Recent Advances in Nanotubes for Orthopedic Implants

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Abstract

With ever-growing interest and rapid development, the field of biomedical engineering has quickly assumed great importance as one of the pillars of modern science. Especially significant has been the stress given on research and development in the field of orthopedics with implants being a subject of much interest. Among much new advancement in the field of development and design of orthopedic implants, one of the most important ones has been the inculcation of nanotechnology for more viable and reliable implant design. The application of nanotechnology in implants has seen the extensive use of nanotubes i.e. nanoporous structures with tubular shape. In this review paper, we take a look at the various aspects of these three basic applications of nanotubes in orthopedic implant development. We aim to unravel some of the unique features, which make nanotubes an interesting addition to the already diversifying field of implant design.Nanotubes have been used in orthopedic implants in three principal aspects: 1) The use of drug-loaded nanotubes on the implant surface as novel reservoirs for systematic and controlled drug release in order to act as prophylactic or to prevent certain specific post-implantation infections. 2)The use of certain nanotubular structures with high mechanical properties as reinforcing agent in conjunction with bone-simulating bio-active materialsto create durable prosthetics. 3) The use of metal oxide or alloy nanotubes on implant surface promoting greater osseointegration and cell viability due to the alteration from a slightly hydrophobic surface to an extremely hydrophilic surface. Also, owing to prevalent concerns regarding the cytotoxicity and malignancy inducing characteristics of certain nanotubes, the safety aspect forthe use of nanotubes in the orthopedic implants is also discussed.

Keywords: TiO2 nanotubes; silver nanoparticles;orthopedic infections; titanium; osteoblasts; antibacterial; antimicrobial; anodization.

Introduction

Implants have been known to be used for the aid of normal human body functioning since the middle ages[1]. The installed medical devices ranged from nails to screw to plate to entire limbs made out of wood or metals. Common applications of implants are for orthopedic reconstruction like repairing fractures[2], providing joint arthrodesis[2], fixing non-unions[2], complete or partial joint arthroplasty reconstruction of the spinal cord[2]. These devices, among a host of different functions, primarily aim to enable proper alignment of bones and facilitate usual functioning in the course of physiologic loading by giving the structure mechanical stability. Thus the implants provide relief from pain and render natural use of the replaced/reinforced body part. This is considered to be critical for the process of recovery and resumption of normal functioning of the body parts[3].The biological aspects of bone repair are assisted to an extent by decreasing unwanted shear stress due to greater stability fostered in the vicinity of the bone fractures. Also, devices that minimize the extent of micromotion at the junctions between bone and implants in apatite-free joint replacements, and undesired motion between opposed bone surfaces in spinal reconstruction aid in bone formation and remodeling[4,5]. The ultimate clinical outcome is squarely dependent on both the mechanical and biological aspects of bone healing, which are also very closely interlinked. Orthopedic implants have historically been designed as mechanical installation with

consideration of the biological repercussions of the implant being sidelined as byproducts of stable fixation, internally or externally, of the fixture to the ambient bones or soft tissues. Especially in case of fracture fixation it has been largely accepted that bones, if provided with adequate support and stabilization are prone to self-heal. This approach is indicative of a rather dangerous notion however as only in the US per year there are around 100,000 cases of nonunion and in excess of 500,000 fractures with unusually slow union[6]. Osseointegration of the implant with the surrounding bone is not always possible in cementless joint replacements ultimately culminating in implant migration and possible loosening[7].

