lymphoma including determination of the number and size of the colonies in order to determine clastogenicity and gene mutation.

The bacterial reverse mutation test (Ames test according to the OECD guideline 471),<sup>255</sup> measures the increase in the frequency of isolated mutations following gene mutations triggered by tested products. The test might provide false-negative results with nanomaterials.<sup>149-150</sup> Indeed, nanomaterials seem to have difficulty diffusing through the bacterial membrane and prokaryotes are incapable of internalizing foreign substances by endocytosis. Moreover, some genotoxic mechanisms of action are due to the interaction of nanomaterials with the mitochondria, which cannot be detected in the bacterial assay. Consequently, this assay may produce false negative results. It may have to be replaced by another *in vitro* assay.

Other *in vitro* assays are possible: the comet test and the *in-vitro* micronucleus assay.

The comet assay measures unrepaired lesions in DNA strands and DNA alkali-labile sites. As the comet assay includes reparable lesions that have not yet been repaired in its positive results, the findings obtained with this method show more significant genetic damage levels. It is therefore less reliable than the micronucleus assay in the genotoxic evaluation but is still suitable for nanomaterials in order to highlight primary DNA lesions. In this case, since a large proportion of the genotoxic effects of nanoparticles are likely to be related to the production of reactive oxygen species, the traditional protocol may have to be supplemented by using enzymes to identify oxidative lesions of DNA, for instance, by treating cells with the fpg or hOGG1 protein.

The micronucleus test quantifies the clastogenic or aneugenic effects resulting from chromosomal damage (chromosome fragment or whole chromosome loss) or from interference with mitotic mechanisms (loss of chromosomes). It measures the lesions that remain after cell division, i.e. only cells that have not been repaired.

The *in vitro* micronucleus test seems to be also suitable for nanomaterials. Preference should be given to human cells in primary cell culture such as lymphocytes. If the chosen protocol uses cytochalasine B to identify the dividing cells, no interference between this compound and the endocytosis and/or exocytosis of nanomaterials should be checked beforehand. Such interference has been noticed with ultra-superparamagnetic iron nanoparticles.<sup>256</sup>

Regardless of the *in vitro* tests selected, when a primary cell line or continuous mammalian cell is used, information concerning the phenotypic expression of the latter must be documented, especially its p53 status and its ability to tackle the reactive oxygen species (SOD, GSH/GSR, GST, GPX, etc.). Moreover, since the intra- and extracellular flows of nanoparticles depend on the cell ability of endocytosis and exocytosis, they should be documented for all the cell lines used. Deficient cell lines must not be used.

Moreover, these nanomaterials often have to be internalised in cells in order to observe a toxic effect.<sup>149</sup> The internalisation rate varies considerably, depending on the internalisation process and the nanomaterial. Although it is sometimes necessary to wait for several cell cycles, Dombu et al. recently showed that polysaccharide nanoparticles penetrated the human bronchial epithelial cells within 3 minutes and reached equilibrium after 40 minutes.<sup>257</sup> The protocols should therefore be adapted to suit the endo- and exocytosis kinetics of nano-objects in the cell line selected.

#### ● ***In vivo* genotoxicity tests**

Any *in vivo* test must be chosen based on the most appropriate criterion identified by *in vitro* tests. Experiments to show that the test substance has reached the target organ should be attempted.

If this is not possible, a second *in vivo* test in another target organ may be required to check the absence of any *in vivo* genotoxicity.

The *in vivo* tests currently used include the rodent erythrocyte micronucleus assay, metaphase analysis in rodent bone marrow or the unscheduled DNA synthesis test on mammalian hepatocytes. The selection of the most appropriate test system must be justified and documented. If other *in vivo* tests were performed in order to obtain additional information on genotoxicity, this decision must be justified and documented.

*In vivo* tests should use routes and exposure conditions that best reflect clinical conditions in terms of level of exposure, frequency of administration and cytotoxicity but should also indicate the inflammation level triggered. In particular, the recruitment of cells involved in inflammation, such as macrophages and polynuclear neutrophils which can display their own specific effects by producing free radicals or interfering with repair mechanisms, must be taken into account.

#### • **Recommendations regarding genotoxicity tests**

As for any type of medical device, several tests should be carried out in order to evaluate genotoxicity, namely at least two different *in vitro* tests and one *in vivo* test.

As a general rule, if the device proves to be genotoxic, its development should not be continued, and therefore no carcinogenicity issue needs to be addressed. The toxicological profile must always be weighed against the expected benefits of using nanomaterials in order to justify their use.

### **III.B.6. Immunotoxicology, delayed-type hypersensitivity and irritation**

The EN ISO 10993-10 standard<sup>244i</sup> focuses on irritation and delayed-type hypersensitivity tests whilst the technical specification ISO/TS 10993-20<sup>244d</sup> provides details on the principles and methods relating to immunotoxicology tests for medical devices.

The cells involved in the immune response such as dendritic cells or macrophages also contribute to nanomaterial transportation and elimination. Immunotoxicity analyses should therefore take these cell interactions into account in order to gain a better understanding of the potential impact of those nanomaterials. Consequently, the development of cell models to study the influence of nanomaterials on these cells in particular and on the immune system in general must be documented and justified. Sensitisation tests are generally carried out via the topical route.

Buehler's sensitisation test does not appear to be very sensitive and is therefore probably not suited to nanomaterials, thus it is not very often used.

Conversely, the LLNA or (*Local Lymph Node Assay*) is often used for nanomaterials, for example to evaluate the TiO<sub>2</sub> nanoparticles in cosmetic products for topical application. The differentiation of irritant substances and allergens may, however, be an issue in LLNA testing as false-positive results may also be observed.<sup>244i</sup> In addition, nanomaterials may induce an adjuvant<sup>116-117</sup> or immunosuppressant effect on sensitisation reactions triggered by other substances by either exacerbating them or altering them.

