Preface

Nanomaterials

New materials promise novel applications. This particularly holds for nanoscaled materials which can exhibit novel materials properties that appear upon reducing the dimensions of their bulk counterparts. Indeed, it is the fundamentally different physical and chemical properties of nano-sized materials which not only promise new nanotechnological applications but attract scientists' attention to their fundamental scientific importance. In particular, nano-sized materials have novel properties and applications in the fields of optics, magnetism, electricity, catalysis, and biomedicine. For biomedicine, besides the fundamentally novel properties, it is also the mere size reduction which opens a new field of applications: nanoparticles conform to the dimensions of living cells, and can be internalized. Therefore, control and sensing at the *cellular* level can be envisaged by applying intracellular probes by transferring nano-sized biocompatible devices into the cells. These devices (particles) must meet the demands of targeted investigation of relevant cell parameters as well as manipulation of the cells.

One of many possible examples of biomedical application of nanomaterials is cell killing by the use of magnetic nanoparticles exposed to external magnetic fields. Magnetic fields only very weakly interact with organic materials and do not cause known adverse effects. External magnetic fields can be used to "pull" or "push" magnetic nanoparticles into deep layers of (human) tissue (e.g. solid tumours). After being internalized, magnetic nanoparticles can hence be used for various purposes without need for surgery. External static magnetic fields fix ferromagnetic nanoparticles at a precise position; gradient fields move them and alternating (AC) fields lead to local heating. The latter effect can be utilized for so-called "hyperthermia", i.e. a therapeutic anti-cancer treatment which raises the temperature of tumor tissue in vivo [1]. This method applies the fact that a cancer cell-killing effect is caused when a temperature above 41–42°C is maintained in the target volume. One outstanding example of the use of ferromagnetic nanoparticles is "magnetic fluid hyperthermia" (MFH), i.e. the controlled heating of tumour tissue, which is commercially used by one of the associated industrial partners of our Network [2]. In MFH therapy, magnetic nanoparticles are infiltrated deep into tumour tissue and inductively heated by applying AC magnetic fields.

Carbon Nanotubes

The use of nanoscaled functional materials requires that the materials are shielded within a protective coating until they reach their site of action. A promising way to provide such a shield appears to be the coating, e.g., of the magnetic iron with a carbon shell by insertion of the magnetic material inside carbon nanotubes and thereby protecting the biological environment and the filling material from each other. Degradation of filling materials is avoided and their potential toxicity and adverse effects are suppressed.

Carbon nanotubes (CNTs) are hollow carbon structures with one or more walls, a small diameter on the nanometer scale and a large length in comparison. They exhibit a well-ordered arrangement of carbon atoms linked via sp^2 bonds which renders them among the stiffest and strongest fibres known. In addition to mechanical properties, they exhibit superior electrical and thermal conductivity. Depending on the numbers of walls, the diameter is between 0.8–2 nm for single-walled CNTs and 2–100 nm for multi-walled ones. Their lengths range between hundreds of nanometers and up to tens of micrometers and even longer, yielding very high aspect ratios.

The mechanically and chemically stable carbon shells can be opened, filled and closed again without losing their stability. Experiences in filling CNTs range back to their discovery in 1991 [3]. Since then, extensive work has been performed to synthesise CNTs and to functionalise them both exohedrally, i.e. by attaching functional elements to the outer shell, and endohedrally by filling with various materials. CNTs can be filled with metals, semiconductors, salts, organic materials, fullerenes, etc., either during the synthesis process or through subsequent opening, filling, and closing of the CNT. The container feature of CNTs allows, in principle, simultaneous filling of CNTs with different materials thereby combining multiple functionalities in one kind of carrier. In this way, CNTs provide a smart carrier system on the nanometer scale which can be filled with tailored materials to address specific purposes.