Physicochemical compatibility and mechanical stability are specifically important in the case of bone implants. Orthopedic implants use distinct materials because of specific advantages those materials have for particular applications. A material which possesses all the desirable properties at once has proven to be rather elusive. Ceramics like aluminum oxide or zirconium oxide and metallic alloys such as nickel-titanium are usually selected due to their high stiffness and strength for hard tissue replacement. But a potential pitfall of this application is possible bone atrophy resulting from substantial difference between stiffness of the implant and that of the host tissue which leads to the phenomenon of stress-shielding[8]. Additionally, these materials are bio inert in nature since active stimulate of bone-formation process is not accomplished by them. Moreover, introduction of implants into the body brings with it the risk of microbial infection more so in case of fixation of joint-revision surgeries and open-fractured bones implant infections are caused by bacterial attachment to the surface of the implant and consequent biofilm production in the vicinity of the installed implant[9]. Orthopedic devices need to foster integration between host tissues and the implant surface as well as oppose colony formation by microbes. The conundrum lies in the fact that the biomaterial surfaces which allow greater degrees of implant cell integration, proliferation and growth also provide congenial environment for microorganisms which have similar attachment mechanisms as host cells. Moreover, the implant layers which are specifically intended to resist bacterial infestation and creation of biofilm are less likely to have effective osseoconductive and osseointegrative properties. These various problems plaguing the effective installation and use of orthopedic implants have been recently addressed effectively and extensively through the emerging field of Nanotechnology. Nanotubes specifically have been found to be of immense usefulness in this context. This paper will review the progress that has been made during the last decade on the use of nanotubes to not only improve the osseointegration properties of implant materials but also the antimicrobial and drug loading/release by such nanostructures.'

An Overview on Implant Systems and Their Challenges

Man-made medical devices utilized to replace missing biological structure, support damaged biological structures or enhance existing biological structures are called implants. Biomaterials as an industry as well as a subject of academic interest have seen rapid progress over the past few years due to progress in technology, increased spending power and higher life expectancy. The turnover of the biomaterials device market was in excess of $100 billion back in 2008 and has been projected to cross the $250 billion mark by 2014[10]. From a financial perspective most important among biomaterial devices are orthopedic biomaterials. This provides great impetus to the field of research concerning production and furtherance of orthopedic related biomaterials. Orthopedic biomaterials constitute implants and arthroplastic artifacts that are usually composed of metals, metallic alloys, polymers and ceramics[11]. Another important section of orthopedic materials includes scaffolds that help in effective regeneration of host tissue. These are mostly composed of biodegradable polymers[12]. Figure 1 shows a typical him implant made of Ti-6Al-4V at the stem section and CoCrMo alloy at the head. A socket made of Ti with inner coated Polyethylene is in direct contact with the CoCrMo alloy when inserted in the body.
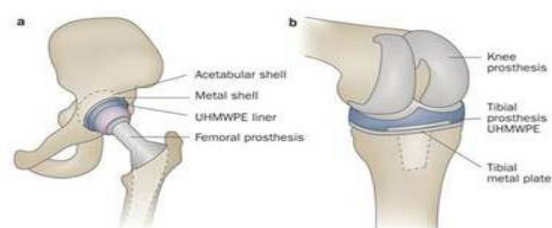

Figure 1: (a) A hip replacement prosthesis, and (b) A knee replacement prosthesis. UHMWPE refers to ultra-high-molecular-weight polyethylene. [13]

The very first problem plaguing implants and their successful installation is the lack of osseointegration between the device and the surrounding body environment (Figure 2). Most traditional implant materials are either bioinert or not biocompatible. Functionalizing these may lead to a conditional biocompatibility but even then the rate of osseoconduction is relatively low and causes immense pain and frustration to the implant recipient [14]. This lack of integration comes from the difference in structure and properties between bones/tissues and implant material. Bones and adjoining tissues have a composite hybrid network of three level of architecture ranging from nano-scale to the micro and macro-scale. This is usually absent in the implant materials leading to repressed rates of cell adhesion and proliferation. Stress shielding due to different values of strength and stiffness of the device and body material is another source of failure of the implant functionality [15].

Inveterate infections are primary causes of post surgery problems that often require further operation in spite of apparently normal functioning of orthopedic implants from a mechanical and biological perspective [16]. About 5% of patients having orthopedic fracture and subsequent reconstructive devices suffer from infection totaling to over hundred thousand incidents per annum in the US itself [17]. In case of operations for complete replacement of the hip,

the percentage of operation-site infection is somewhere between 0.2 and 2.2 [18]. In-situ infections for implants constitute one of the most major reasons behind post operative trauma and even mortality, and also come at a rather dear price to the implant recipients and the medical community.