The Magnusson and Kligman test, which involves an intradermal injection during the first induction, is more sensitive<sup>244i</sup> since it allows the material to cross the skin barrier. The possibility of a nanomaterial triggering a delayed-type hypersensitivity reaction and immunotoxicity following skin exposure depends on its ability to cross the epidermal layer and interact with the proteins in the body. These interactions are crucial for investigating sensitization because the protein-nanomaterial complex is often recognized by the immune system and influence the biological effect.

Regarding irritation or intracutaneous reactivity tests, the current guidance documents seem applicable. However, concerning skin irritation, since protocols involving intradermal administration were designed for homogeneous solutions, caution must be exercised when interpreting the results of experiments carried out with suspensions containing insoluble nanomaterials. In fact, the latter could trigger positive artefact responses, which are not related to the intrinsic or chemical characteristics of the nanomaterial but solely because of its physical presence in the dermis.

The presence of nanoparticles (especially carbon black and titanium dioxide) has also triggered artefacts during the *in vitro* release of pro-inflammatory cytokines.<sup>258</sup> Since this phenomenon is related to the adsorption of cytokines by nanoparticles, a multiparametric evaluation is therefore required.

### **III.B.7. Haemocompatibility tests**

The haemocompatibility evaluation (EN ISO 10993-4<sup>244g</sup>) includes five parameters: haematology, thrombosis, coagulation, platelets and the complement system. These are precisely the end points that have to be tested when determining the haemocompatibility of nanomaterials. In fact, studies have shown that nanomaterials translocated into blood because of their nanoscale. Some have a pro-thrombotic potential and are capable of activating platelets. The haemocompatibility evaluation should therefore be carried out primarily for implantable medical devices containing nanomaterials in direct or indirect contact with blood and/or if there is a possibility that the free nanometric particles will translocate into the blood stream. A specific American ASTM standard for the *in vitro* haemocompatibility evaluation of nanoparticles is currently available, called ASTM E2524 - 08 *Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles*.<sup>259</sup>

### **III.B.8. Systemic toxicity tests (acute, subacute/subchronic and chronic)**

The EN ISO 10993-11 standard<sup>244j</sup> provides a framework for acute, subacute/subchronic and chronic systemic toxicity testing and offers a wide range of exposure routes that can be used for the evaluation of the effects on the whole body over time.

Systemic toxicity studies for devices containing nanomaterials should be as thorough as possible including an evaluation of clinical, biological and anatomopathological parameters. It may be necessary to adapt the test protocols and exposure periods depending on whether the nanomaterials are soluble or insoluble and on their long-term biopersistence. Depending on the type of nanomaterials, additional histological examinations may be carried out. Techniques more appropriate to nanomaterials can be used such as transmission electron or confocal microscopy.

As we have seen in this report, some organs or systems are potentially affected by free nanomaterials, even those protected by physiological barriers. These systems and potential target organs therefore require specific investigation, such as:

- the cardio-respiratory system (lungs, heart and blood vessels, etc.),
- the nervous system (brain, spinal cord and nerves, etc.),
- the digestive system (liver, stomach, pancreas and intestine, etc.),
- the lymph system (thymus, spleen, lymph nodes and bone marrow, etc.),
- the uro-genital system (kidneys and reproductive organs, etc.).

### **III.B.9. Carcinogenicity, reprotoxicity and neurotoxicity**

Finally, additional evaluations (carcinogenicity, reproductive/developmental and neurotoxicity, *etc.*) must be carried out depending on the risks associated with the use of the medical device in normal and failure mode.

Although the paucity of data available at the present time cannot clearly confirm whether or not nanomaterials trigger carcinogenicity, neurotoxicity, teratogenicity or reprotoxicity, the risk cannot be ruled out. In particular, since toxicokinetic studies have demonstrated the accumulation and biopersistence of free nanomaterials, this risk should be considered when analyzing the risks of the medical device according to its intended use. Additional tests should be carried out for nanomaterials, as required, with possible modifications to existing tests currently used.

Up to now, the issues of carcinogenicity and reprotoxicity for medical devices, described in the EN ISO 10993-3 standard,<sup>244f</sup> are most of the time addressed by clinical data and scientific publication documentation.

Generally, genotoxicity can trigger carcinogenicity. Nevertheless, carcinogenicity can exist without genotoxicity. Both risks must therefore be considered independently in the risk analysis. Thus,

due to the lack of clinical hindsight, the risk of carcinogenicity must be addressed in the medical device risk analysis, considering the intended purpose of the medical device and the conclusions drawn from the toxicokinetic evaluation.

Similarly, as nano-objects can cross the placental barrier, the risks of teratogenicity should be assessed according to intended use of the medical device.

No reference is made to neurotoxicity in the EN ISO 10993 standard series. The ability of free nanomaterials to cross physiological (membrane and nuclear) barriers and the blood-brain barrier in particular, must not be overlooked. The risk of neurotoxicity is worth investigating depending on the intended use of the medical device and its route of exposure.

### **III.C. Benefit/risk analysis**

Any medical device that comes into contact with tissues and biological fluids always triggers a reaction. The body response can vary in severity - being localised *in situ* or causing remote damage. A medical device that performs an appropriate host response in a specific application is said to be biocompatible.

It is therefore important to remember that the risk exists for any foreign particle introduced into the body. Just like any other substance, some nanomaterials can cause inappropriate responses when used as intended; whereas this is not the case with others (same material in a bulky form, for instance). The risk is determined by the intrinsic toxicity of the product combined with the degree of exposure (quantity, type and duration of contact, etc.). Both concepts are inextricably linked and are essential for deciding on the potential toxicity of a medical device.