Beyond the shielding effect against a biological environment, the carbon coating offers an interface for further exohedral functionalisation with suitable (bio-) molecules. A major task of such functionalisation is the stable dispersion of the nanoparticles in aqueous media which still is a major challenge. Both non-covalent and covalent modification strategies can be applied among which the former preserve the pristine CNT structure while covalent modification introduces partial damage of the outer wall but in general yields better dispersion. Dispersion becomes even more crucial if ferromagnetically filled CNTs are envisaged which exhibit an increased tendency to agglomerate due to magnetic interactions. In addition, exohedral functionalisation is needed to make the highly symmetric carbon structures compatible with real biological environments. Nowadays, functionalisation is also aimed at performing dynamic tasks such as target recognition, target transformation, transport, or electrical conduction in the living cell. Some of these functions can be provided by biomolecules, such as DNA or proteins (enzymes, antibodies). Importantly for any therapeutic or biomedical usage, functionalised CNTs can effectively cross biological barriers such as the cell membrane and penetrate the individual cell. Recently, it was shown that DNA-wrapped SWCNTs were enveloped by cancer cells and they were used to deliver a lethal dose of microwave radiation to the cancer cells [4]. In contrast to such non-covalent wrapping of CNTs with biomolecules, any covalent chemical exohedral functionalisation needed to improve the biocompatibility of CNTs will structurally perturb the external wall. This however in the case of multi-walled CNTs does not affect the overall carbon shielding. A variety of methods exist for exohedral functionalization of carbon nanotubes, some of which have been successfully applied for the conjugation of proteins, drugs and fluorescent dyes and active tumor targeting in vivo.

Although a very large number of toxicity studies has been published no clear picture evolves for CNTs in general. There are many conflicting reports mainly from in vitro studies. One reason for this ambiguity is the fact that—similar to other nanoparticles—a large number of specific factors govern the toxicity of CNTs such as their shape and size (diameter, length), the number of shells (single- or multi-walled CNTs), agglomeration state, homogeneity, and surface chemistry. In particular, the concentration of CNTs is not necessarily a main parameter. Ambiguity also results from a lack of standardisation and of thorough characterisation of, e.g., defects of the outer shell or potentially toxic contaminants. Such non-carbon material originating from the synthesis process of pristine empty CNTs can amount to several percent of the total mass. These factors may account for much of the reported differences so that details of synthesis, choice of catalyst particles, washing procedures and dispersion methods have to be considered thoroughly.

The successful application of CNTs for biomedical application faces a variety of severe problems to be solved. These include:

- Synthesis of CNTs with tailored functionalities and uniform morphology
- Modification of CNTs to become compatible to biological systems, in particular long-term stable dispersed in aqueous solution
- Detailed study of interaction with biological environments (immune response, toxicity, interaction with the single cell)
- In vivo testing for actual therapeutic and diagnostic purposes such as imaging (contrast agents, markers), sensing (nanoparticle-based diagnostics) and cancer treatment (hyperthermia, targeted drug delivery)

The Book

As described above, extraordinary physical and chemical properties render carbon nanotubes promising candidates as biomedical agents for diagnostic and therapeutic applications. Filling the nanotubes with tailored materials forms packages in which the active content is encapsulated by a protecting carbon shell. The nanoparticles are efficiently internalized by cells. Chemical alteration of the external surface confers biocompatibility and the potential to target specific cells or tissues. This book explores the potential of multi-functional carbon nanotubes for biomedical applications by combining diverse and detailed contributions from chemistry, physics, biology, engineering, and medicine. The overview of the current state-of-the-art addresses different synthesis and biofunctionalisation routes and shows the structural and magnetic properties of CNTs relevant to biomedical applications. Particular emphasis is given to the interaction of CNTs with biological environments, i.e. toxicity, biocompatibility, cellular uptake, intracellular distribution, interaction with the immune system and environmental impact. The insertion of NMR-active substances allows diagnostic usage as markers and sensors, e.g. for magnetic imagingand contactless localtemperature sensing.The potential of CNTs for therapeutic applications is highlighted by studies on chemotherapeutic drug filling and release, targeting and magnetic hyperthermia studies for anti-cancer treatment at the cellular level.

The book is presented in four sections:

I Fundamentals: Synthesis of Multifunctional Nanomaterials and their Potential for Medical Application

Raffa et al. introduce the *Physical properties of carbon nanotubes for therapeutic applications*, emphasizing the unique, unprecedented combination of electrical, magnetic, optical and chemical properties which holds great promise for the development of a new class of CNT-based drugs and therapy. Raffa et al. provide a review of the physical properties of CNTs and discuss the current state of the art as well as future perspectives of applications of CNTs in the field of biotechnology.

The applications of *Carbon nanotubes in regenerative medicine* are reviewed by Paratala and Sitharaman. The focus of regenerative medicine is on developing methods that can be applied to create functional tissues, to repair or replace tissues/organs lost due to trauma or disease. In this respect, the structural and mechanical properties of CNTs make them applicable for use as composites for tissue engineering. CNTs can act as delivery vehicles for drugs and gene therapy and thus are suitable for therapeutics in regenerative medicine. Further, as discussed in this chapter, they also show promise as contrast agents for non-invasive in vivo molecular imaging.

