international symposium

NANOTECHNOLOGY IN THE FOOD CHAIN

OPPORTUNITIES & RISKS

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European Food Safety Authority

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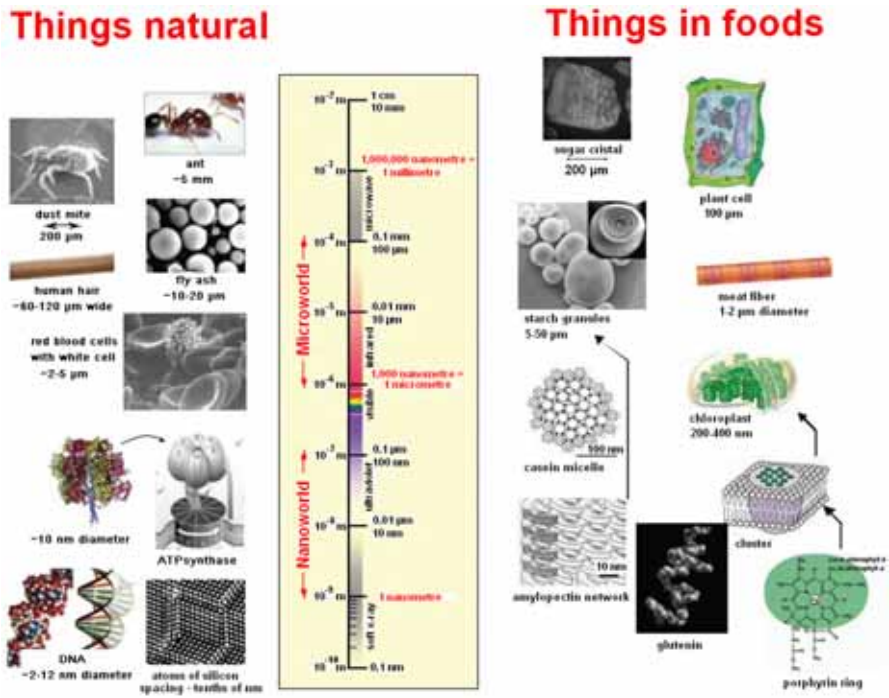
Preface

Nanosciences and nanotechnologies are highly promising and rapidly progressing disciplines in research and industrial innovation. The term “nano” refers to the measurement of size; a nanometre (nm) is a millionth of a millimetre. By way of illustration, a nanometre is about 1/50,000th the width of a human hair, and a sheet of normal office paper is about 100,000 nm thick. A nanoparticle (NP) is usually considered to be a structure between 0.1 and 100 nm (1/1,000,000 mm).

The potential benefits of nanotechnology have been recognized by many industrial sectors, and products based on nanotechnology or products containing NPs are already manufactured such as in the field of microelectronics, consumer products (e.g. personal care products, paints, automotive industry) and the pharmaceutical industry. Also with respect to food and agriculture, a number of promising applications are emerging, such as smart packaging, nanosensors for pathogen detection or registration of storage conditions, nanoformulations of agrochemicals, nano-encapsulation / nano-delivery of food ingredients, etc. Besides engineered or manufactured NPs, nano-sized particles occur naturally in many foodstuffs. For example, food proteins are globular structures between 10-100 nm (e.g. casein micelles in dairy products range from 300-400 nm) and most polysaccharides and lipids are linear polymers of 2 nm in thickness. Fat globules can be considered as natural NPs as well, ranging in size from 100 nm to 20 µm, whereas fat globule membranes have a thickness of 4–25 nm. The homogenization of fat globules can be considered as a sort of “nanotechnology process” decreasing the average diameter and increasing the number and surface area of the fat globules. Additionally, stabilized foams/emulsions are two dimensional nanostructures, one molecule thick at the air/water or oil/water interface and three dimensional nanostructures are formed when food biopolymers assemble into fibrous networks.

Although nanotechnology or NPs have the potential to bring significant benefits to both the industry and consumers, they may also introduce potential risks for human health and the environment. Due to their small size, surface reactivity and translocation possibility across biological membranes as well as potential

interactions of NPs with the surrounding matrix and unexpected effects resulting from this, specific data for risk assessment purposes are required.



Representation of the difference in scale between nano-sized vs. micro-sized materials and structures in foods (Aguilera, 2009, based on "The Scale of Things" chart developed by the Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy, http://www.er.doe/gov/bes/scale_of_things.html).

In response to the rapid developments in the field of nanotechnology, numerous national, European and international discussion initiatives and projects have been undertaken, and generic data requirements and guidance for risk assessment of NPs have been presented in various reports over the last five years.

In 2009, the FAO/WHO convened an Expert Meeting on the application of nanotechnologies in the food and agriculture sectors, where potential food safety implications were discussed in order to identify further work that may be

required to address the issue at a global level. The OECD (Organisation for Economic Co-operation and Development) established two working parties, namely a Working Party on Nanotechnology (WPN) that advises upon emerging policy issues of science, technology and innovation related to the developments of nanotechnology, and a Working Party on Manufactured Nanomaterials (WPMN), which focuses on testing and assessment methods (e.g. Sponsorship Programme where data regarding the physical-chemical properties, environmental degradation and accumulation, environmental toxicology, and mammalian toxicology of nanomaterials are gathered).

At the European level, the European Commission (EC) launched the European Strategy for Nanotechnology and the Nanotechnology Action Plan, addressing the technological and societal challenges and strengthening the research and innovation efforts, with emphasis on sustainable development, competitiveness, health, safety and environmental issues[1]. In addition, the Commission is reviewing current regulation to determine whether NPs are adequately covered with respect to the safety of consumer products and the food chain (e.g. REACH, Novel Foods Regulation, etc.).

In the area of food and agriculture, the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENHIR) of EC DG Health & Consumer Protection advices in a number of opinions about a definition for and the risk assessment of products of nanoscience and nanotechnologies (SCENHIR, 2007a&b, 2009, 2010). Also, the European Food Safety Authority (EFSA) published a scientific opinion on potential risks arising from nanoscience and nanotechnologies on food and feed safety (EFSA, 2009), and is currently working on a guidance document to provide practical recommendations for the risk assessment of NPs for use in food. For now, a case-by-case risk assessment is recommended.

On the national level, different initiatives are taken as well, ranging from public debates to written opinions (e.g. AFSSA, 2009; BfR, 2010; FSA, 2010, 2008; FSAI, 2009; UK House of Lords, 2010; VWA, 2008).

As “nano” is an emerging issue in the food chain, the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) started a self tasking initiative to gather information and knowledge on the subject in order to be able

[1] http://ec.europa.eu/nanotechnology/index_en.html

to give recommendations to be incorporated in the Belgian food safety control program. The organization of an international symposium during the Belgian EU Presidency in collaboration with the EC and the EFSA was considered to be an excellent opportunity for contributing to the European debate between stakeholders, public, politicians and policy regulators.
This symposium on "Nanotechnology in the Food Chain: Opportunities & Risks" presents the current knowledge regarding the applications, opportunities and risks of nanotechnology in the food chain ("from farm to fork"), with a notice for the remaining gaps in knowledge, legislation and control. The proceedings present the abstracts of presentations held and posters presented during the symposium in Brussels on 24 November 2010.

Finally, the FASFC wishes to thank the EC and EFSA for the successful cooperation during the organization of this event, the theme of which being of major importance and of pertinent interest as is indicated by the international character of the participants list.

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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
ADME	Absorption, Distribution, Metabolism, Excretion
AFM	Atomic Force Microscopy
DEG	Differentially Expressed Genes
DLS	Dynamic Light Scattering
EC	European Commission
EFSA	European Food Safety Authority
ENMs	engineered nanomaterials
EU	European Union
EVOH	copolymers of polyvinyl-alcohol with ethylene
EWOD	electrowetting-on-dielectric
FAO	Food and Agricultural Organization
FASFC	Federal Agency for the Safety of the Food Chain
FDA	Food and Drugs Administration
GI tract	gastrointestinal tract
GM	genetic modification
GPSD	EU Directive 2001/95/EC of 3 December 2001 on General Product Safety
HAS	human serum albumin
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IPR	intellectual property rights
MG	malachite green
MP	microparticle
MIPs	molecularly imprinted polymers
NGO	non-governmental organization
NP	nanoparticle
OECD	Organisation for Economic Co-operation and Development
PAA	poly(acrylic acid)
Paap	polynucleotide analogue antisense probe
PCL	polycaprolactones
PEO	poly(ethylene oxide)
PHA	polyhydroxyalcanoates

PHBV	poly(hydroxybutyratehydroxyvalerate)
PLA	polylactic acid
PP	polypropylene
PVOH	polyvinyl-alcohol
REACH	Registration, Evaluation, Authorisation of Chemicals (Regulation (EC) No 1907/2006)
R&D	Research and Development
SAM	Significance Analysis of Microarray
SEM	Scanning Electron Microscope
SCENHIR	Scientific Committee on Emerging and Newly-Identified Health Risks
SME	small and medium enterprises
SPR	surface plasmon resonance
TEER	Trans Epithelial Electric Resistance
TEM	Transmission Electron Microscopy
WHO	World Health Organization
QCM-D	Quartz Crystal Microbalance with Dissipation Monitoring
XPS	X-ray photoelectron spectroscopy

Program

8:30	Registration and coffee
9:30	**General introduction** **S. LARUELLE** (Federal Minister of Agriculture) **A. HUYGHEBAERT** (Chair Scientific Committee Belgian Food Safety Agency)
	SESSION 1 Chairs: J. Mast, K. Dewettinck
9:50	**Theme 1: Definitions of nanotechnology - Terminology & classification**
	Items to be addressed: • Introduction to nanotechnology • Classification, terminology & nomenclature • Physico-chemical characteristics • Analysis & detection (issues regarding reference material, matrix interference, size, novel laboratory practices, …) Main questions to be answered: • What are the characteristics of nanoparticles, nanofibres, nanostructures, etc.? • What is a good 'working' definition for a transparent discussion between stakeholders? • How to analyse/detect engineered nanoparticles in food ? What are the hurdles? **J. BRIDGES** (Chair SCENHIR) *Introduction to nanotechnologies*
10:20	**Theme 2: Applications of nanotechnology in the food chain (part 1)**
	Items to be addressed: • General information regarding R&D, the market situation • Applications and latest developments (smart packages, precision farming, interactive foods, nanodelivery systems, biofortification, etc.) • Nanoparticles naturally occurring in food • Industry's point of view (cases) Main questions to be answered: • What are the (potential) applications of nanotechnology in the food chain?

	• What are the potential/commercial available and future applications? • What is the impact?
10:20	**Q. CHAUDRY** (Fera, UK) *Food applications of nanotechnologies – An overview of potential benefits and risks*
10:45	**J. LAMMERTYN** (K.U.Leuven, Belgium) *Case 1: Nanotechnology in food diagnostics*
11:05	Coffee break
11:25	**Theme 2: Applications of nanotechnology in the food chain (part 2)**
11:25	**J. LAGARON** (CSIC, Spain) *Case 2: Nanotechnology trends to enhance biopackaged food, food quality and safety*
11:45	**M. KNOWLES** (Chair CIAA Nanotechnology expert group) *Case 3: Nanotechnology: a challenge for the food and drink manufacturing industry*
12:05	**Questions and answers about themes 1 & 2**
12:20	Lunch and poster exhibition
	SESSION 2 Chairs: G. Maghuin-Rogister, L. Pussemier
14:00	**Theme 3: Toxicological aspects of nanotechnology in the food chain**
	Items to be addressed: • Toxicological properties (acute/chronic toxicity, genotoxicity, …) • Toxicokinetics & -dynamics • Toxicity at the level of cells, animal tissue, etc. (*in silico - in vitro – in vivo*) Main questions to be answered: • How to determine the toxicity of nanoparticles? • What are the uncertainties regarding the toxicity of nanoparticles (e.g. metric dose)? • What is known about the oral toxicity of nanoparticles (absorption, bio-availability, intestinal toxicity, ...)? • What are the health & safety issues of nanoparticles in food?

14:00	**Y.-J. SCHNEIDER** (UCL, Belgium) *Toxicodynamic aspects of nanoparticles in food: interactions with the intestinal barrier*
14:20	**H. BOUWMEESTER** (WUR, The Netherlands) *Microarray analysis of effects of silver nanoparticles on an in vitro translocation model of the human intestinal epithelium*
14:40	**Theme 4: Risk assessment - EFSA's point of view**
	Item to be addressed: Risk assessment of the use of nanotechnology in the food chain. Main questions to be answered: • What are the health & safety issues of nanoparticles in food? • Which risk assessment issues need to be addressed for nanotechnology in food? • Is there a prioritisation with respect to research needs? **C. L. GALLI** (Chair EFSA Nanotechnology WG) *The potential risks arising from nanoscience and nanotechnologies on food and feed safety*
15:05	**Questions and answers about themes 3 & 4**
15:20	Coffee break
	SESSION 3 Chairs : B. De Meulenaer, J.D. Piñeros-Garcet
15:40	**Theme 5: Communication, perception & participation of the consumer**
	Items to be addressed: • Consumer's perception & participation regarding nanotechnology and food • Communication regarding nanotechnology and food • Consumer's expectations (on product information, etc.) • Ethical considerations, social aspects Main questions to be answered: • What is the consumers' viewpoint on the promises, potential problems, and wider implications of nanofood for the individual and for society? • Where are the ethical borderlines?

	G. GASKELL (London School of Economics, UK)
16:05	**Theme 6: Regulatory aspects**
	Items to be addressed: • International – European – national level • Legislation regarding the food chain (REACH, labelling, etc.) • Regulatory challenges of nanotechnologies • International standards (?) • Official controls (?) Main questions to be answered: • Are nano-applications in the food chain covered by the current legislation? • What are the lacunas in legislation? • Is there a need for a specific "nano-legislation"? • Are there any barriers in legislation? • How to control nano-applications in the food chain? • Quid labelling? **E. POUDELET** (Director Directorate Safety of the Food Chain, EC DG Health & Consumer Protection) *Regulatory aspects of EU food legislation*
16:30	**Questions and answers about themes 5 & 6**
	GENERAL DISCUSSION & CONCLUSIONS Chairs: A. Huyghebaert, L. Pussemier
16:45	**Round-table discussion:** industry – risk assessor – risk manager – consumer

GENERAL INTRODUCTION

The annual scientific event, organized by the Scientific Committee (SciCom) of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) has a particular dimension as it takes place in the framework of the Belgian Presidency of the European Council. In addition, the symposium is organized in cooperation with the European Commission and the European Food Safety Agency.

As a scientific independent advisory body to the FASFC, the SciCom has as a major task to perform risk assessment studies. Already for several years, SciCom organizes, every year an event in order to discuss new developments, to identify new challenges and to reflect on its own activities.

The title “Nanotechnologies in the Food Chain” is indeed particularly promising in this respect. As a novel technology it offers a broad range of opportunities for innovation. On the other hand there are uncertainties with respect to the safety of some applications.
Nanotechnology is an exciting field but has an impact on the whole food chain. The approach chosen covers different aspects including applications in the food chain, toxicological aspects, risk assessment, regulatory issues, consumer perception and communication.
Consumers are quite reluctant to accept novel technologies in the food chain, especially if information on risk assessment is lacking. Taking into account experiences in other fields with new developments, there is a need for a transparent communication to interested parties in the whole food chain.

Networking is also an important objective of a scientific symposium. There is indeed ample opportunity to exchange views with colleagues with a variable background and from different horizons.
It is hoped that this symposium will contribute to our knowledge of the rapidly developing field of nanotechnology.

On behalf of the SciCom I want to thank:

- the executive officers of the FASFC for their continuous support of the activities of the SciCom,
- the EC and the EFSA for their contribution to the development of the programme,
- the programme committee, chaired by Dr. ir. L. Pussemier in a very efficient way,
- the staff members of het Scientific Secretariat of the SciCom for their invaluable assistance.

Prof. Em. dr. Ir. A. Huyghebaert
Chair of the Scientific Committee

THEME 1:

DEFINITIONS OF NANOTECHNOLOGY – TERMINOLOGY & CLASSIFICATION

Introduction to nanotechnologies

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Introduction

Products of nanotechnologies have the potential to bring benefits to the everyday life of EU citizens and to the environment. The field is expanding rapidly. Nanomaterial innovation has been anticipated to develop in four stages (Roco, 2006):

- passive nanostructures;
- active nanostructures;
- systems of nanosystems;
- molecular nanosystems.

Most applications so far can be regarded as first phase i.e. passive nanostructures. Applications already span many industrial sectors ranging from medicines, food and cosmetics to textiles, building materials and electronics. It has been estimated that globally 60% of nano based products are in the cosmetics and personal care product sectors with far fewer in the food sector (http://www.nanotechproject.org/inventories/consumer/). Indeed in the EU it is claimed that there are no nanotechnology based products to which consumers are exposed in either foods or cosmetics.

In view of the increasing widespread applications, exposure of humans and the environment to the products of nanotechnologies is likely to become both

frequent and extensive. It is therefore essential to examine the possibility that adverse effects to human health and/or the environment could arise from some outputs from the nanotechnologies and to identify the measures that should be put in place to minimise or prevent such impacts.

Definitions

The starting point must be to identify what is meant by the nanoscale. One widely used definition is a size range with a lower limit of approximately 1 and an upper limit of 100 nanometres. However, in respect of impacts on human health and/or the environment, there is no good scientific evidence in favour of either the lower or upper limit. However, the state of the science is insufficient to support a different definition It should be noted that around 1 nm, there is ambivalence between molecules, nanoclusters and nanoparticles. In general molecules ought to be excluded. However, exceptions need to be made for certain specific entities such as fullerenes, graphene and complex hybrid molecular structures. It is also the case that this definition does not capture aggregates and agglomerates of primary particles.

Based on their origin, three types of nanoscale materials (natural, by-products of human activity, engineered or manufactured) can be distinguished. Since the nanotechnologies are only concerned with the third category, further attention will be confined to this type. An engineered or manufactured 'nanomaterial' is a categorization of a material by the size of its constituting parts. It may be considered to include biological materials that are commonly used and processed and thus can be considered to be “engineered” or "manufactured", e.g. in the food and pharmaceuticals industry. Therefore, a modification of the definition might be necessary for regulatory purposes for sector uses such as food/feed and pharmaceuticals. Development of a more suitable definition depends on an understanding of the key physico-chemical and biological properties that influence the adverse effects of nanomaterials.

Relevant physico-chemical properties

Size

This aspect has not surprisingly had the greatest attention to date. There is sufficient evidence that reduction of size at the nanoscale results in changes in some properties of the material as a consequence for example of the increase in surface-to-volume ratio. These nano specific properties raise concerns on their potential for harm to humans and the environment. Based on the likelihood of exposure and uptake by biological organisms a particular focus of attention from a risk assessment view point is required for those nanomaterials that either exist as, or may be converted to nanoparticles (3 dimensions in the nanoscale) or nanofibres, nanorods or nanotubes (two dimensions in the nanoscale).

Surface properties and chemical reactivity

The chemical reactivity increases with increasing surface area. However, this property may or may not be associated with an increase in biological activity or toxicity. The design of nanomaterials often includes the application of coatings and other means of modifying surface properties.