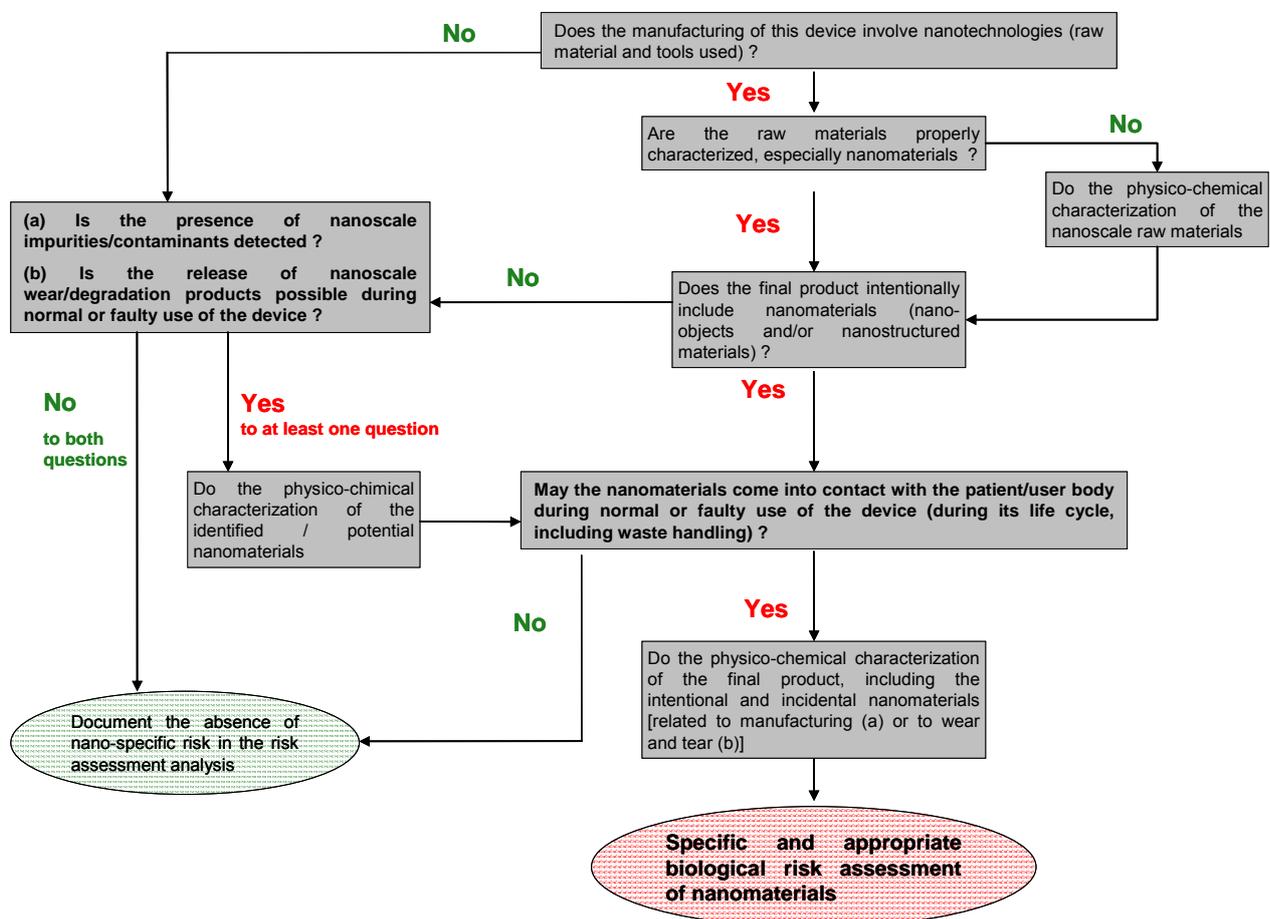
A smaller size does not necessarily mean greater toxicity. Although other factors are involved such as the elimination rate, surface chemistry or, quite simply, the inherent toxicity of the chemical species studied. Unfortunately, current techniques are still unable to pin down accurately the factors responsible for the adverse effects observed. This is why a critical analysis of the results obtained in the biological risk evaluation of the final device containing nanomaterials is absolutely essential in order to avoid any premature conclusion or misinterpretation.

A risk analysis must begin with a detailed description of the medical device and as detailed a physico-chemical characterization as possible regarding the nanomaterial contained in the device. Particular attention must be paid to free nanomaterials and degradation products / potential nanoscale wear and tear particles. Generally, any biocompatibility evaluation of a medical device must include a two-part analysis:

- raw materials: check that they are not harmful;
- implementation: check that the process for implementing the final medical device (manufacturing, cleaning, packaging, sterilisation) do not leave any manufacturing residues that may be harmful once the device comes into contact with tissues / biological fluids.

The risk analysis should take into account the physicochemical parameters of the nanomaterial used in the final medical device as well as the intended purpose (essentially the exposure route) since the toxicological profile may vary considerably depending on these factors. This profile should be weighed against the anticipated benefits of incorporating the nanomaterial in the device. This analysis of the benefits *versus* risk ratio must then be compared with the alternatives available. The use of the nanomaterial is justified only when the overall analysis confirms that the actual benefits outweigh the potential risks.

A tree diagram is recommended to help decision-making when analyzing the risks of medical devices containing nanomaterials or using nanomaterials (see Figure 3).



**Figure 3. Analysis of the nano-specific risks for medical devices**

The term “intentional nanomaterials” is used when the latter are intentionally incorporated in the design of the medical device. Otherwise, we refer to non-intentional nanomaterials.

*Adapted from the studies carried out by the European Work Group, New Emerging Technologies.*

### III.D. Other reference materials

The report on nanotechnologies drafted by the Royal Society and Royal Academy of Engineering<sup>55d</sup> in 2004 in the United Kingdom is one of the first reports to assess the opportunities and risks associated with nanotechnologies - an obvious sign that the regulatory authorities are looking closely at this issue. Some reports by regulatory authorities have been published since. These documents identify actions and priorities related to nanotechnologies. All call for more research in nanomaterial characterization and in understanding their effects on health, safety and environment.