The production of uniform nanoparticles with controlled particle size and morphology is one of the main challenges in applied nanoscience. In this respect, remarkable progress in synthesis of carbon nanostructures has been made in the last two decades. Aspects of synthesis are addressed by Lukanov et al. who discuss various functionalisation routes of *Filling of Carbon Nanotubes with compounds in solution or melted*. The filling of CNTs with a particular functional material represents a remarkable example of matter manipulation at the nanometer scale aimed at designing a tailored nanosized device. These authors review different approaches to filling. The confinement of matter inside CNTs can lead to significant structural modifications, depending on both the bulk structure of the confined material and the CNT inner diameter, which likely causes modifications of the physical properties (electrical, optical, mechanical, thermal) of the composites.

The capacities and potential for nanofabrication of a tailored magnetic material relevant to magnetic imaging and magnetic hyperthermia applications is highlighted by the encapsulation of materials such as Fe, Co, Ni. Within this context, the synthesis of CNTs filled with magnetic materials has been widely investigated, especially with iron due to its excellent ferromagnetic characteristics. A detailed overview of diverse preparation routes of Fe-CNTs is given by Borowiak-Palen et al. *Filling Carbon Nanotubes: containers for magnetic probes and drug delivery*. Here, the effects of varying parameters in the chemical vapour deposition (CVD) synthesis method on the structure of the final material is shown, which varies in respect of the amount of iron encapsulated in the cavity, tube diameter and the number of graphitic walls forming the CNTs. The filling of hollow CNTs through wet chemistry reactions (as a post-synthesis route) and CVD process (filling during the synthesis of CNTs) is also addressed in this chapter, with the particular example of exploiting the potential of CNTs nanosized containers filled with therapeutic drugs, exemplified by the chemotherapeutic cisplatin.

II Magnetically Functionalised Carbon Nanotubes for Medical Diagnosis and Therapy

Sobik et al. describe, in the chapter *Magnetic nanoparticles for medical diagnosis and therapy*, the intrinsic properties of ferromagnetic CNTs and their potential to provide accurate medical imaging and medical therapy (magnetic hyperthermia, targeted drug delivery, etc…) at the cellular level. The unusual magnetic and magnetization properties of these "nanowires" (extremely thin, long assemblies of iron atoms within CNTs) are illustrated. These properties are compared in situations mimicking free suspension in body fluids, or immobilization in solid tissues.

In the chapter *Feasibility of magnetically filled carbon nanotubes for biological applications: From fundamental properties of individual nanomagnets to* *nanoscaled heaters and temperature sensors*, Lutz et al. present a detailed overview of fundamental magnetic properties and magnetisation reversal of individual nanostructures and of ensembles. In addition, heating effects in applied AC magnetic fields are highlighted. This effect is of great interest for thermoablation treatment, i.e. the killing of cells (for example within solid tumours) by direct local heating, The heat output and other relevant properties of various magnetic materials are compared, together with discussion of the control of heat output. NMR studies show that a non-invasive temperature control by virtue of a carbon-wrapped nanoscaled thermometer is feasible.

Anuganti and Velders (*Nuclear Magnetic Resonance Spectroscopy and Imaging of Carbon Nanotubes*) review the potential use of CNTs as contrast-enhancing agents for Magnetic Resonance Imaging, in vitro and in vivo with a detailed investigation of different types of CNT structures and properties by solution-state NMR, Solid State NMR and High-Resolution Magic-Angle-Spinning (HR MAS) NMR spectroscopy.

III Interaction with Biological Systems

Current developments in nanoparticle technology provide a vast variety of new particles, with different morphology and surface chemistry. If these are to be used in biology and medicine, constant and continued study of how they interact with biological materials, especially human tissues, must be carried out, to assess their benefit versus any potential risk. Despite various industrial applications of CNTs and their large scale synthesis relatively little is known in detail about the interaction of CNTs with biological environments. In the chapter *Exploring carbon nanotubes and their interaction with cells using atomic force microscopy*, Lamprecht et al. discuss how atomic force microscopy can be used to study some of the fine details of the interactions of functionalized CNTs with mammalian cells. This technique has the advantage that it does not require extensive processing or chemical treatment (fixing) of the sample before measurements are made, so that systems can be studied close to physiological conditions.