Nanoparticles have the potential to generate free radicals and active oxygen. This is an important property in view of the favoured theory regarding the toxicity of nanoparticles that they mediate at least some of their effect through the generation of active oxygen.

Solubilisation and other changes

Like other particulate matter, nanomaterials can:

- be solubilised or degraded chemically;
- form agglomerates or stable dispersions depending on solvent chemistry and their surface coating;
- have the ability to react with proteins (Linse et al., 2007).

Behaviour in biological systems

It is too early in the development of the nanotechnologies to identify general rules that can confidently be applied to predicting the risk from individual products other than the focus of concern should nanomaterials that have two or three dimensions in the nanoscale. Consequently all aspects of the life cycle from the production phase to the waste treatment at the end of the life cycle of nanomaterial products need to be considered. Very few risk assessment studies

have so far been published. Therefore one has to view extrapolation of the findings summarised below with caution.

Exposure and toxicokinetic aspects

It appears that for some types of nanoparticles size may be a limiting factor for absorption across the intestinal wall whereas for others similar absorption occurs up to 500 nm. From studies using metal particles it appears that there is increasing distribution among body organs with diminishing particle size following oral administration to rodents. Inhalation studies indicate that there is also the potential for uptake across the lung. So far it has not been possible to identify the key characteristics of nanoparticles that influence the extent of uptake nor those that facilitate persistence. In some studies nanoparticles have been found in the brain and in the foetus after oral or air borne exposure.

Hazard aspects

It cannot be assumed that a nanomaterial will necessarily have different hazard properties compared to its constituents, nor is it the case that nanoparticles of comparable size will have similar toxicity. Rather some may be virtually not toxic while others are clearly toxic. Although most of the existing toxicological and ecotoxicological methods for hazard identification are likely to be appropriate, they may not be sufficient to address all the hazards of nanomaterials. A particular concern with some *in vitro* techniques for example is whether they are able to take up the nanoparticles.

It may be the case that the standard tests may need to be supplemented by additional tests, or replaced by modified tests, as it cannot be assumed that current scientific knowledge has elucidated all the potential adverse effects of nanoparticles.

Strategy to assess the risk from individual nanomaterials

In the absence of sufficient information to identify the risks from individual nanomaterials from their physico-chemical characteristics, the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has advised that each nanomaterial must be assessed on a case by case basis. The SCENIHR has proposed an exposure driven tiered approach.

Tier 1 → to identify whether the manufacture, use and/or end of use disposal or recycling could result in exposure of humans or environmental species and ecosystems. For nanomaterials where all the nanoparticles are bound permanently into a much larger three dimensional structures only standard toxicity tests need to be applied.

Tier 2 → to characterise the nature, level and duration of any exposure. Examination of the physicochemical properties can be used to assess whether solubilisation, aggregation or decomposition is likely to occur or whether adsorption of chemicals onto the surface is likely. Measurement or modelling may be used to estimate exposure.

Tier 3 → to identify the hazardous properties of any of the forms of the nanomaterial to which significant exposure is likely. As noted above, for many aspects of the hazard assessment the methodology used for chemicals may be used.

Tier 4 → to characterise the hazard and the risks. The primary need is to identify the dose-response relationship and the threshold level for any adverse effects.

It should be possible in the near future to move to a category based approach. An important barrier to progress is the problem of access to the relevant data by risk assessors.

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assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials.
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THEME 2:

APPLICATIONS OF NANOTECHNOLOGY IN THE FOOD CHAIN

Food applications of nanotechnologies – An overview of potential benefits and risks

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Introduction

This presentation is aimed at highlighting the current and projected applications of nanotechnologies for food and related sectors. Advancements in nanotechnologies are promising to bring a range of benefits to whole of the food chain, in terms of new processes, materials and applications for efficient food production, less use of agrochemicals; hygienic food processing; improved food tastes and textures; less use of fat, salt, and preservatives; improved absorption of nutrients and supplements; and innovative packaging concepts. The presentation will also discuss potential implications of the use of engineered nanomaterials in food products for consumer safety and how such developments are likely to be regulated.

Main applications and potential benefits

Recent advances in nanosciences and nanotechnologies have led to a lot of interest in the control and manipulation of material properties at the nano-scale. The new materials, products and applications derived from nanotechnologies are anticipated to bring lots of improvements to the food and related sectors,

impacting agriculture and food production, food processing, packaging, distribution, storage and developments of innovative products. A number of recent reports have identified the current and short-term projected applications of nanotechnologies for food and related sectors (Chaudhry et al., 2010; Chaudhry et al., 2008). The main driving principle behind these developments seems to be aimed at enhancing uptake and bioavailability of nano-sized nutrients and supplements, and improving taste, consistency, stability and texture of food products (Chaudhry et al., 2010; Chaudhry et al., 2008). A major area for current nanotechnology applications in the food sector is for food packaging. The new nanoparticle-polymer composites can offer a number of improvements in mechanical performance as well as certain functional properties, such as antimicrobial activity to protect the packaged foodstuffs. Food packaging applications of nanotechnologies are estimated to make up the largest share of the current and short-term predicted nano-food market (Cientifica, 2006).

Other main applications relate to health-food sector, where nano-sized supplements and nutraceuticals have been developed to enhance nutrition, and to improve health and well-being. Compared to this, most applications relating to the mainstream food and beverage areas are at the R&D stage, and only a few products are currently available. These applications include development of nano-structured (also termed as nano-textured) food materials. This relates to processing foodstuffs to develop nano-structures and stable emulsions to improve consistency, taste and texture attributes. Nano-textured foodstuffs can also enable a reduction in the use of fat. A typical product of this technology would be a nano-textured ice cream, mayonnaise or spread, which is low-fat but as “creamy” as the full-fat alternative. Such products would offer ‘healthy’ but still tasteful food products to the consumer. Examples include ongoing R&D in Taiwan (Hwang & Yeh, 2010) and Japan (Tsukamoto et al., 2010) on development of micronized starch, cellulose, wheat and rice flour, and a range of spices and herbs for herbal medicine and food applications.

Another area of application relates to the use of nano-sized additives in food products. The main claimed benefits include better dispersibility of water-insoluble additives (e.g. colours, flavours, preservatives, supplements) in food products without the use of additional fat or surfactants. This is also claimed to enhance taste and flavour due to the enlarged surface areas of the nano-sized

additives, and enhance absorption and bioavailability in the body compared with conventional bulk forms. Currently available examples include vitamins, antioxidants, colours, flavours, and preservatives. Also developed for use in food products are nano-sized carrier systems for nutrients and supplements. These are based on nanoencapsulation of the substances in the form of liposomes, micelles, or protein based carriers. These nano-carrier systems are used to mask the undesirable taste of certain additives and supplements, or to protect some them from degradation during processing. The nano-encapsulated nutrients and supplements are also claimed for enhanced bioavailability, antimicrobial activity, and other health benefits. An example application, currently under R&D, is that of a mayonnaise which is composed of an emulsion that contains nano-droplets of water inside. The mayonnaise would offer taste and texture attributes similar to the full fat equivalent, but with a substantial reduction in the fat intake of the consumer (Clegg et al., 2009).

Certain inorganic nano-sized additives are also finding applications in (health)food area. Example of these include transition metals (e.g. silver, iron), alkaline earth metals (e.g. calcium, magnesium), and non metals (e.g. selenium, silica). The use of inorganic nano-additives is claimed for enhanced tastes and flavours due to enlarged surface areas. An example is nano-salt, the use of which would give more salt particles on a product (e.g. chips/ crisps) and allow the consumer to taste the salt more when added at a lower level. Food supplements in this category are also claimed for enhanced absorption and improved bioavailability compared with conventional equivalents.

Food packaging is currently the major area of application of metal and metal-oxide nanomaterials. Example applications include plastic polymers with nano-clay additives for gas barrier, nano-silver and nano-zinc oxide for antimicrobial action, nano-titanium dioxide for UV protection, nano-titanium nitride for mechanical strength and as a processing aid, nano-silica for hydrophobic surface coating, etc.

Nano-silver is finding increasing applications as an antimicrobial, antiodorant, and a (proclaimed) health supplement. Although the current use of nano-silver relates mainly to health-food and packaging applications, its use as an additive in antibacterial wheat flour is the subject of a recent patent application (Park, 2006). Nano-silica is known to be used in food contact surfaces and food packaging applications, and some reports suggest its use in clearing of beers

and wines, and as a free flowing agent in powdered soups. The conventional bulk forms of silica and titanium dioxide are permitted food additives (SiO_2, E551, and TiO_2, E171). There are concerns that their conventional forms may contain a nano-sized fraction due to natural size range variation (EFSA, 2009). Nano-titanium dioxide is used in a number of consumer products (e.g. paints, coatings) and its use may extend to foodstuffs. For example, a patent (US Patent US5741505) describes nano-coatings applied directly on food surface to provide moisture or oxygen barrier and thus improve shelf life and/or the flavour impact of foods. The materials used for the nano-coatings, applied in a continuous process as a thin amorphous film of 50 nm or less, include titanium dioxide. The main intended applications described in the patent include confectionary products, although there is currently no known product or application which incorporates the technology.

Nano-selenium is being marketed as an additive to a tea product in China for a number of (proclaimed) health benefits. Surface functionalised nanomaterials that can add a certain functionality, are also being developed. Functionalised nanomaterials are currently mainly used in food packaging applications (e.g. organically-modified nanoclays), to bind with polymer matrix to offer mechanical strength or a barrier against movement of gases, volatile components (such as flavours) or moisture. As nanotechnologies converge with other technologies (e.g. biotechnology), the use of functionalised nanomaterials in food applications is also likely to grow in the future. Other applications nearing market include nano-coatings (e.g. of TiO_2) for photocatalytic sterilisation of surfaces and water, nano(bio)sensors for food safety, and nano-barcodes for food authenticity (Chaudhry et al., 2010). Water treatment, filtration, and desalination using nanotechnologies also offer a lot of benefits in terms of safe use/ re-use of water.

Potential risks

The main likely route of entry of micro- or nano-sized particles to the gut is through the consumption of food and drinks, although entry through clearance of lungs is also known to take place. At present, there are a number of knowledge gaps in regard to ADME (absorption, distribution, metabolism, excretion) properties and effects of nanomaterials. It is known that physicochemical

properties, behaviour, and interactions of nanomaterials may differ from bulk equivalents. In some cases, changes in physicochemical properties may also lead to a change in the effects on biological systems. Studies have indicated a deviating toxicity profile for some nanomaterials compared to their conventional equivalents (Nel et al. 2006; Donaldson et al. 2001). An important factor is the increased ability of free nanoparticles to penetrate biological barriers (Geiser et al., 2005; Oberdörster et al., 2004), which adds a new dimension to particulate toxicology. This can potentially enable free insoluble and biopersistent nanoparticles to reach new targets in the body, which are otherwise protected against the entry of larger particulates. For example, translocation from the gastrointestinal (GI) tract has been found to be greater for nanoparticles than the larger particles (des Rieux et al., 2006; Hoet et al., 2004; Hillyer & Albrecht, 2001; Desai et al., 1996). Following oral administration, the translocation and distribution of metal nanoparticles to different organs and tissues has been reported (Hillyer & Albrecht 2001; Kim et al., 2008). Studies have also shown that nanomaterials can interact with various entities, such as proteins (Šimon & Joner, 2008; Nemmar et al., 2002), and such interactions may alter their uptake and distribution in the body (Lynch & Dawson 2008; Cedervall et al., 2007; Dobrovolskaia, 2007; Michaelis et al., 2006).

An important point to consider in regard to potential risk is that nanomaterials are likely to undergo a variety of transformations in food/feed, and in the GI tract. For example they may agglomerate, react or bind with other components of food/feed, solubilise on reaction with stomach acid or digestive enzymes, or be excreted from the body. Due to such transformations, they may not be available in free particulate forms for translocation across the GI tract. Any risk will thus be dependent on whether nanomaterials added to food remain (or become) available in free and insoluble particulate forms in the GI tract. The presence of nano-structures, which are digested in the GI tract, should not raise any special safety concerns, and evaluation of foods containing natural nanostructures needs to focus on the digestibility and bioavailability aspects. Similarly, if food additives formulated in nano-carriers are released in the GI tract as a result of the digestion of the carrier system, their risks will not be any different from the conventional forms. If, on the other hand, a nano-carrier can remain intact and deliver a substance into the circulatory system, the tissue distribution of the encapsulated substances may be different from that of the

conventional equivalent. The main consumer safety concerns, however, relate to insoluble nanomaterials, that are unlikely to be assimilated in the GI tract and can be biopersistent in the body. The likelihood of translocation of nanoparticles of such materials to various cells and tissues in the body may lead to a risk to consumer health, such as oxidative damage and inflammatory reactions (Donaldson et al., 2004; Li et al., 2003; Oberdörster, 2000). Nanomaterials are also known to adsorb or bind different substances on their surfaces (Šimon & Joner, 2008), and may carry potentially harmful chemicals and foreign substances to unintended parts of the body. Certain metal(oxide) nanomaterials are known to have antimicrobial activity. There is, however, no published research at present on how their long-term intake via food and drinks might affect the gut natural microflora.

Any risk arising from nanotechnology-derived food packaging would be dependent on the migration behaviour of nanomaterials from the packaging. The results of the few migration studies so far (Bradley et al., 2010; EFSA, 2009) and modelling estimates (Šimon et al., 2008), suggest that any significant migration of nanomaterials from polymer packaging is unlikely. This provides some reassurance in the safety of nanotechnology-derived food contact materials, although more research is needed to assess the potential impacts on health and the environment.

Regulatory controls

The developments in nanotechnologies are not taking place in a regulatory vacuum, as the existing regulatory frameworks will require pre-market evaluation for nanotechnology-derived food products.

The EU's Food Law Regulation 178/2002 sets out the general principles and requirements of food law within the EU and provides for the establishment of the European Food Safety Authority (EFSA). Other cross-cutting horizontal regulations are e.g. the EU Directive 2001/95/EC of 3 December 2001 on General Product Safety (GPSD, in force since 14 January 2004, replacing Directive 92/59/EC). This legislation embodies the main principle that only safe products can be placed on the market. Due to its broad and horizontal scope, GPSD applies to risks that are not covered by other specific EU provisions on products. Thus it applies to products containing engineered nanomaterials, with

the onus of ensuring safety of such products resting with the person who places them on the market. The industrial scale production of nano-sized chemicals used in food packaging is covered under chemical legislation, such as the European regulation REACH (Registration, Evaluation, Authorisation of Chemicals - Regulation (EC) No 1907/2006, in effect from 1 June 2007), which requires registration of all substances that are produced and/ or marketed in the EU above 1 tonne/ year - as such, in preparations, or in articles.

Other pieces of legislation may specifically apply to some nanomaterials that may be used in food production/ protection, such as pesticides, biocides, and veterinary medicines. Environmental regulations are also likely to capture the use of engineered nanomaterials in food packaging, and agri-food production applications.

The main European regulatory controls governing the composition, properties and use of food contact materials or articles stem from Regulation (EC) 1935/ 2004. The principle underlying this Framework Regulation is that any material or article intended to come directly or indirectly into contact with food must be sufficiently inert to preclude substances from being transferred to the food in quantities large enough to endanger human health, or to bring about an unacceptable change in the composition of the food or a deterioration in its organoleptic properties. The Framework Regulation applies to all materials, including plastics, paper, metals, glass, ceramics, rubber, etc. Other relevant frameworks include Directive 2002/72/EC (as amended) on plastic materials and articles intended to come into contact with foodstuffs, and Directive 2005/31/EC amending Council Directive 84/500/EEC on ceramic articles intended to come into contact with foodstuffs.

The use of food additives in the EU is currently controlled by the 'Food Additives Framework Directive' and the subordinate legislation. Subject to adoption by the EC, the Food Additives Framework Directive will be replaced by a common authorisation system in 2010, which will provide for a common basis of controls on food additives (Regulation (EC) No. 1333/2008), food enzymes (Regulation (EC) No. 1332/2008), and food flavourings (Regulation (EC) No. 1334/2008). The adoption of the common authorisation procedure will also bring together all of the existing food additive regulations, and will introduce comitology for the approval of the three categories of substances. The most relevant aspect in relation to the use of nano-sized food additives in the new Regulation is the re-

evaluation of safety assessment, which will ensure that food additives, once permitted, are kept under continuous observation and re-evaluation.
The Novel Foods and Novel Food Ingredients Regulation (EC) 258/97 requires safety assessment of any food product, which does not have a significant history of use, or is produced using a new production process, which gives rise to significant changes in the composition, structure, or function. The legislation is being reviewed in Europe with specific reference to foods modified by new production processes, such as nanotechnology and nanoscience.

Conclusion

An overview of nanotechnology applications in the food and related sectors shows they offer a variety of benefits to the whole food chain – from new or improved tastes, textures, to a potential reduction in the dietary intake of fat and other food additives, enhanced absorption of nutrients, preservation of quality and freshness, and better traceability and security of food products. However, although predicted to grow rapidly in the future, most food related applications are currently at R&D stage. There are also a number of knowledge gaps in our current understanding of the properties, behaviour and effects of nanomaterials, which make it difficult to assess the potential risk to consumers. A careful consideration of the nature of materials and applications can, however, provide a basis for conceptual risk assessment. For example, products containing natural food nano-structures may not require as detailed evaluation as the products containing insoluble/biopersistent nanomaterials. The existence of stringent regulatory controls provides reassurance that only safe products derived from nanotechnologies will be permitted on the market. There is, however, a need for a case-by-case safety evaluation of nanotechnology-derived food products, as recommended in the recent EFSA opinion (EFSA, 2009), before they are placed on the market.

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Case 1: Nanotechnology in food diagnostics

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Introduction

Recent food crises have increased consumers' awareness with respect to food safety and quality. An important requirement to guarantee a high quality and safe food chain is the possibility to measure in a fast, reliable and cost-effective way. Most conventional analytical methods, applied in food chain analysis, display a high sensitivity and accuracy but are however expensive in use. Recent developments in nanotechnology and bionanotechnology allow the design of a novel class of analytical systems including biosensors and micro total analysis systems. They offer some interesting advantages such as a high selectivity and specificity, a low cost of production, a high degree of automation and the possibility to execute the analysis on a miniaturized scale. Because of these characteristics, an increasing number of devices have been reported for use in food diagnostics (Valdez et al., 2009). The medical sector has played a prominent and an indispensable role as driving force in the development of many of these new technologies. A lot of research effort is spent in the development of biosensors for monitoring blood glucose levels in diabetes patients. Later, this knowledge finds its way to other application fields like food and environmental diagnostics.

In this paper we will shortly introduce some aspects of biosensor technology with respect to its potential in food diagnostics. We first elaborate on the basic principles of biosensors. Next we introduce the concept of lab-on-a-chip technology and discuss how nanotechnology opens up possibilities to design sensors with improved sensitivity. In a concluding paragraph, we give an example of an optical biosensor for the detection of peanut allergens in food.