Within the European Commission, various Technical Committees and Agencies have set up working groups dedicated to nanotechnologies and have published reviews and scientific opinions on the subject, which have been summarised in the 2008 report.<sup>260</sup>

The production, use and marketing of chemical substances within the European Union are governed by REACH (*Registration, Evaluation, Authorisation and Restriction of Chemicals*). REACH makes manufacturers liable for evaluating and managing the risks associated with the chemical products they use. Moreover, the manufacturers have to provide suitable safety information for users on their products.

In principle, REACH requirements apply to nanomaterials. However, they have not been given a specific provision yet. In particular, the minimum threshold for the mandatory registration of manufactured substances, which is set at one tonne per annum, by definition eliminates many nanomaterials, which are not produced in such quantities. However, since 2008, reviews have been carried out by the CASG Nano Group (*REACH Competent Authorities Subgroup on Nanomaterials*) to bring REACH legislation into line with the specific case of nanomaterials. As a general rule, manufacturers of medical devices containing nanomaterials should therefore ensure beforehand that the raw materials used have been authorised by REACH.

Outside Europe, the United States, Canada and Australia are also particularly involved. In the United States, the FDA (*Food and Drug Administration*) recognised the importance of nanotechnologies as early as 2006, and set up a Nanotechnology Task Force responsible for examining legislation relating to products containing nanomaterials.

Furthermore, standards are beginning to take into account the specific characteristics of nanomaterials. For example, some ASTM (*American Society for Testing and Materials*) international standards are specific for evaluating the biological risks of nanomaterials including:

- ASTM E2526: Standard test method for evaluation of the cytotoxicity of nanoparticulate materials in porcine kidney cells and human hepatocarcinoma cells (an analysis of their effects on the kidney and liver - the main excretory organs for nanomaterials),
- ASTM E2525: Standard test method for evaluation of the effect of nanoparticulate nanomaterials on the formation of mouse granulocyte-macrophage colonies

(evaluating nanoparticle-mediated stimulation or inhibition on cells obtained from the bone marrow, including macrophages),

- ASTM E2524: Standard test method for analysis of hemolytic properties of nanoparticles.

The OECD, which has been mainly working on the safety of chemical products to date, is also seeking to harmonise, standardise and validate tests and methods for evaluating the health and environmental safety of nanomaterials. Recommendations can be found in the following documents to name but a few:

- Guidance manual for the testing of manufactured nanomaterials;<sup>261</sup>
- Preliminary guidance notes on sample preparation and dosimetry for the safety testing of manufactured nanomaterials.<sup>262</sup>

Finally, a Technical Committee, ISO TC229, attached to the International Organization for Standardization, has been commissioned to draft standards relating to terminology, nomenclature, metrology and instrumentation, including specifications for reference materials, test methodologies, modelling and simulations. They are based on scientific information and practices relating to health, safety and environment. 7 publications are currently available:

- ISO/TS 80004-1:2010: Nanotechnologies - Vocabulary - Part 1: core terms,
- ISO/TS 27687:2008: Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplate,
- ISO/TS 80004-3:2010: Nanotechnologies - Vocabulary - Part 3: carbon nano-objects,
- ISO/TR 11360:2010: Nanotechnologies - Methodology for the classification and categorization of nanomaterials,
- ISO 29701:2010: Nanotechnologies – Endotoxin test on nanomaterial samples for *in vitro* systems - Limulus amoebocyte lysate (LAL) test,
- ISO/TS 10867:2010: Nanotechnologies - Characterization of single-walled carbon nanotubes using near infrared photoluminescence spectroscopy,
- ISO/TR 12885:2008: Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies.

## CONCLUSIONS AND PERSPECTIVES

This report provides practical elements and recommendations regarding the use of nanomaterials in medical devices, both in terms of benefits and potential risks. These studies are mainly focused on the biological evaluation of medical devices containing nanomaterials likely to come into contact with the body of the patient or the user.

The specific properties of nanomaterials, even those which improve the performance of the medical devices and provide considerable proven benefits for patients, raise concerns because they are new. Questions relating to their harmful effects on health are hotly debated. Many research projects attempt to fill any gaps in our knowledge of the potential toxicity of nanomaterials. But current data are incomplete and often inconsistent. Although it is recognised that some nanomaterials can have adverse effects under specific test conditions, it is difficult to refer to a definite health hazard associated with these materials because the risks depend on a large number of factors such as the type of nanomaterial, the exposure route, quantity and frequency of exposure, etc.

Methodologies to characterize and develop all aspects of the potential toxicity of nanomaterials are still at the developmental stage. Interpretation of intra-laboratory data remains difficult, partly due to a shortage of reference nanomaterials. The relevance of some toxicological tests used for conventional materials have yet to be established and then validated. Consequently, given those technical weaknesses, evaluation of the biological risks is still uncertain.

In this context, the risks have to be evaluated case by case. More precisely, subsequently to what has been discussed in this report, an in-depth analysis of the risks associated with medical devices containing nanomaterials is absolutely essential. Given the potential risks triggered by the presence of nanomaterials in the device, a major benefit must be anticipated in order to justify their use.

Current guidance documents concerning medical devices, whether of a regulatory or standard nature, are applicable for evaluating the biological risks of medical devices containing nanomaterials. Indeed, provided a few adjustments to experimental procedures and precautions when interpreting results, they give a basic framework to facilitate the identification and management of all of the risks associated with the use of the medical device. The outcome of this work has led to the drafting of recommendations for manufacturers of medical devices containing nanomaterials to promote the analysis and evaluation of the biological risks during the life cycle of the medical device: from design via application up to recycling after use.

In order to extend its scope, the report will be sent to international bodies (European Commission, ISO, CEN and competent European authorities, etc.). Competent authorities in the medical device sector must monitor this type of medical devices very closely.

In order to guarantee a high level of safety, it will also be possible to modify the classification of medical devices to include those containing nanomaterials likely to come into contact with the body of the patient or the user.