Neves et al. in the chapter entitled *Uptake, intracellular localization and biodistribution of carbon nanotubes* present a detailed, pharmacologically-oriented summary of in vivo studies of biodistribution and circulation of CNTs, together with extensive in vitro studies of uptake into cells and intracellular localisation. It is described how CNTs can be designed to form pharmaceutical complexes, which allow them to enter blood circulation, target cells, deliver payloads, be exocy-tosed and finally eliminated from the body. As nanoparticles for medical applications, CNTs shows promise in offering lower toxicity with enhanced efficacy.

CNTs and other nanomaterials when placed in contact with human (or other mammalian) body fluids and tissues are recognised by the immune system and may also interact with other systems, such as coagulation proteins, other blood plasma components, and cell-surface proteins. Binding of immune system complement proteins activates the complement system and results in strong binding of several complement proteins to the CNT. Such complement activation may influence subsequent interaction of the CNT with cells and tissues and is a predictor of potential toxicity in animal models. Rybak-Smith et al. (*Recognition of Carbon Nanotubes by the Human Innate Immune System*) report on what is known of the molecular events, such as protein binding, that occur when CNTs are placed in contact with human blood or lung fluids, and the biological consequences of these interactions.

Industrial-scale synthesis of CNTs for a wide range of uses is now a reality, on the scale of hundreds of tons per year, and this brings into focus the need to study industrial and environmental toxicology of nanotubes. Flahaut, in the chapter *Toxicity and environmental impact of carbon nanotubes*, provides an overview of current knowledge of the life-cycle of CNTs, from manufacture to final destruction, covering aspects such as accidental uptake into living organisms, potential for toxicity, biopersistence, routes of elimination and degradation.

IV Towards Targeted Chemotherapy and Gene Delivery

More detailed considerations of the application of derivatised CNTs as drug carriers are developed in this section of the book. Heister et al. (*Carbon nanotubes loaded with anticancer drugs: a platform for multimodal cancer treatment*) present an overview of cancer, current treatments and the need for new developments. Strategies for design and targeting of drug carriers are described, followed by a detailed review of the experimental use of modified CNTs, in vitro and in vivo, in the killing of tumours or cancer cells. These authors conclude that "drug delivery is clearly one of the most promising bioapplications of carbon nanotubes and the coming years will reveal the suitability of this rather novel material in comparison to more established drug delivery systems, such as liposomes".

Haase et al. in the chapter *Carbon Nanotubes filled with Carboplatin: Towards Carbon Nanotube supported delivery of the chemotherapeutic agent Carboplatin* present a detailed experimental study of the development of a single system, CNTs filled with the anti-cancer drug carboplatin. The maintenance of the structure of the enclosed carboplatin was confirmed by spectroscopic techniques, and the cytotoxicity of the CNT–cisplatin composites explored using cell lines.

In the final chapter, *Functionalized carbon nanotubes for gene biodelivery*, Sanz-Beltrán et al. describe the design of CNTs derivatised for the delivery of DNA into cells (an approach to gene therapy or to therapeutic use of RNA). Non-covalent functionalization of CNTs with RNA and with amphiphilic polypeptides as model systems, the efficacy of these coatings in forming stable dispersions, and the capacity of the modified CNT to deliver functioning genes to cells is presented in detail.

The experimental work described in these chapters is very wide-ranging, diverse and multidisciplinary. Experimental techniques used range through those of several branches of physics, organic and inorganic chemistry, oncology, immunology, molecular biology, and the structures studied range from metal ions, through protein and DNA macromolecules, synthetic particles, up to animal cells, tissues and whole animal bodies. These illustrate the complexity of work and range of scientific disciplines required for the design of new modes of biomedical therapy.

The majority of the work presented has been done in the multi-disciplinary European Marie-Curie Research Training Network CARBIO (*Multifunctional Carbon Nanotubes for Biomedical Applications*) [5]. The Network provided collaborative training of young researchers in experimental biomedical nanoscience aiming at developing and optimizing multi-functionalised nanocontainers for human medical applications. The project has elucidated the usage of CNTs for previously known applications and has also shown their feasibility for novel applications not encountered before. A particularly high potential is found when multiple functionalities are combined, e.g., drug transport, imaging and local heat generation. The network's results which have been published in more than 100 papers allow better understanding and assessment of possible benefits and potential toxicological and environmental risks of a promising nanomaterial which is a prerequisite for responsible application of nanotechnology.

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