Basic principles of a biosensor

The development of biosensors is a very multidisciplinary research field. It requires the integration of disciplines like biochemistry, microelectronics, biology, surface chemistry and physics at the micro- and nanoscale. In general, a sensor can be defined as a system that generates a specific electronic signal as a result of an external stimulus, allowing the quantification of certain physical (temperature, pressure, mass,…) or chemical (pH, O_2,…) properties. Sensors where biological components such as proteins, oligonucleotides, cells and tissue are included in the system and used for the generation of a specific signal towards the target component, are denoted as biosensors. A schematic representation of the working principle of a biosensor is depicted in Figure 1. A specific biosensor is characterized by three main aspects: (i) biological recognition element, (ii) transducer system, and (iii) integration of this recognition element with the transducer system.

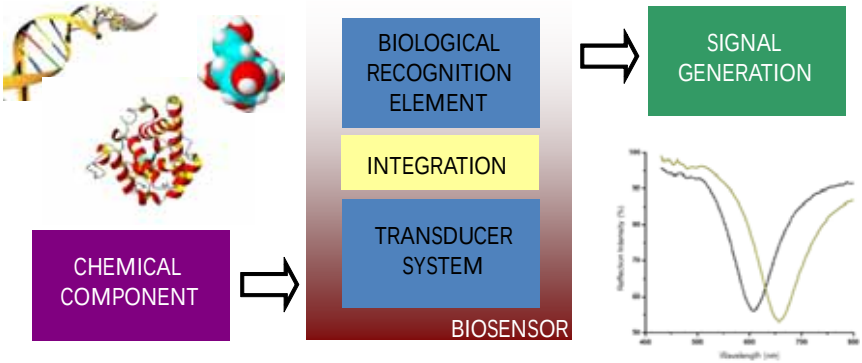


Figure 1. Schematic representation of the working principle of a biosensor. A (bio)chemical component (DNA/RNA strands, proteins, low molecular weight compounds,…) reacts with a biosensor, composed of three essential elements: (i) a biological recognition molecule, specific for the target molecule, (ii) a transducer system, and (iii) the integration of the biorecognition molecule with the transducer system.

The accuracy of a biosensor depends on the specificity and the selectivity of the biorecognition molecule in relation to the target. The biosensor is mostly restricted to the quantification of one specific (or one specific class of) target(s).

There is a broad spectrum of biological recognition molecules available to act as capturing agent for the target molecule. Enzymatic biosensors generate a signal by means of an enzymatic conversion of the target molecule into an optically or electrochemically detectable component. Examples include the detection of pesticides and herbicides in fruits and vegetables and drinking water (Mello et al., 2002). Antibodies are commonly used for the detection of antigens in the so-called immunoassays. These assays are reported highly performant for the detection of toxins and pathogens in food. DNA and RNA bioreceptors are used for the quantification of complementary DNA or RNA strands (e.g. pathogen detection in food). In the last few years, the possibility to use cell structures and tissue as specific recognition molecules, has been investigated for the detection of toxic compounds in food samples (Mello et al., 2002).

Aptamers are a recently new and promising class of biorecognition molecules. Aptamers are oligonucleotides which can be designed with a receptor function for a multitude of target molecules. Selection of these molecules happens through an iterative *in vitro* selection procedure. Aptamers are considered mainly as an interesting alternative to antibodies in immunoassays. Compared to antibodies, aptamers are very stable and can be produced in large quantities with very reproducible characteristics. In addition, they can easily be chemically modified to improve the bioreceptor performance in food samples.

A crucial point in the design of a biosensor is the integration of the bioreceptor with the transduction system. This happens through surface chemistry. Nanotechnology has contributed substantially in creating and characterising bioreceptor layers. The choice of the immobilisation strategy is crucial to create a stable and sensitive biosensor surface that avoids aspecific binding of unwanted food components. The latter results in false positive results. Different immobilisation strategies and surface characterisation methods have been described in the literature ranging from covalent linkage, cross-linking, adsorption, adsorption-cross-linking or encapsulation. Depending on the requested performance of the biosensor one of these methods is selected.

As mentioned above, a transducer translates the interaction between a bioreceptor and its target into an electric signal. A multitude of bioreceptors has been described in the literature. In general, differentiation is made between three different groups of transducer systems: electrochemical, piezo-electrical and optical systems. The electrochemical transducer systems, based on

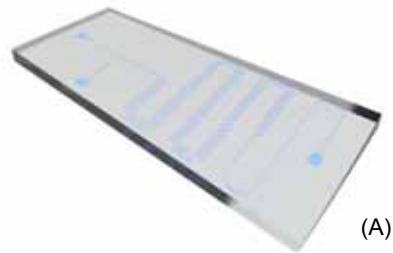
potentiometry, amperometry and conductometry are very popular. They are easy to miniaturize and are (relatively) cheap. Piezo-electric systems react quickly and are conceptually simple: with the capturing of the target molecule by the bioreceptor, the resonance frequency of the biosensor is changed according to the amount of captured molecules. Piezo-electric systems are considered as very sensitive systems. Advances in the field of nanotechnology allow the miniaturization of optical components, such as optical waveguides, light sources, and detector systems. As a consequence, optical transducer systems gain importance in biosensor design (Ligler, 2009). A very sensitive technology for the monitoring of the biomolecular interactions is based on the principle of 'surface plasmon resonance' (SPR).

Lab-on-a-chip technology

An important trend in biosensor technology is the integration of the aforementioned biosensor concepts into miniaturized analytical devices or 'lab-on-a-chip' systems. This term refers to the implementation, miniaturization and the automation of laboratory operations on a portable microchip, typically in the range of millimetre to centimetres. In these chips, small amounts of liquids (µL or nL) are transported, mixed, and separated at the microscale. These microscale operations allow the development of fast and sensitive diagnostic assays in medical as well as in food diagnostics. The way the elementary fluid manipulations are executed on the chip, differentiates 'lab-on-a-chip' systems in two groups, namely continuous flow (figure 2A) and digital or droplet based (figure 2B) systems.

In *continuous flow systems*, fluid is pumped through the micro- or nanochannels by external or internal micropumps. With the aid of microvalves and –mixers, fluids are mixed to initiate the reaction or improve the binding of the target to the bioreceptor. In some applications, transport is achieved by means of electrokinetics, eliminating the need for moving parts such as valves. In *digital or droplet based systems*, liquids are not transported as continuous flows but as individual droplets in a microchannel or even on a two dimensional surface by means of the electrowetting-on-dielectric principle. Both systems have their specific advantages and disadvantages but depending on the application one of both systems is recommended. The main advantage of lab-on-a-chip technology

is the degree of miniaturization, resulting in a strong improvement in analytical performance, cost reduction and high-throughput sampling. This makes lab-on-a-chip a very attractive technology to substitute/complement conventional analytical techniques used in food diagnostics.


(A)

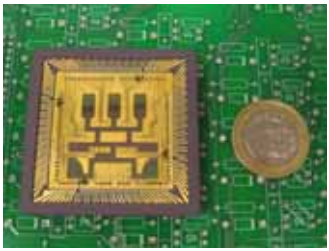

(B)

Figure 2. Typical examples of the two different lab-on-a-chip systems. Figure A represents a continuous system where fluid is pumped continuously through the microchannels and Figure (B) represents a digital system where droplets are transported on a matrix of individual electrodes.

Nanoparticles in biosensors

Materials with nanoscale dimensions (<100 nm) exhibit special magnetic, mechanical, electrical and optical characteristics, which make them interesting for use in biosensor development. With respect to their composition most nanoparticles used in biosensor design consist of noble metals such as gold, silver and platinum. For example, gold nanoparticles have a strong absorbance in the ultraviolet and visible light wavelength range. However, also polystyrene and silica nanoparticles, quantum dots, carbon nanotubes, branched nanoparticle, dendrimers, and nanobarcodes have proven their value in sensor development (Gomez-Hens et al., 2008).

With respect to their specific function in biosensors, nanoparticles can be broadly classified as quantitation tags, signal transducer systems, functional substrates or as functional tags (although there exists quite some overlap between the different classes). Quantitation tags are used for signal enhancement or for the visualization of specific (sub)cellullar structures. The use of nanoparticles as quantitation tags has the advantage that no extra labelling reagents, such as fluorophores or radioactive components, are necessary.

Besides the quantitation, the particles can also serve as initiators of the signal transduction mechanism. In this function, changes of the relative location of the nanoparticles (e.g. aggregation of the particles as a result of the biomolecular interactions) lead to a change in the measurable signal. Nanoparticles also act as functional substrates. With the integration of different nanoparticles in one assay, multiplexing becomes possible allowing the analysis of more than one specific component. In this function additional fluorophores are needed to visualize the outcome of the reaction.

A special and interesting subclass of nanoparticles are the magnetic nanoparticles, consisting of a metal or metallic oxide core, coated by a protecting shell layer. This layer is necessary to stabilize the particles but also serves as a biocompatible layer where the different biorecognition molecules can be efficiently immobilized. Magnetic particles are mainly used for separation and preconcentration purposes, although hybrid magnetic particles, combining sample manipulation and sensing properties are considered as very promising materials.

Biosensor applications in the food industry

As an example of an innovative biosensor technology, an optical biosensor for peanut allergen detection is presented. Studies of the World Allergy Organization indicate that approximately 5 to 6% of the total world population exhibit allergic reactions after intake of food. Clinical symptoms vary from mild to fatal reactions and also the sensitivity of an individual person is strongly variable. The only way to prevent allergic reactions is to avoid the intake of allergen contaminated food. As such, reliable product information is essential to protect those people. The detection of specific allergens is not straightforward because they are only present in limited amounts and they are masked by the complex food matrix. Because a good reputation is of vital importance in the food industry, a lot of companies are interested in sensitive and reliable detection technologies for food monitoring and accurate labelling purposes. Therefore an innovative optical biosensor combining antibody/aptamer technology with surface plasmon resonance technology, was developed at the MeBioS Biosensor group (K.U.Leuven) for the detection of peanut allergens in food (Pollet et al., 2009). A schematic representation of this biosensor is shown

in Figure 3. The biosensor couples the advantages of optical fibre technology to the use of aptamers as specific biorecognition elements. This biosensor allows fast, accurate and label-free screening of different food products with respect to the presence of food allergens.

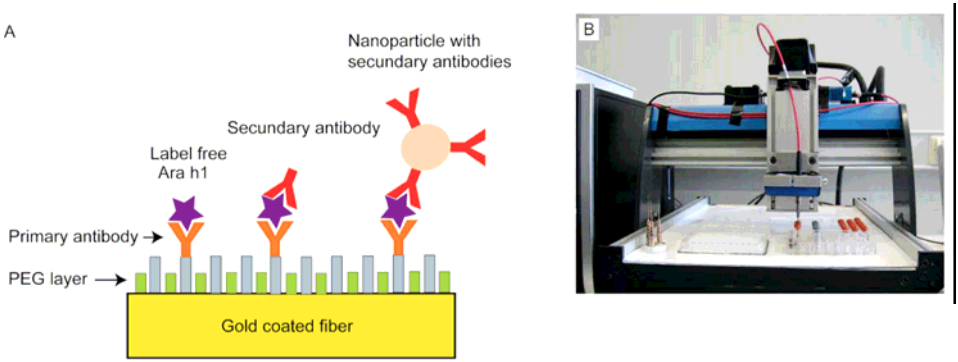


Figure 3. Schematic representation of the working principle of the fiber optic SPR sensor (A). The bioreceptormolecules are immobilized on the nanoplasmonic sensormodule, built around an optical silicafiber, coated with a thin (50 nm) gold layer. When allergens bind to the bioreceptors at the surface, the refraction index changes which is monitored by an optical detector. Nanoparticles are used for signal enhancement. Experimental set-up (B).

Conclusion

As a conclusion, we can state that recent developments in nanotechnology and bio-nanotechnology boost the development of new and innovative biosensor platforms, which can be applied for the quantification of a broad range of chemical components related to food quality and safety. The challenge now is to transfer the devices, from the research lab to the real world. Hereto, the integration of the biosensor concept in the so-called 'lab-on-a-chip' systems can be the first step to facilitate this transfer. The integration requires an interdisciplinary approach with expertise from nanotechnology, material physics, surface chemistry, microfluidics, molecular biochemistry and bio-engineering sciences.

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Case 2: Nanotechnology trends to enhance biopackaged food, food quality and safety

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In this presentation, the current situation of novel nanobiostructured packaging materials is described, together with the global challenges to be faced and the possible strategies to overcome some of the pending issues in this exciting and potentially world changing research and development.

Introduction

In the last decades there has been a significant increase in the amount of plastics being used in various sectors, particularly in food packaging applications. In fact, the largest application for plastics today is packaging, and within the packaging niche, food packaging amounts as the largest plastics demanding application. This is so, because plastics bring in enormous advantages, such as thermosealability, flexibility in thermal and mechanical properties, and they permit integrated processes, lightness and a low price. However, polymers do also have a number of limitations for certain applications when compared with more traditional materials like metals and alloys or ceramics. The chief limitation being their inherent permissiveness to the transport of low molecular weight components, which leads to issues such as food oxidation by penetration of oxygen, migration of toxic elements from the plastic and scalping of food components on the packaging with the consequent losses in food quality and safety attributes. From these, the potential migration of polymer constituents and additives is perhaps the most widely recognized issue regarding packaged food safety. In spite of this, plastic materials continue to expand and replace the conventional use of paperboard, tinplate cans and glass, which have been typically used as monolayer systems in food contact materials. Initially, most plastic packaging were made of monolayer rigid or

flexible materials but, as the advantages of plastic packaging became more established and developed, the increasingly demanding product requirements found when plastics had to suit more and more food products led, in conjunction with significant advances in plastic processing technologies, to more and more complex polymeric packaging formulations. This resulted in complex multicomponent structures such as the so-called multilayer packaging-based systems widely used today, which in many cases can make use of metalized layers. Still, there are significant advantages in terms of costs, ecopackaging strategies and other issues such as ease of recycling in developing simpler, more environmentally friendly packaging formulations. As a result, strong efforts in material development and in blending strategies have been carried out over the last decades to reduce complexity in food packaging structures while tailoring performance.

In addition, the substantial increase in the use of plastics has also raised a number of environmental concerns from a waste management point of view. As a result, there has been a strong research interest, pushed by authorities at national and international levels, and a concomitant industrial growing activity in the development and use of biodegradable and/or biobased materials. The term “biodegradable” refers to materials that can disintegrate and biodegrade through processes such as composting into mostly carbon dioxide and water, hence reducing plastic waste. “Biobased” sustainable materials on the other hand, apart from being typically biodegradable albeit not necessarily, consume carbon dioxide during their production, hence creating the potential for the new concept of “carbon neutral materials”

Amongst biobased materials, three families are usually considered: polymers directly extracted from biomass, such as the polysaccharides chitosan, starch, carrageenan and cellulose; proteins such as gluten, soy and zein; and various lipids. A second family makes use of biomass-derived monomers but uses classical chemical synthetic routes to obtain the final biodegradable and/or renewable polymers, including thermoplastics and thermosets. In regard to thermoplastics, this is the case of polylactic acid (PLA) and the non-biodegradable sugar cane ethanol-derived biopolyethylene. The third family makes use of polymers produced by natural or genetically modified micro-organisms such as polyhydroxyalcanoates (PHA) and polypeptides.

Amongst non-biobased materials, i.e. using either petroleum-based monomers or mixtures of biobased- and petroleum-based monomers, there are also a number of biodegradable resins such as polycaprolactones (PCL), polyvinyl-alcohol (PVOH) and its copolymers with ethylene (EVOH) and some biopolyesters. Nevertheless, it seems clear that although biodegradability can help reduce plastic waste, from a “green house” perspective, biobased sustainable materials, the so-called bioplastics, are currently considered the way to go and may be the only alternative in the future as fossil resources become exhausted.

Furthermore, in order to reduce both energy consumption during the production of bioplastics and potential competition with agricultural resources for foods, and to provide additional raw material sources, the valorisation of food by-products is also the current trend. Food processing effluents or solid wastes are only partially valorised and are mostly disposed in landfill sites where, since they are amenable to putrefaction, they have to be treated according to the restrictions identified by, for instance, the international Landfill Directive. These by-products are rarely and mostly in recent years being used as a source of high added value components such as food ingredients, but they present great potential value for their use in the production of bioplastics. Our research group is involved in an ambitious FrameWork-7 European Union funded collaborative project, with acronym ECOBIOCAP (“eco-efficient biodegradable composite advanced packaging”), devoted to this very relevant area of research.

In spite of the significant potential of bioplastics to substitute petroleum-based materials to help reduce environmental impacts, these materials still present a number of property and processing shortages that prevent their use in many applications, particularly in the food packaging field. The reasons for this are generally related to their lower barrier properties to gases and vapours, their strong water sensitivity, lower thermal resistance, lower shelf-life stability due to aging, migration and a number of processability issues still associated to the use of bioplastics. In this context, nanotechnology brings in significant opportunities to minimize the latter drawbacks.

Nanotechnology for packaging applications

Nanotechnology is, by definition, the creation and subsequent utilization of structures with at least one dimension in the nanometre length scale that creates novel properties and phenomena otherwise not displayed by either isolated molecules or bulk materials. Since Toyota researchers in the late 1980s found that mechanical, thermal and barrier properties of nylon-nanoclay composite material improved dramatically by reinforcing with less than 5% of nanoclay, extensive research work has been performed in the study of nanocomposites for food packaging applications. The term nanocomposite refers to composite materials containing typically low additions of some kind of nanoparticles. Specifically in the food biopackaging sector, nanocomposites usually refer to materials containing nanofillers, typically 1 to 7 wt.-%. For reinforcing purposes, a good nanofiller-matrix interaction is highly desired, which is often one of the major challenges faced when developing new nanocomposite materials. It has been observed that the interactions matrix-filler significantly improve when reducing the size of the reinforcing agent. Macroscopic reinforcing components usually contain defects, which become less important as the particles of the reinforcing component are smaller. Therefore, shifting from micro- to nanosized particles incorporated into the polymeric matrices leads to better performance of the composite materials.

Current nanotechnologies of value in the food packaging area are nanoclays, cellulosic nanomaterials, electrospun nanofibers and nanocapsules, carbon based nanomaterials, nanoparticles of metal and metal oxides, and nanoparticle containing carriers. These nanomaterials are used as an efficient gas, UV light and vapour barrier, to enhance mechanical and thermal properties, to reduce migration issues, and to provide controlled release, and active or bioactive functionalities to packaging.

The high surface-to-volume ratio of many nanoscale structures which favours this improved performance of packaging materials, also becomes ideal for applications that involve chemical reactions, drug delivery, controlled and immediate release of substances in active an functional food packaging technologies and energy storage in, for instance, intelligent food packaging.

Risk assessment of migration

Regarding inherent nanoparticle hazard assessment, due to their small size, nanoparticles are generally much more reactive than their corresponding macro-counterparts. On the other hand, as a result of this, much smaller filler loadings are required, and hence added to the matrix, to achieve the desired properties. The large surface area of nanoparticles allows a greater contact with cellular membranes, as well as greater capacity for absorption and migration. Therefore, assessment of the effects of nanoparticles in food packaging materials such as migration to foods and potential bioaccumulation, needs to be considered in the expected dosages. Currently, data on toxicity and oral exposure of nanoparticles are extremely limited and controversial when it comes to the studied dosages. In addition, the small size of many nanoparticles causes them to take on unique chemical and physical properties that are different from their macroscale chemical counterparts. This implies that their toxicokinetic and toxicity profiles cannot be extrapolated from data on their equivalent non-nanoforms. Thus, the risk assessment of nanoparticles should be performed on a case-by-case basis. However, it is also very important to differentiate between three-dimensional nanoparticles (spherical or otherwise 3D nanoparticles such as nanometals), bi-dimensional nanoparticles (nanofibers, with only nanodimensions in the 2D cross-section) and the least concerned, one dimensional nanoparticles (nanoclays with only one nanodimension in the thickness direction).