The constantly evolving world of nanotechnologies poses a real challenge for scientists and regulatory authorities alike. The diversity of the nanomaterials, their properties which diverge from conventional materials, and the fact that they are brand new, raises many questions, which cannot always be answered at the present time. We are on a learning curve and the tools we need to improve our understanding are still in their infancy. Co-ordinated, international, multidisciplinary action is expected from the relevant competent institutions in order to promote knowledge and innovation whilst ensuring better risk control. The French Health Products Safety Agency pays specific attention to the new medical devices containing nanomaterials launched on the French market.

# RECOMMENDATIONS TO MANUFACTURERS ON THE BIOLOGICAL ASSESSMENT OF MEDICAL DEVICES CONTAINING NANOMATERIALS

The following recommendations must be read along with the related scientific report, entitled “Biological assessment of medical devices containing nanomaterials” and published by the French Health Products Safety Agency (Afssaps).

Overall, the current existing regulatory and guidance documents (harmonized directives, guidelines, standards...) are considered to provide a suitable framework for the biological risk assessment of medical devices containing nanomaterials, which are likely to come in contact with the patient/user’s body. Nevertheless, there is a need to formulate and clarify some nano-specific recommendations and guidelines.

- ***Assessment of the benefit/risk ratio:***

The toxicological profile must be contrasted to the expected benefits resulting from the inclusion of nanomaterials in the medical device. Then, this benefit/risk ratio has to be weighed against those of available alternatives. The use of nanomaterials seems to be justified only when the comprehensive analysis provides sound evidence for a favorable balance.

- ***Information disclosure and transparency:***

In order to ensure information transparency on the presence of nanomaterials, it is mandatory to explicitly mention in the “Instructions For Use” document the use of nanomaterials in the medical device, which are likely to come in contact with the patient/user’s body.

- ***Identification and characterization of the materials used:***

As a rule, similarly to any medical device, the responsible manufacturer has to make sure that its raw materials are properly characterized and authorized by the prevailing REACH European regulations. Special attention shall be given to the characterization of nanomaterials (nano-objects and nanostructured materials), whose physico-chemical properties may change over time and during the product life cycle.

For this very reason, the physico-chemical characterization of the final product containing nanomaterials must be performed before any biological risk assessment. Likewise, final product batch to batch consistency and reproducibility are crucial, in order to ensure the validity of the biological risk assessment performed.

Since medical devices containing nanomaterials, which are likely to come in contact with the patient/user's body, may wear out, deteriorate over time and release nanosized particles, biodegradation must be properly addressed in the risk analysis of the medical device, in its intended use conditions. Should its assessment be required, comprehensive determination and characterization of the released nanoparticles will be necessary in the physiological conditions similar to the standard conditions of use. The release kinetics, quantity and fate of the free nanoparticles in biological media have to be evaluated.

The most relevant physico-chemical parameters to assess the biological risks of a nanomaterial are the following: size and size distribution, morphology, aggregation/agglomeration state, solubility/dispersability, specific surface area, composition (including chemical composition and crystalline structure, amongst others), surface charge, surface chemistry. Those parameters considered for the biological risk assessment have to be carefully contemplated with regard to the medical device containing nanomaterials and its intended use, because the toxicological profile can greatly differ according to its physico-chemical characteristics. It is recommended to indicate the method and the measurement uncertainty for each parameter measured. Moreover, measures should be performed on an appropriate number of samples.

A qualitative and quantitative evaluation of impurities has to be carried out, especially their physico-chemical, biological and toxicological characterization must be provided. In case of unavailability, this should be justified. Since nanomaterials are prone to adsorb impurities, it is highly recommended to routinely check for their absence before batch release (pyrogenicity, etc.).

• ***Caveats in the biological risk assessment:***

Generally, toxicity is specific to the tested nanomaterial and cannot be generalized or extrapolated, even within the same chemical family. Furthermore, *a priori* the concept of equivalence is not acceptable, because difficult to prove.

According to the prevailing regulations, biological risk assessment is performed on the final product. This approach stays applicable to medical devices containing nanomaterials. However, there might be situations where biological risk assessment on the final product seems satisfactory, while the biological evaluation on the nanomaterials alone is not. Therefore, and in case the risk analysis reveals a likelihood of contact between nanomaterials and the patient/user's body, it may be required to carry out a separate biological assessment on the nanomaterials alone, especially to perform tests related to major risks such as genotoxicity and carcinogenicity.

Relevant toxico-kinetic studies on free nanomaterials and/or nanosized degradation particles are highly recommended. The methodology can be adapted from testing protocols of drugs (ADME type– Absorption, Distribution, Metabolism, Excretion). Biodistribution studies should be designed with an appropriate labelling (e.g. radioactive or fluorescent), which should not modify the physico-chemical and biological properties of free nanomaterials and which should stay firmly attached to nanomaterials during the whole time of study.

The conventional dose metrics, namely mass and surface, may not be the most appropriate metrics for the biological evaluation of medical devices containing nanomaterials. If other dose metrics (specific surface area, number of particles...) seem to establish more informative results, closer to reality, then these adaptations are recommended and should be documented in the risk assessment analysis. Likewise, it might be more suitable to perform the extractions according to the specific surface area instead of mass, when preparing medical device samples for biological assessment.

Experimental conditions for the biological risk assessment should be as close as possible to the clinical conditions, for example regarding exposition route, quantity and frequency of exposure or aggregation/agglomeration state.

Special attention should be given to the reproducibility, reliability and sensitivity of the *in vitro* toxicological tests selected, before drawing any hasty conclusion. Particularly, caution should be taken because of potential interferences of nanomaterials with test protocols relying on colorimetric or fluorescent agents, such as those in cytotoxicity testing. In such cases, corroboration of several test results coming from different methodologies is required for a scientifically sound interpretation.

Evaluation of haemocompatibility must be performed on medical devices containing nanomaterials in direct or indirect contact with blood. Moreover, if the toxico-kinetic study reveals a potential translocation of free nanosized particles originated from the medical device into the systemic blood circulation, then haemocompatibility should also be evaluated.