Implant infections may result from characteristics of the body of the implant recipient like hormonal disorders or congenital medical problems/allergies and surgical procedures[18]. The location, nature and structure of the implant itself including dimensions, composition, surface characteristics and purpose are significant parameters[17]. Considerable reductions in the occurrence of implant-associated infections have been observed through the use of prophylactic systemic antibiotics. However, localized treatment with antibiotics or bactericidal substances can be effected through other mechanisms. For example, bone cements loaded with antibiotics have been found to be effective as anti-infection agents in case of arthroplasty[19].

Figure 2: (a) Radiograph image on a patient shows the signs of loosening and fracturing of femoral component of implant: American Academy of Orthopedic Surgeons (AAOS)type III femoral defect. [20] (b) Radiograph of a hip spacer of a 63year old man with late periprosthetic infection of the left hip[21].

Nanotubes for Medical Implants

Nanotubes are cylindrical hollow structures, which have diameters in the range of 1-800 nm and very high length to diameter ratios to the extent of 132,000,000:1[22]. Carbon nanotubes (CNTs) (Figure 3) are also can be considered for biomedical implants because of their mechanical, thermal, or electrical properties[22]. Recently, specific interests have been generated over the fabrication of nanotubes made of metal oxides. The most commonly used nanotube materials are titania (Figure 4), alumina, silicon, boron nitride, manganese dioxide, tungsten disulphide, zinc oxide, molybdenum disulphide and tin sulphide[23].
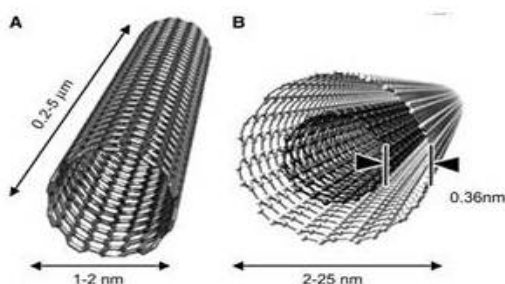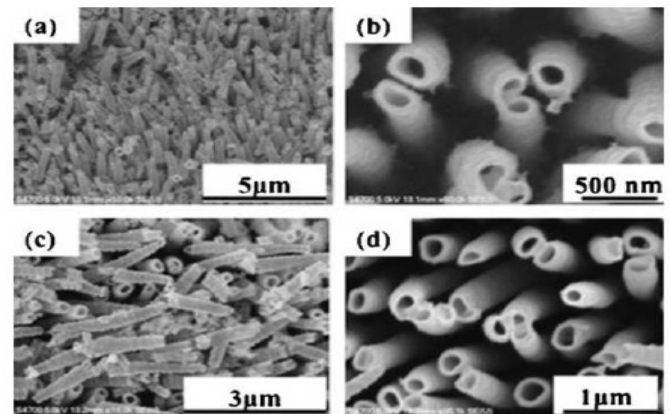

Figure 3: Conceptual diagram of single-walled carbon nanotube (SWCNT) (A) and multiwalled carbon nanotube (MWCNT) (B) delivery systems showing typical dimensions of length, width, and separation distance between graphene layers in MWCNTs[24].

The SEM images of (a) as-synthesized TiO2 nanotubes arrays and (b) close up of nanotubes shown in [a]

Figure 4: Titanium dioxide nanotubes prepared by Pt electrode (a)–(f). In general, titania nanotubes have lengths between 7 and 10μm andinner diameters ranging from 60 to 130 nm. (a) and (b) are amorphous, (c) and (d) are crystalline, (e) is as-synthesized and (f) is annealedTNT[25].