Nanoclays should be considered aside because in essence they are heat stable microparticles, which remain such all along the process of production and commercialization and to a significant extent also as two-dimensional microparticles within the biopolymer matrix during service. In any case, the general risk assessment of migration products resulting from packaging materials has posed and continues to pose a difficult challenge. As a general rule, nanocomposites within the European Union must currently comply with the EFSA total migration limit of 10 mg/dm^2, with the functional barrier stringent migration level of 0.01 mg/kg of food or food simulant and/or with the specific migration levels for their constituents in case they comprise food contact components (Commission Directive 2007/19/CE that modifies Directive 2002/72/CE). The existing information about actual migration to food or food simulants is very scarce but suggests that no specific relevant issues are to be

expected with nanoclays in food contact. Nevertheless, more research is needed in this area, not only investigating the migration and potential toxicity of nanoclays, but more importantly also of other nanoparticles used in food packaging structures.

Conclusions

It is envisaged that the potential strategies to overcome the above and other pending issues will come from focussing the research efforts and political strategies on the following items:

- Boosting the creation of nanotechnology industry-based platforms with solid knowledge of the problems to solve and of the legislation and commercialization barriers ahead. Open innovation and collaborative action towards more rapid product development will strongly benefit the technology. Development and commercialization of commodity products are a must. Thus, nanotechnology will only contribute to widespread the use of bioplastics through the balancing of their properties if they become a commodity in terms of pricing and volumes;
- Focussing R&D efforts in order to provide real value for nanobiocomposites, i.e. developing the underpinning science and technology to understand and control the composition/properties/ processing/aging relationship of nanobiocomposites;
- Developing new bioplastics and tailor-made reinforcing nanobioadditives that make use of only biobased products and resources, particularly derived from valorisation of food by-products;
- Establishing clear and knowledge-based legislation worldwide that defines nanoproducts and enables a clear assessment of the liability of existing ones in the various application fields and that provides concise guidelines for the clearance route of new developments. It might be that there is no need to change legislation to accommodate many existing nanomaterials and, therefore, it is all related to complying with the current global legislation for most of these. But then this has to be clearly stated to industries and society to boost implementation. According to the FDA, products on a case-by-case scenario and not technologies have to be regulated, and perhaps this should be the right approach;

- Deepening our understanding regarding the life cycle analysis of nanobiocomposites;
- Deepening our understanding about the potential toxicity of nanomaterials (current and under development) and of their nanobiocomposites. This should be carried out through the characterization of the stability of nanobiocomposites during processing and shelf-life, full migration studies and assessment of issues related to the various disposal channels.

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Case 3: Nanotechnology: a challenge for the food and drink manufacturing industry

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Introduction

The Food Industry has pragmatically and responsibly adopted technological advances in its past allowing for modernisation and growth . Driven by needs to deliver real consumer benefits, it has always scrutinised the potential of new and emerging technologies, always cognisant of their safety-in-use and consumer acceptance. Technological applications from nanotechnologies are being treated in the same pragmatic way. Their potential to deliver novel and innovative benefits for our consumers has to be carefully examined against the requirements for safe use and technological applicability. As a consequence, the food industry is leading its own stakeholder dialogues and actively participating in those organised by others.

Nanotechnology applications could bring a range of benefits to the food sector, including new tastes, textures and sensations, less use of fat, enhanced absorption of nutrients, improved packaging, traceability and security of food products. Nanotechnology-derived food products are set to grow worldwide and it is debated that a variety of food ingredients and food contact materials is already available in some countries. It has been suggested that a number of companies are currently applying nanotechnologies to food. A Friends of the

[2] CIAA – the Confederation of food and drink industries of the EU – represents the food and drink manufacturing industry, the largest manufacturing sector, major employer and exporter in the EU. The European food and drink industry reported a €965 billion turnover in 2008 and directly employs 4.4 million people. Our members are major food producers, federations and sector associations that represent small and medium sized businesses as well as large companies. As such, there are 310,000 companies in what is a fragmented industry given that 99% of those companies are SMEs. The CIAA membership is made up of 26 national federations, including 3 observers, 26 European sector associations and 20 major food and drink companies.

Earth Report, published in 2008, claimed that this number could be as high as 400-500 products worldwide (FOE, 2008).
In reality, the current level of applications in the European food chain is at an elementary stage. There is a need to distinguish between those applications that exist and those that are commercialised, particularly in the EU and the US. Some examples of existing applications are antibacterials in packaging, such as plastic food containers for domestic use, and supplements like nano silver and nano co Q10.

It is expected products will be increasingly available in the coming years.

Nanoparticles naturally occurring in food

While the common use of the term 'nanotechnology' may be new, food is naturally and traditionally made up of nanometre scale particles and humans have been exposed to nanometre scale particles since their existence.
Food and drinking water naturally comprises particles in the nanometre scale. Humans inhale and ingest many millions of organic and inorganic nanoscale particles every day in their food and drinking water and it is estimated that people inhale around 10 million nanometre scale particles in every breath.
Many traditional foods contain naturally occurring nanoparticles, such as protein structures, such as in the case in dairy products.

CIAA and Emerging Technologies

The application of nanotechnologies in the food industry itself is at an early stage. However, the Food industry supports the contribution nanotechnologies will bring to food products in order to confer consumer benefits, including:

- improving nutritional quality of foods,
- a longer shelf-life of fresh and processed products bringing better quality at end of shelf-life,
- and a better knowledge of storage history and potential safety issues (sensors).

With this in mind, it is likely that packaging applications will come first. Nanotechnology will contribute to the development of stronger, lighter and less wasteful packaging. Other potential benefits include:

- food safety improvements through the use of anti-microbial surface cleansers,
- a greater range of 'Healthier option' food choices, and
- better quality food by the improvement of flavour, texture, and appearance.

In looking at nanotechnology applications, it is important to highlight the principle requirement for prior regulatory approval, without which no applications can be placed on the market. This requirement is to be based on a detailed set of guidelines for safety evaluation, and is expected to be developed by the EFSA.

Clearly, innovation represents the key challenge for tomorrow for Europe's food and drink industry. Innovation provides a window of opportunity, enabling the food and drink industry to move forward. It is central to meeting the major economic, social and environmental concerns of our time, making the industry more competitive. However, the industry in Europe currently spends only 0.37% of its expenditure on R&D, which is wholly insufficient.
As an innovative and progressive industry, the food sector is interested in science-based research and developments, including the application of nanotechnologies. CIAA members, together with other stakeholders and academia, are therefore actively supporting and carrying out research in this area. The food industry is actively involved in the European Technology Platform Food4Life, which is run under the auspices of the CIAA (http://ec.europa.eu/research/biosociety/food_quality/projects/171_en.html).

In touching upon the benefits of nanotechnology in terms of R&D, one can foresee improved delivery systems of functional ingredients and movement from micro-encapsulation to nano-encapsulation. Nanotechnology will bring great benefits in the field of process engineering, enhancing surface coating and reducing bio-film development as well as nanofilters for water purification.

Potential Applications

Food belongs to one of Europe's highest regulated areas and nanotechnology could be used in various applications, directly or indirectly linked, but to the benefit of food production and to consumers. This should be done on a case-by-case basis and risk assessment might need to be adapted to address specific nanotechnology-linked questions.

The Food4Life Strategic Research Agenda, submitted under the European Technology Platform, indicates potential uses of nanotechnology that could be of interest to the food industry in the years to come. From 2015–2020 we could envisage research into:

- tailor-made food products, with a particular focus on the relationship between physical/chemical properties and structure,
- improving process and packaging design as well as process control, and
- improving understanding of process-structure-property relationships.

Further potential application for nanotechnology in the food chain could include application in the food contact material area, for example:

- addition of nano-clays to "traditional" polymers (= nanocomposites), e.g. montmorillonite for the enhancement of gas barrier properties,
- addition of nanoparticles to coatings for antimicrobial, corrosion resistant surfaces,
- nanostructured coatings for the enhancement of barrier properties,
- intelligent packaging : nanosensors, labels, …

Challenges Ahead

But Nanotechnology is not without its challenges.

Firstly, there is a need for greater legal certainty and clear definitions. Several definitions/characterisations have been adopted for various purposes but, for obvious reasons, framing a definition for food is more difficult.

Food is naturally nano-structured, so too broad a definition ends up encompassing much of modern food science, and even some aspects of traditional food processing. So, how can we make a distinction?

A distinction can be made between engineered nanomaterials and naturally self-assembled nanostructures. Engineered nanomaterials are covalently bonded,

and thus are persistent and generally rather robust, though they may have important surface properties such as catalysis, and they may be prone to aggregate. Examples of engineered nano materials include titanium dioxide nanoparticles.

Self-assembled nanostructures occur where the molecules are held together by weak forces, such as hydrogen bonds and hydrophobic interactions. The weakness of these forces renders them mutable and transient. Examples include soap micelles, protein aggregates (for example, the case in micelles formed in milk).

A second challenge in terms of nanotechnology focuses on some knowledge gaps in the biological properties of these materials. There is a need for clear EFSA Guidelines on how to conduct safety evaluations in order to address such questions as necessary.

Further challenges include the life-cycle analysis where there is an incomplete knowledge of the fate of nanomaterials in the environment and innovation bottlenecks due to the existing regulatory system.

Based on these problems, there is a need for independent sources of information and education in addition to science-based decision-making. Multi-stakeholder dialogues could also go a long way in helping to address these challenges.

Conclusion

In conclusion, it is evident that the technology has huge potential, but presents huge challenges also.

Ensuring the safety of products is of utmost importance. Adequate safety assessments on a case-by-case basis are needed. If the use of nano gives rise to changes in existing products or processes, the forthcoming EFSA guidance for nanotechnology risk assessments will be invaluable in bringing the EU risk assessment procedure fully up to date.

There is a continued need for openness, transparency and consumer engagement in the development of nanotechnology, though there may be limits to the availability of specific information due to confidential business information, intellectual property rights (IPR) or other legal restrictions. In addition, open

dialogues with different stakeholders are important and the CIAA has so far organised three stakeholder meetings.

The EU plays a key role both in furthering innovation and commercial development in this field and in setting a common regulatory framework that gives the required safety assurances to consumers and stakeholders.

The definition of nanotechnology should distinguish between the natural occurrence of nanoparticles, their presence through conventional processing techniques, and instances where the particle size has been deliberately engineered to behave differently to its conventional counterpart. A workable EU-level definition will be an important step forward.

Moreover, the benefits, uncertainties and actions being taken to address uncertainties about nano should be made clear to consumers.

In the food sector, potential and actual nanotechnology applications may reduce the environmental impact of food packaging and may improve food safety. However, cost to food manufacturers and perceived consumer benefits are central to the uptake of potential applications.

A better understanding of public attitudes towards nanotechnology and other new food technologies is needed - both to determine which applications will be acceptable and to communicate effectively with consumers and other key stakeholders about these applications' potential benefits.

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THEME 3:

TOXICOLOGICAL ASPECTS OF NANOTECHNOLOGY IN THE FOOD CHAIN

Toxicodynamic aspects of nanoparticles in food: interactions with the intestinal barrier

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Introduction

Nanomaterials are not yet officially defined, but widely described as discrete entities in the order of 100 nm or less (Bouwmeester et al., 2009; SCENIHR, 2007; Kreyling et al., 2006) in one (e.g. films), two (e.g. fibers and tubes) or three (e.g. particles) dimensions.

Nanoparticles (NPs) are used in a wide range of applications in science, technology and medicine. For instance, our group has shown their interest in drug and mucosal vaccine delivery (des Rieux et al., 2006). In human food and animal feed, nanotechnologies can concern materials in contact with food, antimicrobial agents, biosensors, ingredients, aroma, texturing agents, … Most of the current approaches still remain research projects, but recent developments have led to the commercialisation of some products containing NPs (AFSSA, 2009). The lack of data required to evaluate the risks associated with NPs “naturally” present in or added to food and feed, makes it urgent to develop accurate toxicity studies.

In case of “classical” substances to be evaluated, the actual concentration of the active soluble molecule, as well as its interaction with cell receptors, enzymes, genes,… are the key parameters. For what concerns nanomaterials, especially those present or added to food and feed, it is crucial to take into account parameters such as the real dimensions (length, especially for fibres), cristallinity, microporosity, state of aggregation and, probably the most important one, the accessible surface and the amount and type of bound material. For instance, the response to monodisperse amorphous silica NPs is governed by different physico-chemical parameters and, furthermore, varies with the cell type: external surface area (macrophages), micropores volume (macrophages), and surface roughness (endothelial and fibroblasts) (Rabolli et al., 2010).
Accordingly, it is of great concern to assess the potential negative impacts nanomaterials - present voluntarily or not in food and feed - may have on biological systems (Bouwmeester et al., 2009; Kreyling et al., 2006,) and, in particular, on the intestinal barrier, which is the site of possible absorption to gain access to the systemic circulation, as well as of initiation of inflammation, immunity, …. Toxicity testing of NPs requires that the end dose-response relationships can be described, for both *in vitro* or *in vivo* tests (Bouwmeester et al., 2007).

Nevertheless, in order to evaluate the fate of nanomaterials present in food, both *in vitro* and *in vivo*, as well as their effects on the gastro-intestinal tract, it is mandatory to have appropriate tools to detect their presence, stability, level of aggregation, … in food matrices, intestinal cells and, eventually, in biological fluids. Currently, several microscopic methods are used to evaluate the some of the characteristics of nanomaterials in these environments, such as:

- *Optical microscopy*. Although this technique lacks the resolution to identify individual nanomaterials, it is widely applied to assess suspensions for the presence of large aggregates of nanoparticles. When the optical signal of a NP can be amplified (e.g. when the particles are autofluorescent or can be fluorescence labelled) optical microscopy can be a valuable tool to examine the distribution of NPs in cells and tissues.
- *Electron Microscopy*. By their high resolution, scanning and transmission electron microscopy are two of the few techniques that allow direct visualization of nanomaterials. Conventional sample preparation techniques

coupled to SEM and TEM imaging and (semi)automatic, threshold based detection of NP in electron micrographs allow the detection of the primary subunits of nanomaterials and measuring the physical characteristics of NP on a per particle basis. These include the size (distribution), shape, aggregation state and the surface morphology of nanomaterials. Different methods for TEM imaging and image analysis in two and three dimensions were examined.

- *Atomic Force Microscopy* (AFM). This technique allows measuring NPs in the Z-direction with a resolution in the order of one nanometre and hence optimally complements electron microscopy with the best resolution in the X- and Y-directions. The deflections of a cantilever with a sharp tip (mechanical probe) are measured when scanning a surface containing NPs. AFM can be operated in a number of modes, depending on the application. These can be divided into static (also called contact) modes and a variety of dynamic (or non-contact) modes where the cantilever is vibrated. As a result, the topography (Z-direction) of a sample is represented in function of its X- and Y-coordinates. In specific configurations, electric potentials can also be scanned using conducting cantilevers.

The size and surface physico-chemical properties can also to be determined after appropriate extraction from complex media through methods that do not affect their state:

- *Dynamic Light Scattering* (DLS), today the most popular method to evaluate the size of particles, is based on a monochromatic and coherent laser light hitting a suspension of NPs and light scattering recorded by a detector;
- *Zeta* (ζ) potential is related to the electric surface properties of nanomaterials, which are crucial to promote their association with other substances (ions, food constituents, other NPs,…). An electrically charged particle is surrounded by an inner shell, strongly bound, and by an outer shell, less tightly bound. The ζ potential is that of this boundary and its value is related to the stability of the nanomaterial dispersion. If high, positive or negative, the particles will tend to, repel each other; if low, the particles will tend to aggregate. The ζ potential measurement is based on the velocity of the particles in an electric field.

Finally, chemical concentration is also an important parameter. Especially in the case of metal nanomaterials, a possible dissolution in the gastro-intestinal environment should be evaluated. In many cases, the assay of the actual concentration in different biological samples is crucial. The total concentration of a given element can be determined in tissues, food and feed by using:

- Atomic Absorption Spectroscopy (AAS).
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
- Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Actually it is important to bear in mind that microscopic and chemical determination approaches are complementary to get all the information needed (what amount is present in my sample and under which precise form?).

Evaluation of NPs intestinal toxicity

When considering the particular aspects associated with nanomaterials in food and feed, as compared, for instance, to NPs in air or water, a key question to be addressed concerns their bioaccessibility, i.e. the proportion of the substance of interest that would be able to be absorbed across the intestinal epithelium. Then, beside a toxicity evaluation at the intestinal level, it is also important to consider the bioavailability of the NPs, i.e. the amount of material that will reach the bloodstream and target organs where it could also exert toxic effects.

For the *in vitro* evaluation, Caco-2 cells are a human intestinal line, which mirrors the absorptive epithelium of the intestine (Koeneman et al., 2009; Oberdörster et al., 2005) and which is widely used in pharmaco-toxicology. The Nanomaterial Toxicity Screening Working Group, from the Risk Science Institute recommended the use of this line for *in vitro* testing of the ingestion of NPs (Oberdörster et al., 2005). Using this cell line, the studies should focus on:

(i) the evaluation of the direct toxicity, i.e. necrosis, apoptosis or, with cultured cells, post-apoptotic necrosis using routine methods (MTT, neutral red, LDH release, …);

(ii) the evaluation of the disruption in tight junctions, controlling the paracellular passage using established procedures (transepithelial electric resistance,

radiolabelled mannitol or fluorescent Lucifer yellow fluxes, confocal immunodetection of tight junctions proteins, …);

(iii) the investigation of the transport of NPs through the intestinal monolayer (Koeneman et al., 2009). In particular, it should be taken into account that NPs would probably cross the intestinal epithelium via the M cells of the follicle associated epithelium. Our group has developed an *in vitro* model of this barrier (des Rieux et al., 2007). Furthermore, the intestinal hydrophilic mucus layer covering, physiologically, the epithelium would also play a key role in the adsorption of NPs on the epithelial cells and their transport across the intestinal barrier. *In vitro* models also exist to mimic this important aspect (Nollevaux et al., 2006);

(iv) the assay of intestinal functions upon addition of NPs of different size, chemical composition, surface properties, …. In particular, effects on phase I & II biotransformations enzymes, phase III efflux pumps, … should be recorded. A particular attention should also be drawn to the development of acute or chronic inflammation by following the NFκB and MAPKinases activation, ILs, MCP-1, … secretion (Van de Walle et al., 2010);

(v) the evaluation of transcriptomic (Sergent et al., 2010) and proteomic perturbations in such a culture system should also be taken into account;

(vi) the effects of food constituents (nutrients, but also xenobiotics, phenolic compounds,…) known to affect some of these properties (Sergent et al., 2008).