As for any medical device, it is essential to carry out several tests to evaluate the genotoxicity of the device, namely at least two different *in vitro* tests and an *in vivo* test. The relevance of the testing protocols with the tested nanomaterials should be ensured (risk of false-negative results from the Ames test, test duration...), adapting them or switching to more appropriate tests if necessary.

Since some scientific studies suggest that nanomaterials may affect the immune system, the risk of delayed-type hypersensitivity and more generally of sensitization must be addressed. Risk analysis has to evaluate the need to carry out immunotoxicological tests.

It is recommended to design systemic toxicity studies on medical devices containing nanomaterials as comprehensive as possible, including the evaluation of clinical, biological and anatomo-pathological parameters. According to the nature of the nanomaterial, additional histological investigations should be performed.

Given the very limited clinical data, the risk of carcinogenicity must be addressed in the risk analysis of the medical device, according to the intended use of the medical device and the results of the toxico-kinetic studies.

Similarly, if there is a potential accumulation of free nanomaterials in some specific biological tissues (reproduction organs, central nervous system...) and/or a potential physiological membrane crossing (placenta, blood brain barrier...), then toxicological effects on reproduction, teratogenicity and neurotoxicity have to be investigated.

To conclude, the current toxicity testing approaches provide an appropriate framework and starting point to address the biological risk assessment of medical devices containing nanomaterials, with adaptations on a case-by-case basis if required. Although more appropriate analytical tools and experimental methods for nanomaterials still have to be adjusted and developed, data on the properties of nanomaterials should be generated and gathered in order to fill the significant knowledge gaps. Safety issues arising from the use of nanotechnologies in the medical device field should be addressed in a cautious and step-wise way, whilst keeping in mind the risk to benefit balance.

As part of its market surveillance activity, the French Health Products Safety Agency (Afssaps) pays specific attention to medical devices containing nanomaterials which have recently obtained the CE marking and launched on the French market. Upstream of CE marking, a close follow-up for the development of such devices may be given within Afssaps' comprehensive approach of innovation support, in order to facilitate rapid patient access to medical innovations whilst providing a framework for the risks induced by these new technologies.

# GLOSSARY

**ADME:** absorption, distribution, metabolism and excretion

**AEC: Atomic Energy Commission**

**AFM:** Atomic Force Microscope

**AFNOR:** French association for standardization

**Afssaps:** French health products safety agency (Agence française de sécurité sanitaire des produits de santé)

**Afsset:** French Agency for Environmental and Occupational Safety (Agence française de sécurité sanitaire de l'environnement et du travail)

**AIMD: Active implantable medical device**

**ASTM:** American Society for Testing and Materials

**ATP:** Adenosine triphosphate

**BET:** measuring technique developed by Brunauer, Emmett and Teller

**CASG Nano:** REACH Competent Authorities Subgroup on Nanomaterials

**CTAB:** cetyl trimethyl ammonium bromide - a cationic surfactant

**CNS:** Central Nervous System

**CNT:** carbon nanotube

**DLS:** Dynamic Light Scattering (a technique for spectroscopic analysis)

**DNA:** **desoxyribonucleic acid**

**DMEM :** Dulbecco's Modified Eagle's Medium, cell culture medium

**ENT:** ear, nose, throat

**Fc:** portion of an immunoglobulin molecule that can be crystallised and which is formed by constant regions of two immunoglobulin heavy chains

**FDA:** United States Food and Drug Administration

**Bis-GMA :** polymer composed of bisphenol A glycidyl methacrylate

**IARC:** International Agency for Research on Cancer

**IgG:** Immunoglobulin G (antibody)

**IL:** interleukin

**INM:** National Metrology Institute (institut national de métrologie)

**INT:** 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride, colorimetric reagent used in a cytotoxicity test

**ISO:** International Organization for Standardization

**IVDMD:** *In vitro* diagnostic medical device

**LDH:** Lactate dehydrogenase

**LLNA:** Local Lymph Node Assay

**MD: Medical Device**

**MEMS/NEMS:** micro/nano-electromechanical systems

**MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, colorimetric reagent used in a cytotoxicity test

**MWCNT:** multi-walled carbon nanotube

**OECD:** Organisation for Economic Co-operation and Development

**PBS :** Phosphate buffered saline

**PDMA:** polydimethylacrylamide

**PEG:** polyethylene glycol

**PLGA:** polylactic-co-glycolic acid

**PHDCA:** polyhexadecylcyanoacrylate

**PNIPAM:** poly(N-isopropylacrylamide)

**REACH:** Registration, Evaluation, Authorisation and Restriction of Chemicals

**RGD:** peptide sequence containing Arginine, Glycine and Aspartic Acid

**ROS:** Reactive Oxygen Species

**SEM:** scanning electron microscope

**SPIO:** Super Paramagnetic Iron Oxide

**STM:** scanning tunnelling microscope

**SWCNT:** single-walled carbon nanotube

**$\beta$ -TCP ( $\beta$ -tricalciumphosphate) :** calcium phosphate

**TEDMA:** polymer composed of tetra-ethylene glycol dimethacrylate

**TEGDMA:** polymer composed of tri-ethylene glycol dimethacrylate

**TEM:** transmission electron microscope

**PET:** position emission tomography

**TGF- $\beta$  (*transforming growth factor*) :** polypeptide cytokine, which acts as an anti-rejection element

**Th(1, 2, 17):** type of immune response

**TLRs :** Toll-like receptors - a group of biological receptors

**THF:** tetrahydrofuran (organic solvent)

**TNF:** tumour necrosis factor, a cytokine involved in immune reactions

**Treg:** T-regulating lymphocyte cells involved in controlling auto-immune reactions

**USA:** United States of America

**UV:** Ultra-violet

**WST:** 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-sodium tetrazol, a colorimetric reagent used in a cytotoxicity test