The reasons for use of nanotubular structures in orthopedic implants can be classified as follows:

(a) Improved levels of osseointegration: The structure of the bones consist of elements at three different dimensional scales: (a) the macrosized cortical as well as cancellous bones; (b) the microscale structures including haversian systems, osteons, and lamellae; (c) the nanoscale features like non-collagenous organic proteins, fibrillar collagen and embedded mineral crystals. A hybrid of micro-scale pits in the implant surface itself and nano-ordered layers of tubes are used to mimic the cellular environment thus favoring rapid bone accrual process (Figure 5).
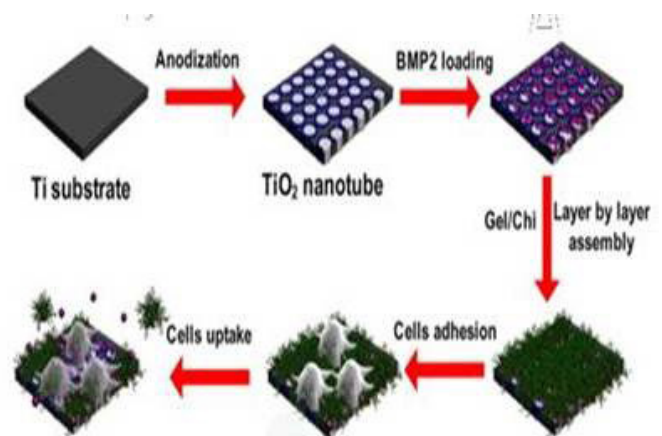

Figure 5: Graphical illustration showing the process of TiO2nanotube layer formation on Ti substrate and its effect on cell uptake and adhesion[26].

(b) Anti-inflammation/anti-microbial functionality: Nanotubes by means of their particular ordered structure have been found to possess antimicrobial properties. Moreover, loading nanotubes with anti-inflammatory drugs or antimicrobial agents can further reduce the risks of post-operative infection. This could be done through anti-inflammatory agents like dexamethasone, drugs such as Indomethacin, Gentamicin and Vancomycinor antibacterial chemicals like silver nanoparticles or zinc oxide nanoparticles (Figure 6). Functionalized nanotubes can target specific cells and then release the loaded drugs in response to triggers like change in pH or temperature.
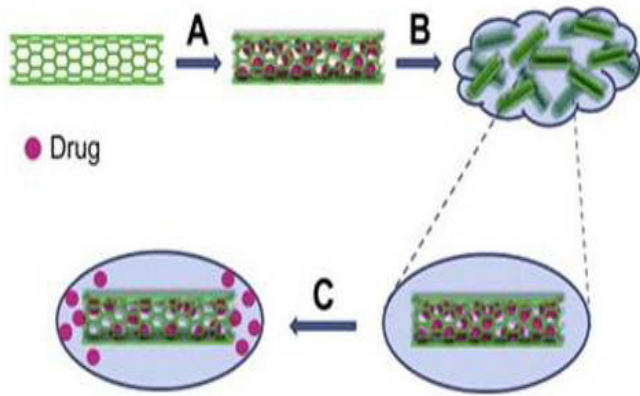
Figure 6: Schematic of the drug loading and release process of CNT nanoreservoirs. A) Drug solution is filled into the interior of acid treated CNTs through sonication; B) Pyrrole is added to the suspension containing CNTs and Dex and electropolymerization is carried out; C) Drug is released from CNT nanoreservoirs to surroundings through diffusion or electric stimulation[27].