For the *in vivo* evaluation of oral exposure to NPs, the physical and chemical properties of the material should be characterized in the form delivered to the animal (mice or rats), as well as after the test - if possible (Oberdörster et al., 2005). The NPs should be characterized in a matrix as close as possible to the food product. After ingestion, the amount of nanomaterial eliminated via the faeces should be determined and compared to the amount of material retained. In case the nanomaterial would be significantly absorbed, histology assays are recommended (Oberdörster et al., 2005). *In situ* detection would be of high interest. This approach would bring more knowledge on the absorption, distribution, metabolism and excretion (ADME) of NPs (Bouwmeester et al., 2007).

Conclusion

Investigation of the possible toxicity of nanoparticles in food is an important issue although there is no clear request from the actual legislation. The physico-chemical parameters of the NPs should be well described and the use of appropriate dose metrics is of high importance for the toxicity testing. The characterization of NPs before and after administration *in vitro* and *in vivo* is also considered the ideal in screening studies.

In this regard, we are involved in a national project aiming at developing methods for risk assessment of NPs in food (RT 10/5 NANORISK, 'Development of methods for assessing toxic effects of ingested engineered nanoparticles'). This project relies on three pillars:

- the detection and characterization of NPs in complex matrices;
- *in vivo* tests on rats (oral intake) using OECD guidelines and focussing on the toxicokinetics, acute and chronic effects;
- *in vitro* tests focussed on the intestinal function. The *in vitro* tests will be conducted on simple matrices before switching to food and feed matrices. Caco-2 cells layers and co-cultures including M cells and/or a mucus layer will be compared and the fate of NPs will be studied. Nano-silver was selected as a first model as it is already used in food for its antibacterial properties. A second model nanomaterial will be selected in a later stage of the project.

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Microarray analysis of effects of silver nanoparticles on an *in vitro* translocation model of the human intestinal epithelium

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Introduction

Engineered nanoparticles are increasingly being used in various food and food derived applications (Chaudhry et al., 2008). The most prominent use is in food packaging materials to increase the barrier properties, to incorporate smart sensors or as a coating with antimicrobial properties. Silver nanoparticles are used most frequently for this purpose.
At present, there are a number of knowledge gaps in regard to the ADME properties and effects of nanoparticles (Bouwmeester et al., 2009). Therefore, we employed an *in vitro* model of the human follicle-associated epithelium to study silver nanoparticle translocation and local effects exerted by these nanoparticles.

In vitro model

In short, Caco-2 cells are co-cultured in a transwell design with Raij B lymphocytes resulting in a co-culture of Caco-2 cells and differentiated M-cells, specialised in particle translocation (des Rieux et al., 2007). Presence of M-cells has been confirmed immunohistochemically by using the M-cell specific marker galactin-9. Translocation studies can only be performed reliably if the integrity of monolayer of cells in the transwell design has been confirmed. For this we used Trans Epithelial Resistance as the first measure. In addition, exposure to Lucifer Yellow confirmed the integrity of the monolayer because of absence of

translocation. In addition, Trans Epithelial Resistance was determined before and after exposure. No significant changes were observed.

Nanoparticles tested

Four groups of silver nanoparticles (Nanocomposix, San Diego, USA) were used: 30 ± 4 nm; 31 ± 5 nm; 69 ± 7 nm and 112 ± 9 nm as determined by TEM; while the hydrodynamic sizes in H_20 as determined by DLS were 47 ± 5 nm; 70 ± 2 nm; 67 ± 4 and 115 ± 6 respectively. Methoxy (polyethylene glycol)-thiol (mPEG-SH) coated silver nanoparticles (4nm and 35nm) were a kind gift from Dr. P. Christian from the University of Manchester (UK). In addition, $AgNO_3$ was used in a reference group. Concentrations used in the translocation experiment ranged from 5 to 25 µl/ml and were shown to be not cytotoxic to Caco-2 cells by WST-1 assay. We detected a considerable dissociation of silver ions from the silver nanoparticles in the exposure medium; 17% from the 30 and 31 nm sized nanoparticles and 6% from the 69 and 112 nm sized nanoparticles.

Gene expression & Translocation

The effects of the silver nanoparticles on gene expression of the Caco-2 and M-cells was assessed by transcriptomics. For this the monolayer was lysed using trizol, subsequently RNA was isolated, purified and Cy5-labeled. Isolates were hybridized on Agilent whole human genome microarrays. Briefly, both the groups exposed to silver nanoparticles and ionic silver resulted in an upregulation of 2 to 80 genes. Functional information could be attained for 79 genes, following Metacore analysis. The genes have been described being involved in processes such as proliferation (23), response to oxidative stress (20), metal ion binding (19), unfolded protein response or ER stress (9), apoptosis (9), cell structure and migration (8), other stress responses (6) and other functions (5). A proportion of the genes play a role in two processes. Specifically metallotheinins genes HSPA6 and HMOX1 were amongst the highest up-regulated genes.

In a follow-up study we assessed the translocation of a selection of the uncoated and coated Ag nanoparticles. This was determined following an exposure of 4 hours. Silver translocation was determined by means of ICP-MS

in samples from the upper and lower compartments and subsequent calculation of the translocation (Paap). In this study we used both two smallest-sized uncoated Ag nanoparticles (20 and 30 nm) and two mPEG-SH coated Ag nanoparticles. In addition, we evaluated the translocation of $AgNO_3$ as a control group. We observed significant overall differences in translocation rates (Paaps). Post-hoc analysis revealed that the coated nanoparticles were significantly more translocated than the uncoated ($p=0.006$). The coated Ag nanoparticles were also translocated significantly more ($p=0.03$) than the Ag^+ from the $AgNO_3$ control group, while no differences between the uncoated nanoparticles and Ag^+-group were observed. Furthermore, translocation of either the coated or uncoated nanoparticles were not size and concentration dependent. TEER was measured before and after exposure to Ag nanoparticles.

Conclusion

In conclusion, for our microarray study we were mainly interested in the primary effects of the silver NPs, and therefore selected a short exposure time of 4 h. Using SAM analysis, 97 genes were found to be significantly up-regulated by at least one treatment. No gene was down-regulated, which is likely due to the short exposure time.

An important aim from the microarray experiments was to detect possible nanoparticle specific effects. However, genes affected by any of the nanoparticles were at least to some extent affected by $AgNO_3$ as well. We detected a considerable dissociation of silver ions from the silver nanoparticles in the exposure medium around the silver ion concentrations of the $AgNO_3$ (1.5 µg/ml) group. It is, therefore, most likely that the gene expression changes are completely caused by the effect of silver ions. This implies that at least in the present experimental setting, nanoparticles themselves have no effect on the gene expression in Caco-2 cells.

The results of our translocation experiment clearly shows the importance of coating of nanoparticles on the translocation.

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THEME 4:

RISK ASSESSMENT – EFSA'S POINT OF VIEW

The potential risks arising from nanoscience and nanotechnologies on food and feed safety

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Introduction

Following a request from the European Commission the European Food Safety Authority (EFSA) published in 2009 a scientific opinion on potential risks arising from nanoscience and nanotechnologies on food and feed safety (EFSA, 2009). In view of the multidisciplinary nature of this subject, the task was assigned to the EFSA Scientific Committee.
This 2009 opinion addresses engineered nanomaterials (ENMs). Food and feed are addressed together, since the basic aspects (applications and potential impacts) are expected to be similar. The opinion is generic in nature and is in itself not a risk assessment of nanotechnologies as such or a survey of tentative applications or possible uses thereof or of specific products.

It is claimed that nanotechnologies offer a variety of possibilities for application in the food and feed area – in production/processing technology, to improve food contact materials, to monitor food quality and freshness, improved traceability and product security, modification of taste, texture, sensation, consistency and fat content, and for enhanced nutrient absorption. Food packaging makes up the largest share of current and short-term predicted markets.

Formulation at the nanosize may change the physico-chemical characteristics of materials as compared to the dissolved and micro/macroscale forms of the same substance. Their small size, high surface-to-mass ratio and surface reactivity are important properties, both for new applications and in terms of the associated potential health and environmental risks.
Current uncertainties for risk assessment of ENMs and their possible applications in the food and feed area arise due to presently limited information on several aspects. Specific uncertainties apply to the difficulty to characterize, detect and measure ENMs in food/feed and biological matrices and the limited information available in relation to aspects of toxicokinetics and toxicology. There is limited knowledge of current usage levels and (likely) exposure from possible applications and products in the food and feed area.

The risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is considered applicable for ENMs. However, risk assessment of ENMs in the food and feed area should consider the specific properties of the ENMs in addition to those common to the equivalent non-nanoforms. It is most likely that different types of ENMs vary as to their toxicological properties. The available data on oral exposure to specific ENMs and any consequent toxicity are extremely limited; the majority of the available information on toxicity of ENMs is from *in vitro* studies or *in vivo* studies using very high doses, acute administration and other routes of exposure. The risk assessment of ENMs has to be performed on a case-by-case basis.
Current toxicity-testing approaches used for conventional materials are a suitable starting point for risk assessment of ENMs. However, the adequacy of currently existing toxicological tests to detect all aspects of potential toxicity of ENMs has yet to be established. Toxicity-testing methods may need methodological modifications. Specific uncertainties arise due to limited experience of testing ENMs in currently applied standard testing protocols. Additional endpoints presently not routinely addressed, may need to be considered in addition to traditional endpoints.
For hazard characterization, the relationship of any toxicity to the various dose metrics that may be used is currently discussed and several dose metrics may need to be explored in addition to mass.

The different physicochemical properties of ENMs compared to conventional dissolved and micro/macroscale chemical counterparts imply that their toxicokinetic and toxicity profiles cannot be fully inferred by extrapolation from data on their equivalent non-nanoforms.
Appropriate data for risk assessment of an ENM in the food and feed area should include comprehensive identification and characterization of the ENM, information on whether it is likely to be ingested in nanoform, and, if absorbed, whether it remains in nanoform at absorption. If it may be ingested in nanoform, then repeated dose toxicity studies are needed together with appropriate *in vitro* studies (e.g. for genotoxicity). Toxicokinetic information will be essential in designing and performing such toxicity studies. For ENMs which are intended to increase the bioavailability of incorporated substances (i.e. ENM carrier systems), the changes in bioavailability should be determined.

Although, case-by-case evaluation of specific ENMs may be currently possible, it is emphasised that the risk assessment processes are still under development with respect to characterisation and analysis of ENMs in food and feed, optimisation of toxicity testing methods for ENMs and interpretation of the resulting data. Under these circumstances, any individual risk assessment is likely to be subject to a high degree of uncertainty. This situation will remain so until more data on and experience with testing of ENMs become available. The limited database on assessments of ENMs should be considered in the choice of appropriate uncertainty factors.
For research needs it is important to develop methods to detect and measure ENMs in food/feed and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.

Current work within EFSA

Currently EFSA has set up a working group of its Scientific Committee to address a new request of the European Commission to provide guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food, feed and pesticides.

The background of the request indicates that the present state of knowledge still contains many gaps preventing risk assessors from establishing the safety, according to standard procedures, for many of the possible food related applications of nanotechnology and thus ensuring that the safety aspects of engineered nanomaterials and nanotechnology bases processes are addressed in a coherent and comprehensive manner.

The purpose of the request by the European Commission is to obtain guidance on risk assessment thus providing the necessary transparency for stakeholders and regulators in order to develop an appropriate approach for the assessment and authorisation of engineered nanomaterials and other nanotechnologies.

It is possible, that even with the current state of knowledge, scenarios may exist for which different risk assessment approaches could be considered. These include, for example, applications where it could be established that consumer exposure would not arise (e.g. food contact materials with no nanomaterial migration) or that nanomaterials are soluble or biodegradable or when a delivery system for bulk substance is in nanoscale (e.g. micelles, nanoemulsions or other types of encapsulation).

The European Commission requested EFSA to prepare a guidance document for the safety assessment of applications involving the application of nanoscience and nanotechnology to food and feed (including food additives, enzymes, flavourings, food contact materials, novel foods, feed additives and pesticides). The guidance should provide practical recommendations for the risk assessment of food related applications of nanotechnology to the extent possible with current knowledge. In the cases where knowledge is insufficient, it should indicate the endpoints and/or parameters that would have to be known in order to carry out a risk assessment. The guidance should indicate, where necessary, the additional requirements in terms of endpoints, tests, and data that would have to be fulfilled to be able to perform conclusive risk assessments. In support of this work, the EFSA considers any relevant document developed for risk assessment in the context of nanotechnologies by scientific advisory bodies at European level (SCENIHR, SCCS, EMEA, ECHA, ECDC, SCOEL, OSHA etc.), EU Member States, third countries and international organisations including documents produced by the OECD Working Party on Manufactured Nanomaterials.

A draft of the guidance document under preparation is scheduled to be published for a public consultation before its finalisation, for the first half of 2011.

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THEME 5:

COMMUNICATION, PERCEPTION & PARTICIPATION OF THE CONSUMER

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Introduction

Although potential applications of nanotechnology in food packaging and food additives have been discussed, as yet, there is relatively little systematic research on public perceptions of the use of nano- particles in the food chain. The current situation is well summarized in the NanoBio-Raise Report ("Nanotechnology and Food")[3].

"there is a huge lack of knowledge among the general public about nanotechnology as such and food applications in particular. Therefore there is a need for genuine public dialogue to hopefully avoid another GM-type situation developing. The seriousness was demonstrated by two "publifocus" conferences involving consumers where several applications of nanotechnology were discussed including food applications held in Switzerland and in Germany at the end of 2006. The sixteen German consumers involved were positive about the opportunities for improved food safety by nano-based quality control but consider food applications of nano-ingredients a very sensitive area. The Swiss consumers were generally positive about nanotechnology but were most concerned about food applications. Both groups asked for labelling of nano-containing products although there is a need to distinguish between "naturally occurring" molecules already present in food and artificially introduced manufactured nano-particles that are not. There is a clear mistrust of (food)

[3] http://files.nanobio-raise.org/Downloads/Nanotechnology-and-Food-fullweb.pdf

producers who may incorporate nanotechnology in products without indicating it on the label. German (Die Welt, ARD, Der Spiegel) and British (Observer, BBC Focus magazine) media have started reporting about food applications of nanotechnology, in some cases very critically".

This paragraph points to some key issues – very sensitive area; natural; labelling; critical media reporting and trust. It also mentions another GM-type situation, which has long been a concern of those developing applications of nano packaging and ingredients. Given that those who ignore history are sometimes condemned to relive the past, it is informative to reflect on the lessons of GM food and on what lies behind the continuing resistance to GM ingredients among wide sections of the European public.

Food; a sensitive topic

Food and genetic modification/nanotechnology (bio-nano) bring together the old and the new. Biotechnology has a short history dating back to the 1970s with the development of rDNA technologies; nanotechnology has an even shorter history. It was only in the early 1990s that so-called vegetarian cheese made with a GM enzyme, and subsequently the FLVR SVR tomato emerged as consumer products. By contrast, the production, preparation and consumption of food are as old as human society. Over the millennia and across the world, food and eating, while arguably the most basic biological function, have evolved to be a central feature of culture - shaping social organisation, the division of labour, and demarcating religions, races, communities, classes and genders.

Food, according to Claude Fischler an eminent French social scientist, is constitutive of both cultural and individual identity through the process of 'incorporation' - the crossing of the barrier between the 'outside world' and the 'inside world' of the body. Food intake is not merely physical. With the food we also absorb beliefs and collective representations – as suggested by the age old aphorism 'you are what you eat'.

In the last half century the Western world has witnessed a change from food shortages to surpluses. Anxieties about having enough to eat have been replaced with concerns related to the ever increasing distance between 'the farm and the fork'. The modern eater is an increasingly anxious consumer, torn between the appeal of cheap, convenient and palatable processed food, and the

repulsion or menace of factory farming and pesticides, and additives to replace natural ingredients. The perceived, and to some extent, real consequences are new and subtle dangers, less visible, understood or controllable.

While culture gave earlier generations principles about what and what not to eat, the clues of texture, flavour and even intuition fail to protect us from the perceived hazards of eating in modern times. The consequent psychological, political and ethical distress resonates in worries about being 'at risk' from pesticides, residues, pollutants and additives. The most common complaint about contemporary processed foods typically is that "one does not know what one eats anymore". It encapsulates the contemporary food consumer's dilemma: "I am what I eat; I don't know what I eat; thus I don't know what/who I am".

With the decline of tradition and culture we see the emergence of individual choice – for some desirable, for others the cause of anxiety, bewilderment and the state of 'gastro-anomie' to use Claude Fischler's term. Individuals are often at a loss as to how to make choices in the general nutritional cacophony - conflicting norms (or normlessness), prescriptions and proscriptions about food. Anxieties about food are evidenced in food scares, pathological eating disorders and a normlessness that some social scientists link to the trend of rising obesity. All in all, in our modern times food is as likely to be seen as a source of stress rather than pleasure.

To 're-identify' with food, to re-appropriate it ("knowing what they eat") and to introduce a new logic into everyday eating, people search for new strategies, seen in the demand for food labelling, legal protection against the use of chemicals and biotechnology, the adoption of individual alternative diets, ranging from more or less rigid vegetarian, organic, low-calorie or low carb etc.

Food anxieties have led to new 'strategies of confidence' including the development of repertoires of trusted food, for example organic food, fair trade, vegetarian or local food; brand loyalty – with brands standing for familiarity and reassurance of safety and quality. This strategy fuels Ritzer's 'McDonaldization' of taste.

In many, if not all cultures, but at the very least in the United States, France, Britain, Switzerland, Italy and Germany, the adjective "natural" associated with food is considered positive. As such it is a common theme in the advertising of food products. Naturalness is affected more, in people's perception, by addition,

even infinitesimal, than it is by subtraction; by process rather than by content; by chemical rather than by physical transformation; by contaminants and above all by genetic modification and potentially by manufactured nano-particles.

15 years of food biotechnology

The history of public perceptions of the Life Sciences, and in particular the troubled history of GM food, provide some interesting insights into the dynamics of public perceptions and offers some useful lessons for those wishing to achieve socially robust innovation, whether in novel technologies or foods. Two of the general lessons are that social and ethical issues should be taken into account at a formative stage of the innovation process and that market success requires more than regulatory approval and the support of producers.

It is a striking fact that the many widely accepted medical applications of the Life Sciences were subject to both scientific and ethical scrutiny. The same cannot be said for GM food which, until 2001 was assessed solely on the basis of scientific risk assessments. The neglect of consideration of the social and environmental impacts contributed, in part, to the extended controversies that continue to this day.

Equally striking, is that the GM industry assumed that with regulatory approval and the support of farmers, the market was secure. What they did not appreciate is that the environment into which GM crops and food was introduced included the public, in the roles of decision makers both as citizens (qua voters) and as consumers (qua purchasers). It was the public in these two roles that influenced national governments, food manufactures and the supermarket chains. Undoubtedly the NGOs and the media played a role in mobilizing public opinion, but there is a good case to be made that these intermediaries were led by public concerns, that had been evident in survey research from the 1980s.

The plural rationalities of the public

Research on public perception of science and technology constructs an image of everyday people, of what they are and what they should be. As Tetlock points out, much research in the aftermath of the so-called ‘cognitive revolution’ has

construed people as *intuitive scientists* or *intuitive economists*, striving to understand the science and maximising utilities respectively.