**XTT:** 2,3-bis-(2-methoxy-4-nitro- 5-sulfophenyl)-2H-tetrazolium- 5-sodium carboxanilide, colorimetric reagent used in a cytotoxicity test

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# APPENDIX I: Extract from the *Bulletin Officiel Santé – Protection sociale – Solidarity* No. 2010-11 of 15 December 2010

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MINISTÈRE DU TRAVAIL, DE L'EMPLOI ET DE LA SANTÉ  
MINISTÈRE DES SOLIDARITÉS ET DE LA COHÉSION SOCIALE

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## ADMINISTRATION

### AUTORITÉS ADMINISTRATIVES INDÉPENDANTES ET ÉTABLISSEMENTS SOUS TUTELLE

AFSSAPS  
Agence française de sécurité sanitaire des produits de santé

Décision DG n° 2010-225 du 13 octobre 2010 portant création à l'Agence française de sécurité sanitaire des produits de santé du groupe de travail sur les dispositifs médicaux incorporant des nanomatériaux

NOR: SASM1030919S

Le directeur général de l'Agence française de sécurité sanitaire des produits de santé,  
Vu le code de la santé publique, et notamment les articles L. 5311-1 et suivants, R. 5212-7 à R. 5212-11 et D. 5321-7 et suivants ;  
Vu l'arrêté du 27 novembre 2007 portant nomination à la Commission nationale des dispositifs médicaux,

Décide :

#### Article 1<sup>er</sup>

Il est créé auprès du directeur général de l'Agence française de sécurité sanitaire des produits de santé un groupe de travail sur les dispositifs médicaux incorporant des nanomatériaux. Ce groupe de travail, rattaché à la Commission nationale de sécurité sanitaire des dispositifs médicaux, est chargé de :

- réaliser un état des connaissances sur les dispositifs médicaux incorporant des nanomatériaux et leur évaluation biologique ;
- proposer des recommandations à destination des fabricants développant ce type de dispositifs médicaux.

#### Article 2

Les membres du groupe de travail sont désignés par le directeur général de l'Agence française de sécurité sanitaire des produits de santé pour une durée d'un an renouvelable.

#### Article 3

Le président du groupe de travail est désigné parmi les membres nommés par le directeur général de l'Agence française de sécurité sanitaire des produits de santé.

#### Article 4

Les travaux du groupe de travail sont confidentiels.

#### Article 5

Les membres du groupe de travail ne peuvent prendre part aux travaux du groupe s'ils ont un lien direct ou indirect avec le dossier examiné.

#### Article 6

Le directeur de l'évaluation des dispositifs médicaux est chargé de l'exécution de la présente décision, qui sera publiée au *Bulletin officiel* du ministère de la santé.

Fait à Saint-Denis, le 13 octobre 2010.

Le directeur général,  
J. MARIMBERT

BO Santé – Protection sociale – Solidarité n° 2010/11 du 15 décembre 2010, Page 66.

## ADMINISTRATION

### AUTORITÉS ADMINISTRATIVES INDÉPENDANTES ET ÉTABLISSEMENTS SOUS TUTELLE

AFSSAPS  
Agence française de sécurité sanitaire des produits de santé

Décision DG n° 2010-226 du 13 octobre 2010 portant nomination au groupe de travail sur les dispositifs médicaux incorporant des nanomatériaux à l'Agence française de sécurité sanitaire des produits de santé

NOR : SASM1030821S

Le directeur général de l'Agence française de sécurité sanitaire des produits de santé,  
Vu le code de la santé publique, et notamment les articles L. 5311-1 et suivants, R. 5212-7 à R. 5212-11 et D. 5321-7 et suivants ;

Vu l'arrêté du 27 novembre 2007 portant nomination à la Commission nationale des dispositifs médicaux ;

Vu la décision DG n° 2010-225 du 13 octobre 2010 portant création à l'Agence française de sécurité sanitaire des produits de santé du groupe de travail sur les dispositifs médicaux incorporant des nanomatériaux,

Décide :

#### Article 1<sup>er</sup>

Sont nommées membres du groupe de travail sur les dispositifs médicaux incorporant des nanomatériaux les personnes dont les noms suivent :

M. FELTIN (Nicolas) ;  
Mme FRAMERY (Sylvie) ;  
Mme HARMAND (Marie-Françoise) ;  
M. POUPON (Joël) ;  
Mme VAYSSADE (Muriel) ;  
M. VILLIERS (Christian).

#### Article 2

M. POUPON (Joël) est nommé président du groupe de travail.

#### Article 3

Le directeur de l'évaluation des dispositifs médicaux est chargé de l'exécution de la présente décision, qui sera publiée au *Bulletin officiel* du ministère de la santé.

Fait à Saint-Denis, le 13 octobre 2010.