(c) Shielding/Scaffolding effect: Use of certain nanotubular structures (like Carbon Nanotubes/Boron Nitride Nanotubes) with high stiffness, shear resistance and compressive strength as reinforcing agent in conjunction with bone-simulating bio-active material like Hydroxyapatite or Polypropylene fumarate to create effective prosthetics allowing for rapid osseointegration (Figure 7).
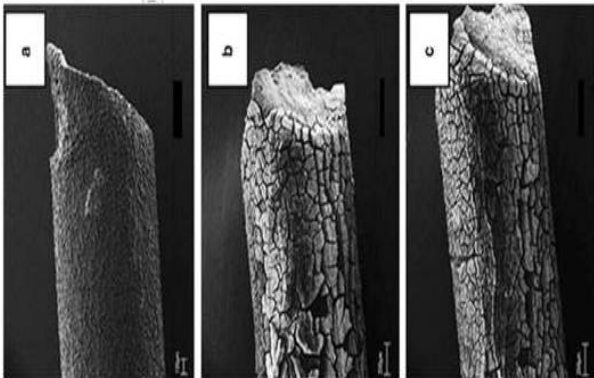

Figure 7: SEM images of HA–CNTs coating on Ti alloy wire with different deposition times (a) 1 min, (b) 2 min, and (c) 3 min[28].

Nanotubes for implant surfaces: The bio-mimicking effect for osseointegration

All complex organisms are composed of an architectural hierarchy ranging from the nano-level through micro-level to the macroscale. Complex macro scale actions are effected by synergistic combination of processes at smaller dimensional scales. It starts with extracellular proteins like collagen which build the tissue structure and have dimensions of about 1.5 nm in diameter and 200 nm in length, a 5 to 10 nm thick encapsulating cell membrane, proteins for cell adhesion that effect this through binding sites of length 5nm situated at the end of their 20nm long body and biochemical compounds like DNA which through changes in conformity can produce structural and functional effects[29]. Cellular activities such as cell attachment, locomotion, growth, gene expression and fate of stem cells are mostly controlled through nanosized features. Hence, development of biomaterials incorporating nano-dimensioned facets has received considerable attention with a view to provide favorable environment for normal cell behavior (Figure 8).

In case of nano-patterned surfaces for implants, the chemical stability and structural properties of the layer is useful for providing greater reactive surface area and higher number of sites for preferential adsorption of proteins. Such biologically inspired nanotube layers are better suited for attachment of osteoblast cells due to the presence of nanoscale holes allowing greater penetration of cell filopodia. The elevated surface roughness and increased surface affinity for calcium also causes preferential deposition of apatitic minerals thus rendering the surfaces the valuable property of bioactivity.

One of the most important parameters governing the successful proliferation of cells on any surface is the cell-cell/Extracellular matrix (ECM)-cell interaction[30]. Nearly 300 different proteins are responsible for forming the core of the ECM. The proteins are polymeric units formed by cross linking of insoluble monomeric singular units which have a fiber like appearance. This ECM constitutes the characteristic environment, which determines cell behavior and proliferation. Changes in the elasticity of the environment have a determining effect on the cell behavior. As the number of sites of adsorption increases there is a subsequent increase in the area over which the force is distributed thereby reducing the localized stress, thus simulating an increase in elasticity. Apart from the interaction between the cell and the ECM cell signaling determines the functioning of the tissues. These intercellular interactions are a consequence of the action of nanoscale molecules, which also regulate the production, properties and composition of the ECM and translocation of biological matter. Disruption in the natural order in the cell and unwarranted remodeling and production of the ECM can result from the disturbance of intracellular interaction mechanisms. ECM contains hydrocarbon in the form of collagen and inorganic carbonate apatite which acts as the mineral constituent of the bone. The nanotubes, owing to their particular dimensional range, are considered to be analogous to the fibrillar protein constituents in the ECM, especially those in the collagen.