With the ideal of intuitive scientists or economists, people were expected to be interested in scientific details and probabilistic conceptions of risk to evaluate technological developments. On the level of science communication, initiatives set out to 'educate' and 'inform' the public. Research on public perception, however, soon highlighted that compared to expert judgment, everyday thinking is not concerned with scientific detail, and when it comes to risk and probabilities, it is 'biased' and prone to errors. Others have argued against such a 'deficit' typification of the public and have empirically demonstrated the functionality and quality of everyday thinking.

However, people are not only self-interested individuals concerned with maximising their utilities. There are at least two more possible metaphors to understand everyday people: *intuitive politicians* and *intuitive ethicists. Intuitive politicians* are concerned with fairness, the balancing of social interests, with distributional and procedural justice and the avoidance of social exploitation. Questions like 'who is affected?' and 'for whom are technologies potentially risky and beneficial? play a role here. *Intuitive ethicists* are concerned with the core values and beliefs that are essential to the fabric of society. These values sometimes need to be defended from challenges which may arise from science and technology amongst other sources. They ask questions such as, 'Should science trump social values?', 'It may be safe but is this the sort of society we wish to live in?'. All 'intuitive experts' are able to evaluate technologies as either good or bad. However, it is only the intuitive scientist/economist that can be expected to base judgments primarily on expected consequences, on utilitarian grounds. Intuitive politicians and ethicists may well base their approach on non-consequential arguments, such as procedural fairness or other core values.

Furthermore, a realistic model of human nature will recognize that people switch between these intuitive logics and may experience tensions between them. Consequently, there is a need to consider what clashes between the logics might arise in everyday life, both on the level of the individual, and on a societal level. When Oscar Wilde described economists as knowing the price of everything and the value of nothing, he referred to one of these tensions. Modern humans, taking both the perspectives of intuitive scientists/economists and ethicists, for example, frequently struggle with competing themes, such as

for example the idea of respect for nature and an urge to conquer or master it (Rozin et al., 2004).

Cultures of risk

The tension between different ways of thinking about the world brings into focus the two cultures of risk - the gap between scientific and societal thinking about the issues of risk and uncertainty. Essentially, confronted by the same putative hazard, the approach adopted in science is, on occasions, strikingly different to the approach taken by intuitive logicians.

The scientific perspective

A simple definition of risk is the probability of an unacceptable loss. The expert view of risk assessment inclines towards probabilistic models that determine the likelihood of positive and negative outcomes multiplied by the potential impacts of these outcomes. Risk assessment is under-pinned by the scientific method. The scientific method assumes that there are 'facts' about the world to be discovered and that knowledge progresses through empirical research, leading over time to a closer approximation to the truth (Jaeger et al., 2001). Research that follows the canons of the scientific method is objective and unbiased by human motivations and agency. Thus, scientific risk assessment is the only recognised approach, all claims about potential risks to human health and safety must be subjected to the same criteria regarding methodology and evidence. In this way risk, supported by the 'methodology of risk assessment' is almost a universal currency – it transcends place and time. A risk is a risk is a risk, whether one is in Britain, Belgium or Burundi. Of course, there may be different levels of risk acceptability, but this is a risk management issue and a political decision.

The public perspective

An alternative definition of risk is that it is a complex, socially narrated concept based on a variety of non-cognitive factors. In the public sphere risks may take on political, ethical and emotional dimensions. For the public, the essence of perceptions of risk are not cold calculating cognitive decisions but rather fears, hopes, pleasure and anger drawing on the intuitive logic of the politician and

ethicist. In different cultures and social milieus within cultures what constitutes risk may be very different. Culture, stereotypes, trust in experts and social values (amongst other things) all play a part in the identification of risks and in the amplification or attenuation of risk perceptions.

The implication of this is that purely science based claims about risk, and in particular the absence of risk, may not be very convincing to the public. Such claims are likely to be least convincing when dealing with a 'sensitive technology' and in situations where benefits from the innovation are in question and the existence and distribution of down side risks uncertain.

The presentation

Having set the scene, the presentation will combine a review of some recent social survey research on public perceptions of nanotechnology and draw out the lessons from agricultural biotechnologies for nano-materials in food packaging and in food ingredients.

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THEME 6:

REGULATORY ASPECTS OF EU FOOD LEGISLATION

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A safe and responsible development of nanotechnology

Nanotechnologies represent both an opportunity in the context of the EU 2020 innovation policy and a challenge to overcome the obstacles, which hinder their development. The acceptance of nanomaterials in consumer goods and in food products in particular will depend on the clear demonstration of their safety and their benefits for society, and also on consumer confidence.

In its 2004 Nanotechnology Strategy, the European Commission already called for an "integrated, safe and responsible" approach to the use of nanotechnologies. This approach implies that significant resources and efforts are devoted to the development of scientific knowledge on the specific properties of nanomaterials to be able to manage accurately the related risks for Health, Safety and Environment (HSE).

Policy makers have a key role to play in managing the potential risks related to the use of nanomaterials in food and the capacity to perform a proper risk assessment is central to this. The Commission has called on the independent scientific committees of the European Union to address some of the critical issues relevant to the assessment of the safety of nanomaterials for food and consumer goods. As a result, several opinions have been adopted or are under development, in particular by the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) and the European Food Safety Authority (EFSA).

In particular, the Commission has asked EFSA to provide guidance by mid 2011 on the risk assessment concerning potential risks arising from applications of

nanotechnologies to food, feed and pesticides. The main objective of this opinion is to streamline the risk assessment of nanomaterials in food products through a classification of the various types of nanomaterials according to their properties and related risks and to identify which scientific data and test results need to be provided by the applicants to ensure a proper and conclusive scientific assessment.

Still, the development of scientific knowledge on the risks related to nanomaterials and for developing tests methods and methodologies to assess these risks is necessary and will need to be maintained in the long run, both at national, EU and international levels. This effort is necessary to ensure that only safe products are put on the market and to keep the pace with the constant development of new generations of nanomaterials.

An adapted legal frame

In its "Communication on regulatory aspects of nanomaterials", the Commission has reviewed the EU legislation, including on food, to check whether it addressed properly the potential risks of nanotechnologies. This review concluded that the current EU legislative framework covers "in principle" the potential health, safety and environmental risks in relation to nanomaterials but that "current legislation may have to be modified in the light of new information becoming available".

As a result of this exercise, the obligation for an EU risk assessment of all substances under nanoform has been introduced in the legislation of food contact materials and additives. Similar provisions are proposed in the ongoing revision of the novel food legislation.

Further, to ensure a harmonised implemention of the EU requirements across various sectoral legislations, the term "nanomaterials" needs to be legally defined and in a scientifically solid manner. The Commission has requested the independent Scientific Committee for Emerging and Newly Identified Health Risks (SCENIHR) to provide the necessary elements to be taken into account for elaborating such definition. These will be examined by the Commission to ensure the definition for food applications results in a secure and technically reliable implementation of the EU food legislation.

Building trust

Building public trust in the safety and benefits of nanomaterials requires, in particular in the food sector, a clear and open communication between all parties: industry, regulators, scientists and consumers. It depends on several interconnected elements:

- Clear and easily available information about the nano-products on the market;
- Understanding of the risk assessment process and of the uncertainty associated with the use of the nanomaterials;
- Involvement of civil society in policy-making through open dialogue and analysis of the risks and benefits of nanotechnology.

Efforts by all players, including mainly industry, to improve transparency on existing and future applications of nanotechnologies in food products or food contact materials are also necessary. The Commission will collect information on the current and future uses in different sectors, in close cooperation with Member States, the industries concerned and other stakeholders. The Commission also organizes among other initiatives, the annual "Nano Safety for Success Dialogue" to share information and views between all interested parties.

Only transparency as well as clear and readily understandable information will help building trust for policy makers and consumers, which will ultimately contribute to the safe and responsible development of nanotechnologies in the food and other sectors.

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General Conclusions

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These conclusions reflect the main messages extracted from the various presentations of the nanotechnology symposium (at least when they were explicitly addressed in the summaries prepared by the speakers), as well as the point of view of the scientific board responsible for the organization of this symposium.

Definitions of nanotechnology (theme 1)

Regarding the first theme devoted to the definition and classification of nanomaterials as summarized in the presentation made by J. Bridges (SCENHIR), one can say that the issue of definitions is of paramount importance and must be given a high priority due to its impact on regulatory aspects. There are several ongoing activities in this field and it is worth mentioning the complexity involved. We indeed need to correctly define what nanotechnology is and what nanomaterials and nanoparticles (NPs) are. Size and shape of NPs must be considered, but the main concern remains the functional changes of those materials due to modified properties at the nano-scale. Natural versus engineered NPs is another crucial aspect for the acceptability by the consumers, as also mentioned by G. Gaskell ("Communication, perception and participation of the consumer", theme 5). Regarding scientific aspects and impact on food safety, the stability of nanomaterials will be very important because rapid destruction of NPs in, for example the GI-tract, will have a large impact on the actual risks for living organisms.

Applications in the food chain (theme 2)

A general overview of some (potential) applications in the food chain was given by Q. Chaudhry (Fera, UK). It is important to note that some of the applications are envisaged during crop production (e.g. for improved targeting of pesticides and fertilizers applications). Regarding possible risks for man and environment, it seems, however, that such applications will mainly impact the environment and the operator's health, and not so much food safety. On the other hand, the applications in the food and feed sectors could have direct consequences on animal and/or human health. At least three domains of (potential) applications could be identified for the food sector. The first one, as described by J. Lammertyn (K.U.Leuven, Belgium) deals with nano-constructions showing some promising applications for food diagnostics (e.g. detection of toxic compounds, allergens, etc. in food). The second domain is the use of nanomaterials in food packaging, where there could exist some risks of migration of contaminants from the package to food. This could alleviate the positive effects expected from such materials, which were illustrated in the presentation of J. Lagaron (CSIC, Spain). A third domain, for which direct contact with food has to be considered, relates to food or feed components, food and feed additives, and food complements that are directly ingested. The aim is to improve the availability of some ingredients eventually leading to an increased intake of essential nutrients or a reduced intake of sugar or fat. Here again, the notions of NPs' stability and of engineered versus natural materials will play a very important role. This means, for example, that a clear distinction has to be made between the application of persistent NPs, such as metallic elements, and some other applications, such as the use of organic carriers for targeted delivery of drugs or nutrients.
As pointed out by M. Knowles (CIAA), nanotechnology presents great potentials for the food industry, but its implementation involves a lot of challenges too.

Toxicological aspects (theme 3)

An overall overview of the toxicological issues was given by Y.J. Schneider (University of Louvain, Belgium).
In the food sector, special emphasis must be put on the oral exposure route, which has been much less studied so far than the exposure via inhalation or

dermal uptake. The passage through the GI-tract needs to be studied in detail and particular attention must be paid to the physicochemical fate of the NPs; their toxico-kinetics and some specific toxic effects, such as oxidative stress and inflammation. In addition, the movement of NPs is not controlled by some well known barriers and, hence, NPs may migrate and accumulate in very sensitive parts of the body, such as in the foetus of pregnant women, the brains and/or other organs. In order to have the most complete picture of toxicological effects, *in vivo* tests are useful but cannot be applied on all the targeted species including humans, taking into account that numerous applications of NPs will be proposed in the near future. Therefore, *in vitro* tools need to be further developed. In this respect, the bio-informatic approach was illustrated in the presentation of H. Bouwmeester (Wageningen University, The Netherlands). These *in vitro* tests need, however, to be validated and critically assessed for their concrete relevance in the real world.

Risk assessment (theme 4)

So, we are naturally moving in the field of risk assessment, which was addressed in the presentation of C.L. Galli (EFSA). The main question asked by the research bodies and risk assessment agencies is the following: do we need a new paradigm or is it possible to assess the risks using the current methodological approach and taking into account specific properties of NPs such as increased mobility and modified physicochemical properties? To be able to carry out a risk assessment, it is necessary to gather information on the potential exposure of the target group in order to be able to build exposure models or exposure scenarios. This will require more information on the occurrence of NPs in food/feed, improved traceability and also adapted detection methods. The nano world is complex and will require special attention during the design of *in vivo* experiments as well as for the extrapolation from *in vitro* results. In addition, we have to face some complex problems linked to the diversity in composition, shape, size, and coating of nanomaterials. The golden rule for risk assessment should be: “case by case approach, sound science and common sense”. It is important to be aware that experimental data required for the different toxicological endpoints will not be available for all NPs currently existing in a commercial or developmental stage or the ones that will be

discovered in the next future, and, hence, some prioritization will always be necessary. In parallel, a realistic estimation of the uncertainties will be required and will be part of the risk assessment process.

The consumer (theme 5)

But, finally, what will be the reaction of the consumers? G. Gaskell (London School of Economics, U.K.) proposed some useful tracks. First of all, clear communication about the costs and the benefits must be provided and the uncertainties must be documented. Consumers are more prone to accept some kinds of materials (e.g. natural NPs such as clay materials) and some types of applications (e.g. smart packaging). Food is a very sensitive topic and labelling will likely be required by the consumers, certainly when engineered NPs are considered. It is thus clear that the participation of the consumer in the decision process is highly recommended in order to ensure his social engagement. This was obviously not the case for the GMO's applications with some rather irrational consequences, at least in Europe.

Regulatory aspects (theme 6)

Last but not least, and probably on top of the current priorities are the regulatory aspects as highlighted in the sixth theme with the presentation of E. Poudelet (EC DG Health and Consumers). As already mentioned above, we need definitions to be able to further progress in the regulatory field. We also need instruments for tracing NPs through their full life cycle, from synthesis, introduction on the market, transport, utilization, to elimination of the waste. Some important recommendations were identified and put forward during a nano event organized two months ago in the framework of the Belgian presidency of the EU Union (*Towards a regulatory framework for the traceability of nanomaterials*, Brussels, 14 September 2010). Regulatory and standardization work will be needed for several other key points as mentioned by several speakers during this symposium, such as the methodology of risk assessment, the validation of methods for the detection and characterization of NPs, the production of reference materials, the integration of these new

methods in current food labs, etc . The Organization for Economic Cooperation and Development is currently very active in this field.

Concluding thoughts

Finally, as general points to feed our discussions and thoughts, I would like to refer to the so- called “Deficit Model” or even “The New Deficit Model” (Simon Brown (2009) *The New Deficit Model*, Nature Nanotechnology (4) 609-611) that clearly illustrates the challenges linked to the development of emerging complex technologies. It seems that when dealing with such complex matters we are only able to see the top of the Iceberg and still we must allow the development of new technologies when they are really providing new opportunities of sustainable development. This will be only possible by adopting rather pragmatic approaches based, among others, on case by case studies and by paying attention to the real needs of our society.

POSTERS

Nanolyse: Nanoparticles in food - Analytical methods for detection and characterisation

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In its 2009 opinion on nanotechnologies in food and feed the Scientific Committee of the European Food Safety Authority makes a series of recommendations: "*.. in particular, actions should be taken to develop methods to detect and measure engineered nanomaterials (ENMs) in food/feed and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.*" (EFSA, 2009).

The above citation illustrates well the current situation with view to the analysis of engineered nanomaterials in food. At the moment, nanotechnology applications for the food sector are intensively investigated and developed. A number of nanomaterials are already in use as food additives or in food contact materials. At the same time, very limited knowledge is available on the potential impact of engineered nanomaterials on consumers' health. Exposure of the consumer to engineered nanomaterials cannot be determined due to the lack of appropriate analytical methods.

This gap is addressed by the FP7 project NanoLyse. The NanoLyse project will focus on the development of validated methods and reference materials for the analysis of engineered nanomaterials in food and beverages. The developed methods will cover relevant classes of engineered nanomaterials with reported or expected food and food contact material applications, i.e. metal, metal oxide/silicate, surface functionalised and encapsulate engineered nanomaterials. Priority engineered nanomaterials have been selected out of each class as model particles to demonstrate the applicability of the developed approaches, e.g. nano-silver for the metal nanomaterials. Priority will be given to methods which can be implemented in existing food analysis laboratories. A dual approach will be followed. Rapid imaging and screening methods will allow

the distinction between samples which contain engineered nanomaterials and those that do not. These methods will be characterised by minimal sample preparation, cost-efficiency and high throughput. More sophisticated, hyphenated methods will allow the unambiguous characterisation and quantification of engineered nanomaterials. These will include elaborate sample preparation, separation by field flow fractionation and chromatographic techniques as well as mass spectrometric and electron microscopic characterisation techniques. The developed methods will be validated using the well characterised food matrix reference materials that will be produced within the project. Small-scale interlaboratory method performance studies and the analysis of a few commercially available products claiming or suspect to contain engineered nanomaterials will demonstrate the applicability and soundness of the developed methods.

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Quantitative analysis of the physical characteristics of manufactured silica nanoparticles (NPs) used in food by advanced transmission electron microscopy

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Manufactured silicon dioxide (silica) nanoparticles (NPs) are chemically inert, pure white and free-flowing with a neutral pH. They do not affect the colour, taste, odor or nature of food. Silica NPs are applied in concentrations of up to 2 % of the end product weight to make granular and powdered food materials free-flowing, as anti-caking agents for food products high in oils or fats and to convert liquids into free flowing powders. Silica NPs are effective across a wide variety of food applications, including cheese, non-dairy creamers, food flavours, powdered drink mixes, seasonings and as tabletting aid for vitamin supplements.

Physical characteristics of silica NPs are important factors to evaluate their effectivity and the possible health risk of their application. By its high resolution, transmission electron microscopy (TEM) is one of the few techniques that allow direct visualization of nanomaterials. Conventional sample preparation techniques coupled to TEM imaging and (semi)automatic, threshold-based detection of NPs in electron micrographs are evaluated to measure the physical properties of silica NPs used in food on a per-particle-basis, and standard operating procedures were developed.

Conventional TEM imaging using a Tecnai Spirit TEM (FEI, Eindhoven, The Netherlands) operating at 120 kV allows directly visualizing the agglomeration state of the silica NP and the structure, size and shape of their composing primary subunits (Figure A). Digital micrographs were made using a 4*4 k Eagle CCD-camera (FEI) and stored in an iTEM database (Olympus, Münster, Germany) together with imaging and sample preparation data and with (intermediate) results.

Threshold-based detection of projected particles in micrographs using the 'Detection module' of iTEM allows measuring hundreds of individual particles simultaneously. These quantitative measurements include the electron density, size, shape, perimeter and surface area. Distributions of these parameters can be expressed on a number basis (Figure B).

Electron tomographic reconstruction allowed visualizing the morphology of the silica NPs in three dimensions. Tilt series of micrographs were recorded semi-automatically assisted by Explore 3D (FEI). These were aligned and reconstructed using Inspect 3D (FEI). Reconstructions were visualized using AMIRA (Mercury). Artefacts inherent to the analysis of projections of NPs were detected and interpreted. An estimation of the surface area, volume (Figure C) and volume specific surface area of NPs in suspension was obtained.