*Le directeur général,*  
J. MARIMBERT

## APPENDIX II: List of medical devices with nanotechnology, identified in this report

Brand name of the medical device	NAME OF THE MANUFACTURER (Country)	Description of the device	Nanotechnology used
Acticoat®	SMITH & NEPHEW (United Kingdom)	Dressing	Silcrist™ metallic silver nanoparticles (size from 15 nm)
NanoFense™	APPLIED NANOSCIENCES	Surgical mask to filter influenza viruses	Silver nanoparticles
	SCOUTBURG (Taiwan)	Surgical mask	Silver nanoparticles
Mipan® Magic Silver Nano	HYOSUNG (Korea)	Textile with a permanent antimicrobial effect	Nano-silver
NanoMask®	EMERGENCY FILTRATION PRODUCTS (USA)	Surgical mask	Magnesium oxide nanoparticles
Aerosil®	DEGUSSA (Germany)	Dental restoration composite	Silicon dioxide nanoparticle (7-40 nm)
Filtek™ Supreme	3M ESPE (USA)	Photopolymerisable dental restoration composite	Silica nanoparticles
Adper™ Scotchbond™ SE	3M ESPE (USA)	Self-etch adhesive	Silanised zircon nano-filler
Ketac™ N100	3M ESPE (USA)	Dental restoration composite	Nano-ionomer
Kappalux Nano	PRODUITS DENTAIRE PIERRE ROLLAND (France)	Dental restoration product containing synthetic resin	Silicon dioxide and zirconium oxide nanoparticles (10-100 nm)
Grandio®	VOCO (Germany)	Dental restoration composite	Hybrid nano-composite
Optiglaze	GC CORPORATION (Japan)	Photopolymerisable dental restoration composite	Silica nano-filler
Nanogel®	TEKNIMED (France)	Injectable bone filling product	Nanoparticle hydroxyapatite (100-200 nm in size).
Nanostim™ / Ostim®	AAP BIOMATERIALS (Germany) / MEDTRONIC (France)	Injectable bone filling product	Nanoparticle hydroxyapatite
PerOssal®	AAP BIOMATERIALS (Germany)	Injectable bone filling product	Nanoparticle hydroxyapatite
FortrOss®	PIONEER SURGICAL TECHNOLOGY (USA)	Bone filling product	NanOss® technology hydroxyapatite nanoparticles with the E-Matrix osteoconductive matrix
Vitoss® Scaffold	ORTHOVITA (USA)	Bone filling product	Calcium phosphate with nanometric porosity
Puretex®	SYBRON IMPLANT SOLUTIONS (USA)	Nanostructured metallic orthopaedic implant	Pure titanium with nanoporous surface nanometric topography
NanoImplant®	TIMPLANT (Czech Republic)	Metallic dental implant	Nanostructured titanium
Nanos™	SMITH&NEPHEW (United Kingdom)	Orthopaedic prosthesis	Bonit® microporous coating containing hydroxyapatite nanocrystals manufactured by DOT (Germany)
Symax™	STRYKER (France)	Joint prosthesis	Bonit® microporous coating containing hydroxyapatite nanocrystals manufactured by DOT (Germany)
NanoTite™	BIOMET 3i	Orthopaedic and dental prostheses	Surface coating of calcium phosphate nanocrystals (20-100 nm)
Debiostent™	DEBIOTECH (Switzerland)	Stent coating	Nanostructured ceramic coating (TiO <sub>2</sub> , ZrO <sub>2</sub> , SiO <sub>2</sub> , IrO <sub>2</sub> , Al <sub>2</sub> O <sub>3</sub> , CaP) with a thickness of 100 nm to 10 µm

Brand name of the medical device	NAME OF THE MANUFACTURER (Country)	Description of the device	Nanotechnology used
Rheo Knee	OSSUR (Iceland)	External knee prosthesis	Iron nanoparticles (100 nm to 1000 nm)
	ALCOVE SURFACE, (Germany).	Stent coating	Nanostructured aluminium oxide coatings (Al <sub>2</sub> O <sub>3</sub> ) with a thickness of 300 nm
Catania™	CELONOVA BIOSCIENCES (Canada)	Bare coronary stent	Polyzene®-F polymer coating (thickness of 40-50 nm)
VestaSync™	MIV THERAPEUTICS (Canada)	Active stent	Ultra-fine hydroxyapatite coating with a porosity of 100-500 nm
ON-Q® SilverSoaker™	I-FLOW CORP.	Anaesthesia catheter	Coatings incorporating silver nanoparticles (SilvaGard™ technology) manufactured by ACRYMED (USA)
AVflo™	NICAST (Israel)	Self-sealing vascular access graft	Electrospun polymer nanofabric
NovaMesh™	NICAST (Israel)	Ventral hernia mesh	Electrospun polymer nanofabric
	Team at the Royal Free Hospital, London (United Kingdom)	Vascular system	UCL-NanoBio™ nanocages forming a patented polymer nanocomposite
	ROSKARDIOINVEST (Russia)	Nanostructured artificial heart valve	Nanostructured carbon coating
Diamaze PSD	GFD GESELLSCHAFT FÜR DIAMANTPRODUKTE MBH (Germany)	Scalpel blade	Nanostructured diamond coating (thickness of 20-40 nm)
Sandvik Bioline 1RK91™	AB SANDVIK MATERIALS TECHNOLOGY (Sweden)	Suture needle	Stainless steel with nanocrystal inclusions (1-10 nm)
Mako	ORTHOSENSOR (USA)	Surgical device providing accurate data on personalised orthopaedic knee implantation	Nanosensors
Nano-cancer	MAGFORCE NANOTECHNOLOGIES (Germany)	Nanoparticle used in the thermal treatment of cancers	Super paramagnetic iron oxide nanoparticles (approximately 15 nm), covered with aminosilanes
NanoXray	NANOBIOTIX (France)	Nanoparticle used in heat-based treatment of cancers	Hafnium oxide nanoparticles (70-100 nm)
AuroShell	NANOSPECTRA BIOSCIENCES (USA)	Nanoparticle used in the thermal ablation of tumours	Gold-coated silica nanoparticles (150 nm in diameter)
Brachysil™	PSIVIDA (Australia)	Medical device for the treatment of prostate cancer	30 µm microparticles of silicon with BioSilicon nanometric pores incorporating radioactive phosphorus <sup>32</sup> P
NanoKnife™	ANGIODYNAMICS (USA)	Tumour ablation technique based on the irreversible electroporation of cancer cells	Inducing irreversible nano-pores in cell membranes
	RETINA IMPLANT (Germany)	Retina implant	Nano-electronic component
Argus™	SECOND SIGHT MEDICAL PRODUCTS (USA)	Retina prosthesis	Nano-electronic component
	American team at the MIT (Massachusetts Institute of Technology)	Subcutaneous device for <i>in vivo</i> monitoring of blood glucose levels	Carbon nanotubes
Verigene	NANOSPHERE (USA)	Gene testing machine for diagnosis	Gold nanoparticles (13-20 nm in size)