During installation of the implant, blood comes in contact with the implant initially. Blood owing to its high concentration of plasma proteins adsorbs to the surface of the implant and the extent of adhesion is controlled by the hydrophilicity of the surface[29].This interaction between the implant and the blood is responsible for plasma protein accrual and is a determining factor in the osseoconduction stage of osseointegration. Nanotubular surfaces can be manufactured to have superhydrophilic surfaces, which in this regard, would significantly improve the interaction characteristics, besides allowing for easier absorption of nutrients over the course of the recovery period. Thus, wettability of the nanotubular surface is another factor, which makes it suitable for heightened cell adhesion allowing for easier osseointegration following implant placement in the body.
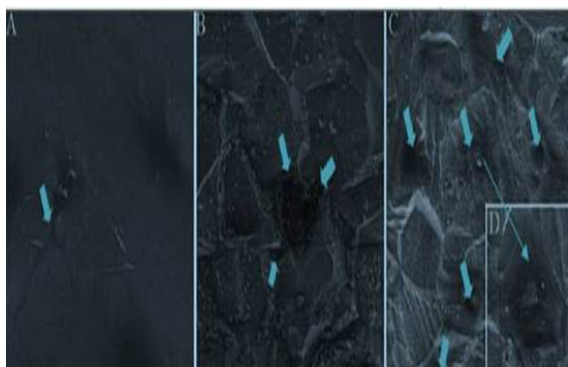
Figure 8: The SEM images after culturing and adhering osteoblasts on three different Ti substrate surfaces for 2 h. (A) Smooth interface. (B) Micro-treated interface. (C) Hierarchical micro/nano interface. (D) Local amplification of single osteoblast's adhesion on micro/nano interface[31].

Nanotubes for Implant surfaces: The antimicrobial and anti-inflammatory properties

Orthopedic implant installations are executed with utmost attention being paid to sterilizing the wound, the implant and the implantation site to minimize the risk of infection. This, however, does not always prove to be enough. According to medical reports, up to 30% of all transcutaneous fracture fixations and 13% of bone supplementation procedures are followed by infection in the recipient's body causing unbearable pain and necessitating both painful and extremely expensive surgery[32].

The chances of infection are determined by the surgical site and the procedure. The pathogen colonization of hardware is enhanced by the host response to implantation. The recipient's body secretes copious quantities of serum proteins which accumulate over the implant material and are responsible for cell proliferation and repair of damaged tissues. However, these serum proteins are also used by microbes for adsorption and virulence[32]. Imparting implants with drug-eluting properties to ensure localized delivery of drugs is a favorable remedy to this problem.

The nanotube approach towards addressing the issue of post-surgery infection can be broadly divided into two categories- functionalized carbon nanotubes loaded with antimicrobial agents and metal oxide/alloy nanotubes loaded with nanoparticles/antibacterial drugs. Carbon nanotubes have been widely studied for their antibacterial drug loading and releasing property and constitute one of the most efficient ways of achieving this goal (Figure 9). Pristine CNT aggregates when in contact with cells cause damage to the cell membrane resulting in cell lysis[33]. However, CNTs have their antibacterial capacity regulated by their physicochemical properties. Thus, functionalization with metallic nanoparticles or peptides has been suggested to improve the antimicrobial activity of CNTs[34]. However, the antimicrobial efficiency is diminished due to the low chemical stability of the groups used for functionalizing. This could be addressed through covalent functionalization with cationic chemical groups. It is common to deploy functional groups which have cationic charge and through strong oxidizing potential produce high oxidative stress in the bacterial membrane. Carbon

nanotubes are prime candidates for such antimicrobial drug release agencies owing to their elevated levels of cellular uptake, increased surface area and the ability to be easily conjugated with different drugs showing superior efficacy, high specificity and reduced side effects. Common mechanism for CNT functionalization involves sonication with lysine with microwave irradiation. Also, it is common to functionalize CNTs with carboxylic and cephalexin linkers. Other approaches for functionalizing CNTs involve coating the surface of the nanotubes with shallow antibacterial films based on layer by layer assembly of biological polyelectrolytes such as anionic poly (L-glutamic acid) and cationic poly (L-lysine).