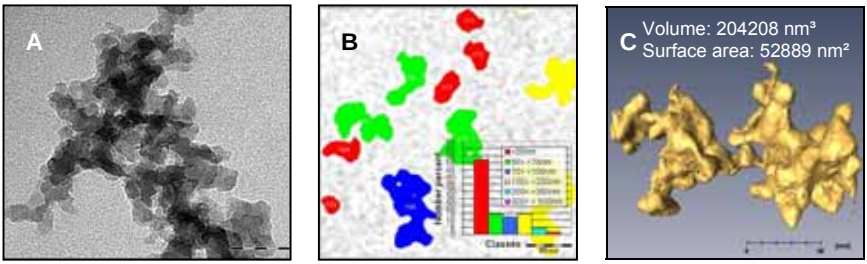

Figure (A) Micrograph showing an aggregate with subunits; (B) Annotated micrograph showing a mixture of NPs categorized by mean diameter; (C) Electron tomographic reconstruction of a SiO_2 aggregate. Bar 50 nm.

Quantitative analysis of the physical characteristics of gold and silver nanoparticles by advanced transmission electron microscopy

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Silver and gold nanoparticles (NPs) are commercially distributed on the internet at an international level as food supplements. The question of toxicological risks arises directly, because from a nutritional physiology point of view, noble metals are not required and because gold NPs have catalytic effects, while silver NPs have a biocidal effect. The latter is the basis of the use of Ag NPs as antimicrobial agents in food packaging, refrigerators and kitchen appliances.

To evaluate the possible health risks of these applications, knowledge of the physical characteristics of these metallic NPs is an important factor. By its high resolution, transmission electron microscopy (TEM) is one of the few techniques that allow direct visualization of such nanomaterials. Conventional sample preparation techniques coupled to TEM imaging and (semi)automatic, threshold-based detection of NPs in electron micrographs are evaluated to categorize the particles as NPs and to measure their physical properties on a per-particle-basis. Standard operating procedures were developed for this purpose.

Conventional TEM imaging using a Tecnai Spirit TEM (FEI, Eindhoven, The Netherlands) operating at 120 kV allows directly visualizing the structure, size and shape and agglomeration state of preparations of gold and silver NPs (Figure A). Digital micrographs were made using a 4*4 k Eagle CCD-camera (FEI) and stored in an iTEM database (Olympus, Münster, Germany) together with imaging and sample preparation data and of (intermediate) results.

Threshold-based detection of projected particles in micrographs using the ‘Detection module’ of iTEM allows to measure hundreds of individual particles simultaneously. These quantitative measurements include the electron density, size, shape, perimeter and surface area. Distributions of these parameters can be expressed on a number basis (Figure B).

Electron tomographic reconstruction allowed visualizing the morphology of the gold and silver NP in three dimensions. Tilt series of micrographs were recorded semi-automatically assisted by Explore 3D (FEI). These were aligned and reconstructed using Inspect 3D (FEI). Reconstructions were visualized using AMIRA (Mercury). Artefacts inherent to the analysis of projections of NPs were detected and interpreted. An estimation of the surface area, volume (Figure C) and volume specific surface area of particles in suspension was obtained and a correlation with the area and volume calculated from the equivalent circle diameter of projected NPs was demonstrated.

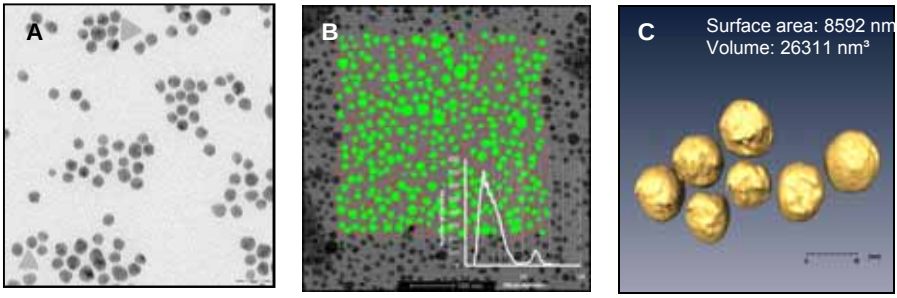

Figure (A) Micrograph showing various shapes of silver NP. Bar 50 nm; (B) Annotated micrograph showing a mixture of 4 nm silver NP (red) and 20 nm silver NP (green). Bar 100 nm; (C) Electron tomographic reconstruction of gold NP. Bar 20 nm.

Nano enhanced biosensors: the next generation of food safety diagnostics

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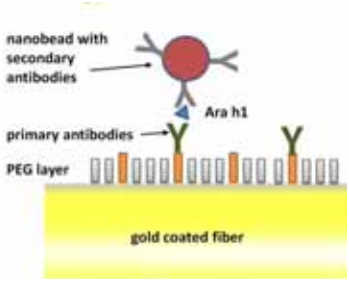
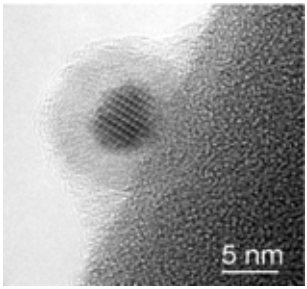
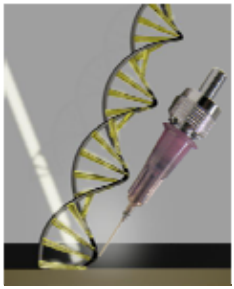
Following several national and international food crises, the customer awareness for food quality and food safety is growing. Driven by public opinion and growing regulations, producers search to control the origin and quality of all ingredients and the end product. An ever increasing challenge for the food industry with both social and economic impact is food allergy detection. Accurate and reliable product information is essential to inform a rising number of food allergic patients. This has created a growing need for reliable and accurate diagnostics, operable in the complex matrix of food.

In the last years, a remarkable progress has been witnessed in the development of biosensors and their applications in areas such as medical diagnostics, drug screening, environmental monitoring, biotechnology, food safety and security. Optical affinity biosensors based on surface plasmon resonance (SPR) present one of the most advanced biosensor technologies. Their ability to monitor the interaction between molecules immobilized to the surface of the sensor and molecules in solution have made SPR sensors a very powerful tool for biomolecular interaction analysis.

Recently, the MeBioS-Biosensor research group (www.biosensors.be) of the K.U.Leuven has developed an innovative technology based on fibre optic surface plasmon resonance. This biosensor has a great potential as reusable, cost-effective and label free biosensor for measuring antibody-antigen, DNA hybridization and DNA-protein interactions as has been demonstrated in a recently published paper (Pollet et al., 2009).







In this research we present the development of a cost-effective surface plasmon resonance probe for peanut allergen detection. Upon the gold surface of the optical fibre, a nanostructured biological layer is deposited. This layer is formed as a mixed self-assembled-monolayer, on which the biomolecules are immobilized. In order to improve the detection limit and to deal with variable matrix effects, nanoparticles (NPs) are used to purify and concentrate allergens from different extracts of food samples (e.g. chocolate). The use of NPs as carriers for the allergen proteins, also strongly amplifies the SPR response, and opens the door towards subnanomolar detection limits.

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Towards improved food diagnostics using micro- and nanofluidics

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In this abstract, we present 'lab-on-a-chip' technology as an innovative analysis platform to execute bioanalytical assays related to food diagnostics. The detection of undesired food constituents in a complex food matrix is a challenging task requiring highly selective and sensitive analytical methods. Within the research field of analytical chemistry, there is a growing tendency to downscale the analysis volume from the µL-scale to the nL-scale (or even lower). At this micro- and nanoscale, special phenomena occur which are studied in the research area of micro- and nanofluidics. Analysis systems with integrated microfluidic channels are often denoted as 'micro total analysis systems' (µTAS) or as 'lab-on-a-chip' systems. The main idea behind 'lab-on-a-chip' is the implementation, miniaturization and automation of some laboratory operations (e.g., sample preparation, mixing, reaction, separation, detection) on a microchip. It allows conducting cheap and sensitive analyses in a high-throughput context. Besides the practical advantages such as portability and minimal operation cost, miniaturization has the principal advantage of improving the performance of the analytical process. This is due to the compactness and the high surface area to volume ratios of microscopic fluid devices which make them an attractive alternative to conventional analysis systems. Furthermore, it is possible to reduce the molecular diffusion time significantly by handling microvolumes of fluids in small channels in comparison to handling large volumes of reactants in ordinary macro devices. As a consequence, (bio)chemical reactions and analyses are realized in a cheap and sensitive way. Different types of fluid flow can be obtained in these systems and three of them will be discussed on the poster. '*Continuous microfluidic*' systems deal with continuous fluid flow through microchannels. This continuous flow is achieved

by means of pressure driven flow (with the aid of an external mechanical pump) or by means of electrokinetic flow (with the aid of a high voltage source). As an alternative to continuous flow, fluids can also be transported as individual droplets also called '*digital or droplet based microfluidics*'. The transport of individual droplets can occur in a surrounding oil phase, pumped continuously through a microchannel ('*droplet microfluidics*'). Another type, '*digital microfluidics*', involves the transport of discrete droplets on a coplanar chip, achieved by the principle of 'electrowetting-on-dielectric' (EWOD). In this concept no intervention of micropumps, microvalves, and microchannels is needed as in the classical microelectromechanical systems.

All the aforementioned analysis platforms have a high potential to implement bioanalytical assays, related to food diagnostics. Current research in our group is now focusing on the implementation on the different analysis platforms of following bioanalytical assays: enzymatic assays, magnetic bead-based immunoassays, cell based assays, ... The poster will present a broad overview of these applications and indicate the advantages of the lab-on-a-chip analysis platform with respect to their use as a tool for food diagnostics.

Monitoring food safety with novel MIP-based sensors: Use of artificial nano receptors as versatile recognition elements

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A variety of molecules in the food chain can be of potential danger to human health. Exposure to these molecules can cause chronic diseases, such as cancer, or acute effects, such as nausea and headache. Therefore, it is of considerable importance to detect small concentrations of such potentially harmful molecules. For example, interest exists in the quantitative analysis of dye-molecules in food samples. This is a result of the fact that in many parts of the world, dyes belonging to the triphenylmethane family are used as an inexpensive method to control fungal attacks and infections in aquaculture. However, this class of dyes is linked to carcinogenesis and respiratory toxicity. Therefore it is extremely desirable to monitor their concentration in fish products before they enter the food chain. An example of a dye belonging to the triphenylmethane family is malachite green (MG), which is currently still being used in aquaculture in many Asian countries. A second example involves monitoring the freshness of food products. Spoiled foods have a high amine concentration due to the breakdown of proteins. One of the most important amines due to spoiling is histamine. Consuming food, which contains high levels of histamine, can cause severe sickness.

In view of the above considerations, we have developed molecularly imprinted polymers (MIPs), which can selectively bind histamine or MG. MIPs are nano-scale synthetic receptors, which mimic the recognition and binding behaviour of natural receptors. These tailor-made and highly selective artificial receptors are stable in a wide variety of environments. MIPs are comparatively low-cost to obtain and have a long shelf life. The building blocks to synthesize a MIP

comprise a careful selection of functional vinyl-type monomers. The MIP synthesis itself is based on a free radical polymerization of a pre-polymerization complex (functional monomers arranged around a target molecule). For the integration of MIPs into sensors, first of all the MIP synthesis has been optimized. To this end, various MIP morphologies have been prepared and tested in sensor setups. Examples include irregular shaped particles (bulk polymerization) and round shaped particles (suspension or precipitation polymerization) in a range of different sizes. In addition, the MIPs have been optimized to be able to bind (*i.e.* detect) their targets in aqueous environments.

Subsequently, the MIPs with the most suitable properties have been incorporated as synthetic recognition elements into both microgravimetric and impedimetric sensor setups. The impedimetric sensor consists of a conjugated polymer layer into which the MIPs are immobilized. Upon binding of the target, a change in the impedance occurs. In contrast, for the microgravimetric sensor upon binding of the target a frequency change is observed due to an increase in mass at the sensor surface. With the sensors it is possible to reproducibly detect low concentrations of histamine and MG in aqueous media. As a proof of principle, for histamine additional measurements have been performed in canned tuna samples.

In conclusion, MIPs are versatile recognition elements in both sensor setups combining the advantages of high affinity and selectivity, usually found for biological receptors, with those of polymeric materials. Furthermore, the possibility to measure in real samples confirms that these MIP based sensors hold significant promise towards actual applications in food science.

Biogenic nanoparticles: an innovative and safe alternative

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The applications of metal nanomaterials are booming in several sectors: electronics, catalysis, synthetic chemistry, textiles, water treatment,… Particles of zerovalent metals are conventionally produced by chemical reduction of metal salts. Introduction of these nanoparticles in the food chain, drinking water or the environment needs to be prevented. Therefore, they are impregnated on a carrier material, such as activated carbon or alumina oxide. Moreover, these carrier materials prevent the particles from aggregation into larger structures. Main drawbacks of the conventional methods are the use of expensive and toxic solvents, carriers, reductants and stabilizers. A new approach is to exploit the metal reducing capacities of bacteria for synthesis of metal nanoparticles. The nanoparticles are then finely dispersed on the bacterial cell surface. In this way, the bacteria serve as reducing agent and as carrier material as well (Hennebel et al., 2009a).

At the Laboratory of Microbial Ecology and Technology (LabMET) of Ghent University, several 'biogenic' materials with nanoscale properties have been successfully developed. *Shewanella oneidensis* is a metal respiring bacterium. In presence of an electron donor, it can efficiently reduce Pd(II) into Pd(0)-nanoparticles (De Windt et al., 2005). These 'bio-Pd' nanoparticles can then serve as catalyst, e.g. in dehalogenation reactions of chlorinated solvents, PCB's, halogenated micropollutants, … Moreover, they can easily be encapsulated in matrices such as polymeric beads or membranes. Several reactor types (membrane reactors, fluidized bed reactor) based on the bio-Pd technology were built, in which a clean and metal-free effluent was obtained (Hennebel et al., 2009b; Hennebel et al., 2009c; Hennebel et al., 2010). Recently, it was shown that *Shewanella oneidensis* is also able to form gold

nanoparticles. They might have interesting properties in catalysis and electronics (De Corte et al., 2010).

Silver ions can be reduced into biogenic silver particles by sugars on the cell wall of *Lactobacilli* (Sintubin et al., 2009). This biogenic silver has a strong antimicrobial activity towards bacteria and viruses. Different filter systems for drinking water hygienisation with this biogenic silver have been operated successfully (De Gusseme et al., 2010).

Biogenic materials can thus serve as a powerful tool in catalysis, drinking water production and disinfection. Attachment to the bacterial cell wall prevents them from being released and taken up by humans, making them attractive as an environmental friendly and safe alternative for conventionally synthesized nanoparticles.

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Immobilization of biogenic silver nanoparticles in membranes for safe disinfection of contaminated water

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Water reuse is becoming essential in increasing the reliability of the water supply. Yet, there is a growing concern about the outbreak of waterborne diseases related to poor treatment of wastewater, meant for reuse or reclamation. Moreover, contamination of drinking water and the subsequent outbreak of waterborne diseases are the leading cause of death in many developing nations. Therefore, the development of innovative drinking water quality control strategies is of the utmost importance in this decade. Significant interest has arisen in the use of silver nanoparticles for disinfection of water. However, the loss of nanoparticles in the food chain should be avoided because of their potential impacts on human health.

In this study, Ag^0 nanoparticles were produced on the bacterial cell surface of *Lactobacillus fermentum*. This unique combination of a microscale bacterium with Ag^0 particles of 11.2 ± 0.9 nm is referred to as biogenic silver or bio-Ag^0 (Sintubin et al., 2009). The bacterial carrier matrix hereby served as scaffold to prevent the particles from aggregation (Hennebel et al., 2009). Consequently, the inhibitory concentrations of bio-Ag^0 were lower than for chemically produced silver nanomaterials. Application of 12.5 mg bio-Ag^0 L^{-1} was sufficient to inhibit the growth of *Escherichia coli* and *Pseudomonas aeruginosa* (Sintubin et al., 2010) and 5.4 mg bio-Ag^0 L^{-1} caused a 4 log reduction of murine noroviruses in one hour (De Gusseme et al., 2010). It was demonstrated that the slow release of silver ions (Ag^+) is the main mode of action of biogenic silver.

We have immobilized bio-Ag^0 in microporous polymeric membranes in order to prevent their loss in the water. Inactivation of UZ1 bacteriophages using these membranes was successfully demonstrated and was related to the slow release of Ag^+ from the membranes. At least a 3.4 log decrease of viruses was achieved

by application of a membrane containing 2500 mg bio-Ag^0 m^{-2} in a submerged plate membrane reactor operated at a flux of 3.1 L m^{-2} h^{-1}. After filtration of 31 L m^{-2}, the concentration of Ag^+ was below the drinking water limit (= 100 µg L^{-1}). In addition, a virus decline of > 3 log was achieved at a high membrane flux of 75 L m^{-2} h^{-1}.

This is the first report to demonstrate water disinfection by immobilization of bio-Ag0 in polymeric membranes. This membrane technology might become a safe alternative for water disinfection or can enhance disinfection efficacy in conjunction with existing techniques.

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Zinc oxide: a promising material for improving future food packaging

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Food packaging has many different functions. One important function is to maintain the food quality by reducing absorption, desorption and diffusion of gases. Barrier properties can be improved by combining packaging materials with other high-barrier materials through coating, blending, lamination or metallization. A recent method for improving polymer properties are nanocomposites: polymers filled with particles with at least one dimension in the nanometre range, e.g. nanoclay. An alternative for nanoclay into the polymer matrix can be the use of inorganic nanoparticles, such as zinc oxide. The incorporation of zinc oxide nanoparticles in either conventional plastics, such as polypropylene (PP), or biodegradable polymers, such as polycaprolacton (PCL), has been the topic of the research here presented. Zinc oxide nanopowders with varying morphologies (i.e. rods, plates, spheres) and dimensions are synthesized by means of solvothermal or hydrothermal methods and subsequently incorporated into the polymer matrix to form a nanocomposite foil. From preliminary results it can be concluded that the morphology of zinc oxide nanoparticles can have an influence on the gas permeability and the mechanical properties as well as on the UV properties of the polymer. Another approach to improve both gas barrier and UV properties is by depositing zinc oxide nanolayers on top of the substrate. Various methods, including vacuum based and solution based routes, exist for the deposition of ZnO coatings. As there is a growing interest to use biodegradable polymers based on renewable materials,

research is carried out on the deposition of zinc oxide nanolayers on these biodegradable plastics, such as poly(hydroxybutyratehydroxyvalerate) (PHBV). In the presented study, the possibilities of sol-gel and sputter deposition are evaluated and discussed.

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Identification of immune-related gene markers following interaction of engineered nanoparticles with human intestinal epithelial cells

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Nanotechnologies offer a variety of possibilities for application in the food domain, such as in food additives, nutritional supplements, packaging, and food storage devices. Besides ingestion, unintended human exposure to nanoparticles (NPs) may occur when NPs are released to the atmosphere during industrial production processes, or in derivates of agricultural products discharged to waste waters or soils. Ingestion of NPs may pose human health risks, but data on oral exposure to specific NPs and any consequent toxicity are scarce, and the implicated biological and molecular processes are largely unexplored.

In this study, the human adenocarcinoma Caco-2 cell line, widely used as an *in vitro* model of the intestinal barrier, was exposed to suspensions of monodispersed, spherical cobalt (7 nm) and cerium dioxide (4 nm) NPs at non-cytotoxic concentrations. A genome-wide transcriptomics study was performed to reveal the genes and processes involved in immune-related effects after NP exposure. Parallel experiments were set up involving cobalt chloride and cerium nitrate exposures to correct NP-specific responses by subtracting those induced by the corresponding ions. Statistically significant changes in gene expression as compared to solvent-treated cells (mean |fold-change|>1.5 (n=3), $p<0.05$) were evaluated after 3, 6, 10, and 24 hours of exposure.