In functionalized state the CNTs are capable of altering the glycolysis pathway in the bacterial cell, which is responsible for producing energy to sustain stress[35]. Apart from this, the miniscule nanotube diameter cause partitioning and partial penetration of nanotubes into the cell wall. Oxidative stress is the other significant factor, which contributes to the antimicrobial effect of the nanotubes[35]. The drug release characteristics from nanotubes are functions of the nanotube dimensions and the drug loading concentration and can be accurately controlled to get release over specified periods of time and in particular quantities. Antibacterial agents like azithromycin, gemifloxacin, and hydroquinone can be effectively dispersed using these nanotubes. Also, nanoparticles of Ag, ZnO, CdS and Ag2S are effective antibacterial agents owing to their strong oxidative nature.
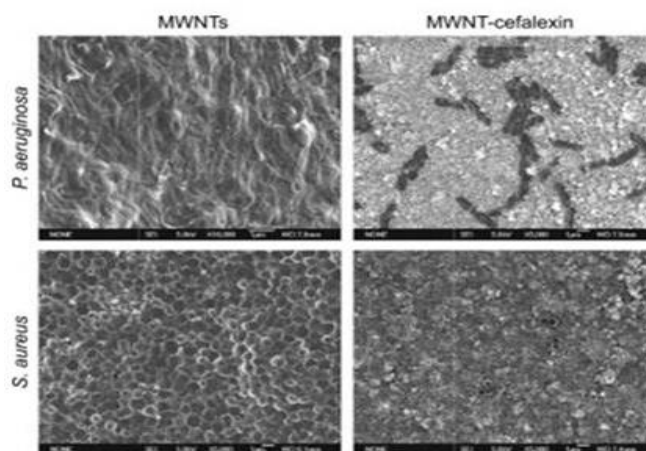

Figure 9: Scanning electron microscopyimages of P. aeruginosa and S. aureus on the MWNTs and MWNT-cephalexin upon 3-hour exposure[36].

In case of metal oxide or metal alloy nanotubes the anti-infection effect comes from four possible sources: the nanostructuring of the nanotubes itself, the functionalizing of the nanotubes with anti-inflammatory agents, nanotubes decorated with antimicrobial nanoparticles and nanotubes loaded with antibacterial drugs. Nanotubes of metal oxides and alloys, especially those made of Titanium, Titanium-Al-V alloy and Zirconia have been widely studied for their inherent antimicrobial properties. Reports31show a substantial decrease in bacterial adhesion and proliferation on the nanotubular surfaces as compared to polished or micro-rough implant surfaces. This is the result of three principal factors: (1) the nanostructuring of the surfaces cause protrusion of nanotubes into the cell wall of the bacteria thus causing cell death by triggering of a stress

response.(2) The presence of fluorine on the surface of the titania nanotubes as a remnant from the fluorine containing electrolyte has antibacterial effect due to the strong oxidizing nature of fluorine that can cause hydrolysis of enzymatic pathways that are responsible for protein adsorption (Figure 10). This can result in muting of genes, which are responsible for cell proliferation. (3)The ability to absorb UV light and subsequent formation of highly reactive radicals such as those of hydroxyl groups or peroxide groups influence the antibacterial nature of the titania. These allow TiO2 to possess bactericidal abilities whilst also enabling them to act as cleaning agents for swift degradation and removal of harmful bacterial excreta.
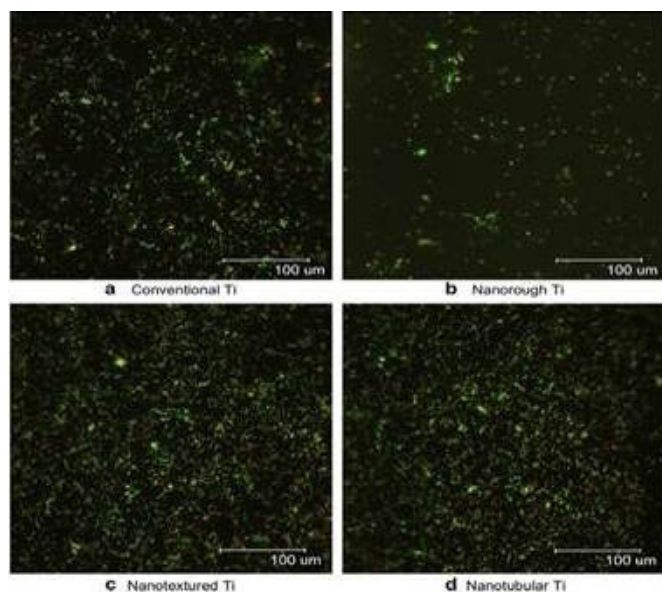