Nanoparticle exposure mainly induced downregulation of gene expression in the Caco-2 cell line. The cell model showed NP-dependent kinetics in its transcriptional response, with the number of differentially expressed genes (DEG) being highest after 3 hours of exposure to cobalt NPs (# 1410), and then

gradually decreasing up to 24 hours. In contrast, cerium dioxide NPs induced a sustained high number of DEG from 6 hours of exposure onward (# 3372). For both NPs, approximately 6% of DEG were related to immune function, and this percentage remained similar over time. In contrast to cerium dioxide NPs, which induced on average 0.4% up- and 5.4% down-regulated immune genes over the entire exposure duration, cobalt NPs induced an increasing portion of up-regulated genes with a maximum of 2.3% after 10 hours.

To allow for identifying candidate gene markers of cell-NP interaction independent of NP type, immune-related DEG which were significantly affected by both cobalt and cerium dioxide NPs in the Caco-2 cell line were selected after correction for ion-induced gene responses. At the different exposure times, twenty three immune-related DEG were observed, which each showed a transient response to NP exposure. The gene encoding protein tyrosine phosphatase, receptor type C (*PTPRC* or *CD45*) was the only one being significantly induced over a prolonged time period (at 6 and 10 hours), and therefore may constitute a promising marker.

Our data suggest that cobalt and cerium dioxide NPs give rise to a distinct immunological response in intestinal epithelial cells, with only few molecular players in common. We identified *PTPRC* gene as a candidate marker that can be used for more targeted toxicity testing. The investigation triggers off additional research to validate the results using different technologies and to test an extended set of NP and/or other cell models.

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Potential toxicity of titanium dioxide nanoparticles

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Titanium dioxide is the most important and widely used white pigment. It is also used among others as an additive in food, cosmetic creams and as an excipient in medicinal products. This additive is authorized for human consumption under the EC No - E 171 (Annex of Directive 94/36/EC concerning food additives).
Titanium dioxide is generally considered chemically inert and nontoxic. However, titanium dioxide nanoparticles can represent a potential health risk as they may cross biological barriers in relation to their nano-size.
As there is an increasing number of studies focused on potential toxicity of titanium dioxide nanoparticles, following brief review could improve our understanding of toxic potential of titanium dioxide nanoparticles.
In recent studies *in vitro* and *in vivo*, interaction of titanium dioxide nanoparticles with biological systems have been investigated. Some of results are presented and discussed.
In vitro studies:
The interaction of TiO_2 and biological systems was demonstrated for example on cells of mouse brain (microglia), rat embryo cells, Syrian hamster embryo cells and human bronchial cells. Neurotoxic and genotoxic effects were observed but results presented in studies are not always consistent.
In vivo studies:
Distribution of TiO_2 nanoparticles to tissues was demonstrated. However the biological barriers should protect the tissues, nanoparticles of TiO_2 can overcome this barriers. Several studies showed that exposure to TiO_2 nanoparticles could result in an accumulation of nanoparticles in the mouse cranial nerve system, in lungs and in testis and cause inflammation, fibrosis and even DNA damage.

Both *in vitro* and *in vivo* studies have provided evidence that titanium dioxide nanoparticles can enter the tissues and that the distribution of the particles is a function of their size and surface. Probably oxidative stress in diverse cell types is responsible for damage or apoptosis of cells.

In this context should be mentioned that the European Parliament has demanded mandatory labelling of all products containing nano ingredients and acknowledged that specific methods to test the safety of nanomaterials are needed.

Nanoparticles: between food handling and skin penetration

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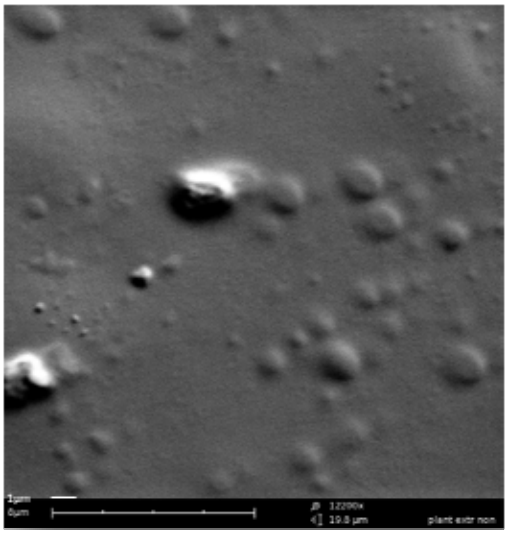
Nanoparticles (NPs) and even microparticles (MPs) are ubiquitous present in the food chain. They occur naturally (e.g. resulting from the biomineralisation process in plants) and/or can be added deliberately (e.g. to increase flavour or colour in the so-called “nanofood”). However, because of proportional larger surface area, NPs have (a) increased chemical reactivity and adsorption properties, (b) can form complexes with cell components like proteins and nuclear materials, (c) possess characteristic pharmacokinetics including their ability to circumvent the immune system.

In the food industry as well as by the consumer itself, food handling is inevitable. Due to skin contact, NPs present on food and food packaging can migrate from these materials and penetrate into the skin.

Depending on the matrix which contains these nano- and microparticles, distinct skin penetration behaviour has been observed (Boonen et al., 2010). The infinite gamma of food/contact matrices (e.g. waxes, oils, …) are thus expected to have different influences on the skin barrier function, resulting in different particulate penetration effects. Eventually, NP and MP might reach the viable epidermal layers or even the dermis. Subsequently, they can induce cytotoxicity in living cells like keratinocytes and Langerhans cells or penetrate further into the dermis, followed by uptake in the lymphatic system and ultimately end up in the systemic circulation. As a result, toxicological aspects of food baring NP and MP, their matrix and skin penetration cannot be ignored and need to be further investigated.



Scanning electron microscopic image of differently sized NP and MP in a plant extract

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Predicting the physicochemical fate of metallic nanoparticles in aquatic environments

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Engineered metallic nanoparticles can be beneficially used in consumer products, agricultural production and environmental remediation processes, but they can also have adverse effects on humans and their ecosystem. Transfer of these particles between aquatic and solid phases significantly affects their exposure to humans, organisms and plants. It is determined by the kinetics of several physicochemical transformation processes that can occur upon release of the particles into the water phase. The particles can be adsorbed from the water phase onto surfaces or they can aggregate into larger particles and precipitate, decreasing their mobility and availability in the water phase. On the other hand, they can also be dispersed by natural organic matter (Yang et al., 2009), and dissolution of material from the particle surface itself into solution can occur (Handy et al., 2008a).

The surface properties of the nanoparticles are known to be one of the most important factors that govern their stability and mobility as colloidal suspensions, or their adsorption or aggregation and deposition in aquatic systems. They are mainly dependent on parameters such as temperature, ionic strength, pH, hardness, particle concentration and size, etc. In addition, occurring redox reactions and/or association of nanoparticles with natural organic matter or surfactants added to maintain the stability of colloidal suspensions, will further increase the complexity of interactions. Accordingly, particles released into different types of aquatic environments (e.g. varying hardness, salinity and redox potential) are expected to behave in various ways, which in turn leads to different exposure of humans, organisms and plants (French et al., 2009; Handy et al., 2008b). In addition, coagulated, precipitated or adsorbed nanoparticles could be transformed and/or remobilised on medium or longer term when

environmental conditions change (e.g. pH, redox potential, hardness, organic matter contents and salinity). Although most factors that could affect the fate in aquatic environments have been identified, lots of technological, (eco)toxicological and biogeochemical studies are currently being confronted with a lack of ability to analyse and predict the physicochemical occurrence of metallic nanoparticles in these aquatic environments under environmentally relevant conditions (e.g. at environmentally relevant concentrations).

Further sustainable development of nanotechnology thus needs the ability to predict the physicochemical fate of engineered metallic nanoparticles released into aquatic environments. Therefore, kinetics of changes in occurrence of metallic nanoparticles as affected by characteristics of the aquatic medium are currently being studied in research projects conducted at the Laboratory of Analytical Chemistry and Applied Ecochemistry of Ghent University (ECOCHEM, Prof. G. Du Laing). This is done by monitoring physicochemical occurrence of a range of engineered metallic nanoparticles after incubating solutions containing these particles under different environmental conditions (e.g. redox, pH, salinity, presence of different types of suspended material and solid phases) in microcosm and mesocosm experimental setups. To be able to conduct these studies, analytical methods based on hyphenation of chromatographic separation techniques (e.g. size exclusion and hydrodynamic chromatography) with the use of a sensitive metal detector (ICP-MS) are being tested and further developed in a first step. Results that will be obtained can be used to assess factors affecting e.g. the transfer of metallic nanoparticles from soils to food crops in agricultural production systems, their long-term catalytic activity and release from packaging materials into food matrices, and their behaviour during digestion in the gastrointestinal tract.

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Mixed PEO/PAA brushes for the control of protein adsorption

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Introduction The creation of smart surfaces with designed functionalities is currently of growing interest in the field of biomaterials. Polymer material presents a large variety of properties which can be tuned to create such smart surfaces. Mixed polymer brushes are of particular interest since they offer the possibility to combine different properties on a same material.

This study aims at creating surfaces showing tuneable properties with respect to protein adsorption, using the combination of a protein-repellent polymer and of a polymer which adopts different behaviours towards proteins depending on the environment. Brushes of poly(ethylene oxide) (PEO) are known to prevent protein adsorption. Brushes of poly(acrylic acid) (PAA), a weak polyelectrolyte, are able to swell or shrink according to the pH and the ionic strength (I) of the solution. Using an appropriate combination of these two parameters, mixed PEO/PAA brushes are expected to either repel proteins, or allow their immobilization.

These smart surfaces have promising applications as food packaging (a modification of the packaging due to food degradation could inform visually the consumer) or as pipe coating in food industry (in order to immobilize some valuable products and to release them by changing the environmental conditions).

Methods The adopted strategy is the “grafting to” approach with thiol-functionalised polymers which self-assemble on gold surfaces. PEO-SH had a M_w of 2,000 and was provided by Polymer Source (Dorval, Quebec, Canada). PAA with a midchain disulfide bond PAA-S-S-PAA (M_n=6,500) was synthesized

as described previously (Van Camp et al., 2010). These polymers were immobilised on gold from ethanol, water, or 50:50 solution of these solvents. The created surfaces were then submitted to human serum albumin (HSA) adsorption (concentration 200 µg/ml, pH adjusted with HCl and NaOH, I adjusted with NaCl).

Polymer assembly was assessed by means of contact angle measurements, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). Polymer assembly and protein adsorption were monitored *in situ* by means of quartz crystal microbalance with dissipation monitoring (QCM-D).

Results & Discussion Exploratory experiments have been performed on homogeneous PEO or PAA brushes in order to study the assembly process as well as HSA adsorption from solutions with different pH and I. The successful assembly of both polymers on gold could be observed by contact angle measurements, AFM and XPS. Brush thicknesses of about 1 nm for PEO and about 3 nm for PAA were measured by AFM. These results are compatible with the level of Au signal detected by XPS. QCM-D measurements performed in real time show the swelling or shrinking of PAA depending on pH and I. These observed effects are in agreement with those obtained previously by means of other techniques by other groups.

QCM-D monitoring of HSA adsorption allowed conditions of PEO assembly to be identified for which HSA adsorption was nearly totally prevented. On PAA, adsorption could be prevented at high pH while it was enhanced when pH was lowered. These effects were modulated by I. Other groups have shown that when protein adsorption occurs on PAA, proteins are adsorbed deeply inside the brush and retain their secondary structure as well as their activity (Hollman et al., 2008; Haupt et al., 2005; Czeslik et al., 2004).

Conclusions & Perspectives Assembly conditions leading to prevention of protein adsorption on PEO and PAA homobrushes were identified, as well as conditions which provide a mild environment for proteins on PAA. Promising results on mixed brushes are obtained.

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Nano-electronics: technological opportunities for applications in the food chain

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As Europe's largest independent research centre in nano-electronics, imec has a strong contribution in the development of deep-submicron CMOS and post-CMOS processes and technologies. But, there's more to nanotechnology than IC processing alone. Indeed imec is entering the fascinating nano world of biology. In that framework, groundbreaking research is oriented towards nano materials (particles, nanowires…) and nano devices (NEMS, image sensors…), bioelectronic applications (biosensors, neurons-on-chip…) and organic electronics.

An example of these technological developments is hyperspectral imaging. In an IWT-SBO project named CHAMELEON, imec is designing the next generation vision system that exploits the benefits of spectral information. The goal is to enable and demonstrate flexible, but domain-specific hyperspectral imaging systems for relevant industrial applications in food.

Traditionally, hyperspectral imaging systems make use of 2D arrays in the 400-1000 nm range, while non-destructive quality assessment of food and agricultural products is typically done with NIR spectroscopy in the 1000-2500 nm range. Therefore, the potential of hyperspectral imaging in the NIR for rapid and non-destructive assessment of quality properties (e.g. sugar, starch and water distribution in fruit, presence of foreign substances in grain, fat content in meat,…) will be investigated.

An industrial fruit grading case is currently being explored. For this application there is a great interest for quality based sorting, but a first challenge is the high image capturing and data processing speed which is required. The current throughput of commercial grading lines is 10 fruits per second per lane. Current imaging systems were found not to be suited to achieve this throughput. Hence,

this application asks for a smart camera system with on-chip processing and data reduction to reduce the bandwidth requirements for data transfer from camera to grading computer and the computational effort needed for the grading decision algorithms. A second challenge for optical quality inspections in the agro-food industry is the fact that biological products are often layered and typically one is not so much interested in surface quality, but rather in the quality of the deeper layers. Also this can be tackled by this hyperspectral imaging approach.

Imec wants to demonstrate the hyperspectral scatter imaging system in real-life settings for grading fruit according to firmness and sugar content.

In a project with Flanders' FOOD, which is the innovation platform for the Flemish food industry, the potential of these and other nano-based innovations for application in the food chain (food safety and quality diagnostics, traceability, packaging,…) are scanned. The joint project is called 'Intelligence For Food'. Interested companies are still welcome to join.

Hightech Europe: fostering innovation in food processing

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Nanotechnology in addition to biotechnology and ICT shows strong innovative power within high-tech food processing technologies. Although excellent research has been carried out, this has not always led to substantial innovations on the European market. This European innovation paradox is at least partly due to difficulties in knowledge transfer. Especially for possibly sensitive developments such as in nanotechnology, it is of paramount importance that complete and clear information is transferred, not only from science to industry, but also to consumers and legislators.

The approach of HighTech Europe, the first European food processing Network of Excellence is the establishment of a European innovation window for the food processing sector. The network will serve as the instrumental strategic incubator for the development of new concepts and ideas enabling competition of the European agro-food industry.

To inform SMEs on potential cost-efficient innovations, the network will launch an Online Interactive Technology Portal. In this portal, a Lighthouse Watcher scans through scientific findings for innovation sources (with a special focus on nanotechnology, biotechnology and ICT), characterizes the underlying scientific principles and identifies target food engineering operations (the so-called Science Cube approach). These potential innovations are matched with industrial needs that are mapped within the project.

Furthermore, existing regional knowledge transfer chains are interlinked into a Knowledge Transfer Tube to optimize R&D findings towards industry and to provide a Europe-wide overview of industrial needs. Sharing of R&D tasks, facilities and personnel is investigated. In addition, new routes for implementation are explored, including a Knowledge Auction, where knowledge providers present their unique findings to knowledge buyers. Successful

implementations of high-tech innovation (again with a special focus on nanotechnology, biotechnology and ICT) in food or feed industry along a knowledge transfer chain will be awarded with the European Food Implementation Award.

The final goal of the project is to set up a European Institute for Food Processing to strengthen the European food industry for global competition. Building blocks for this institute developed within the project include a sustainability action plan, innovation guidelines, fundraising, knowledge transfer schemes, and an Agenda for the White Book on food processing technologies. The latter will allow decision makers to objectively weigh up pros and cons of technologies.

Evaluation and adaption of *in vitro* testing for the assessment of the toxicity of nanomaterials

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Nanoparticles and nanostructured materials have been broadly developed in the past decade and are used in an increasing number of applications and industries (drugs, food, sport, leisure, luxury, surface treatment...). The emergence of materials with new properties, some similar to atmospheric ultrafine particles, raises the question of their safety and especially the adequacy of assessment tools and prevention of risk with these new materials.
Risk assessment is particularly difficult in the case of nanomaterials as many uncertainties remain regarding the potential dangers of these new agents. The data on the toxicity of nanomaterials is scarce.
Since late 2006, we are developing a program that participates in the evaluation of the potential risks posed by nanomaterials to human health. The evaluation of the robustness of *in vitro* testing methods (cell viability, oxidative stress and genotoxicity) for hazard assessment of nanomaterials is one of the subjects of research in the toxicology laboratory. If thorough characterization of the material is crucial in the hazard evaluation, the question of the interactions of the particles with the different test systems used for *in vitro* toxicology is also of great importance. With particles in suspension a classic endpoint quantifier like fluorescence can easily be perturbed and more simply, the particles can interact with the test reagents and induce bias in the outcome of the evaluation. It is therefore important to establish robust methodologies to evaluate the effects of nanoparticles on living systems. We are presently participating in a program where the robustness of genotoxicity methods is assessed with, among others, food grade nanomaterials.
This research supports a proposed scientific expertise into broader programs that focus on strategies to define the boundaries of existing risk assessment and propose new methods (Working Party on Manufactured nanomaterials, OECD,

or the European joint action "Nanogenotox") or for the implementation of new regulations (REACH).

Consumer's attitudes towards nanotechnology and other food technologies in Catalonia

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Understanding emerging trends in public perceptions on nanotechnology applications in food production is important in order to know its acceptability among consumers. The present study reports attitudes towards food applications of nanotechnology in connection with the acceptability of various new food technologies. We have used a qualitative research approach in order to investigate the factors that may contribute to accept or refuse certain new food technologies in Catalonia (Spain): genetically modified foods, high-pressure processing and nanotechnology. Focus groups were conducted with forty-eight consumers recruited from Catalonia.

The analyses revealed that perceptions on nanotechnology and other technologies are generated with low knowledge. Subjective knowledge is shown to be an important factor in explaining attitudes. Public controversies about technologies appear as a negative factor of acceptability. As other studies report, consumers rely on cognitive shortcuts or heuristics to make sense of issues on which they have low levels of knowledge. Also, general attitudes concerning food production, science and technology have a strong impact on public attitudes about nanotechnology and genetically modified organisms and trust in institutions appears as a positive factor for consumer confidence. However, when risk perception exists, these technologies are only accepted if perceived as necessary to solve any problem or to obtain any benefits.

The results show that information about nanotechnologies in Catalonia is lower compared to other technologies and controversy about them is not observed. As a result, public perception of food production is not influenced by nanotechnology. Most of the consumers are confident about its benefits but more research is expected. However, food applications raise fear among consumers concerned on food production, and analogies with genetically

modified foods are made. Therefore, if controversy appears in the media, there is some risk of refusal by consumers.

Notes