Figure 10: Fluorescent micrographs of decreased S. aureus colonies on (b) nanorough Ti compared to all other substrates and increased bacteria colonies on the (c) nanotextured and (d) nanotubular Ti compared to (a) conventional Ti after 1 hour. These micrographs were representative of S. epidermidis and P. aeruginosa[37].

Inflammation and its aftermath in the period following implant installation surgery constitute serious challenges for the wellbeing of the patient and effectiveness of the implant. Chronic inflammatory response to debris from the implant or osteogenic cell stress is also another major cause of concern in this context[38]. Deficiency of cellular anti-oxidant capacity is responsible for many pathological inflammatory conditions, which results in high release of reactive oxygen species (ROS). ROS are known to cause osteoclast damage through lowering of the bone mineral density[39]. Most metallic or alloy implant surfaces despite their biocompatibility cause increase in intracellular ROS levels which can potentially lead to chronic inflammation and reduced bone regeneration[40,41]. Thus osseoconduction and regeneration in the implant vicinity can be positively influenced through the use of pharmacological anti-oxidizing agents for surface modification of the implants.

Antimicrobial agents such as selenium/silver nanoparticles, ZnO nanoglobules, quantum dots of metal salts, organic molecules like Chitosan are being extensively studied for their potential role in limiting the occurrence of postoperative infection[42]. Among these silver nanoparticles, ZnO, and chitosan are specifically important. Silver in mo-

lecular state are inert but upon hydrolysis in the body stream release Ag+ ions. Silver ions catalyze the oxidation of the hydrogen atoms present in the thiol groups of enzymes thus releasing water while causing respiratory arrest and death of the cell due to the formation of disulfide bonds between two thiol groups (Figure 11) [43]. Also, the hydrogen bonding among two anti-parallel strands is disrupted when Ag+ enters the cell and intercalates between the purine and pyrimidine base pairs thereby denaturing the DNA molecule. Inclusion of the silver ion in the cell of the bacteria causes high amount of oxidative stress, which can result in the extermination of the cell.
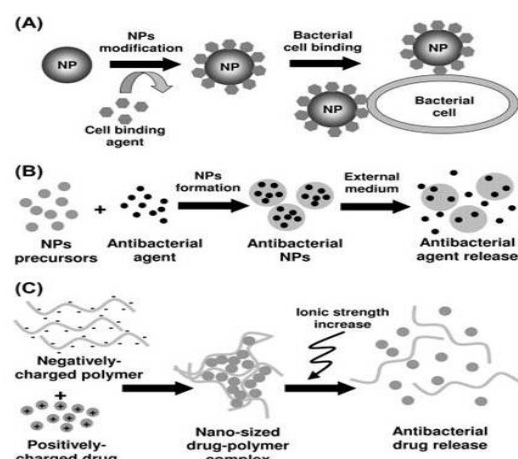
Nano-sized drug formulations with antibacterial activity: (A) NPs binding on bacterial cell, (B) drug release from organic NPs matrix and (C) drug release from nano-sized complex.

Figure 11: A schematic drawing showing the various mechanisms of antibacterial activities exerted by silver nanoparticles[43].

ZnO is a metal oxides possessing substantial photocatalytic and photo-oxidizing capability and hence the capacity to undergo strong chemical interaction with biological specimen[44]. ZnO works as an antibacterial agent by means of producing highly volatile functional groups called reactive oxygen species. The bacterial cell wall is attacked by these ions and under the influence of electrostatic agitation between the zinc nanoglobules and the surface of the cell increased levels of stress are produced in the membrane ultimately leading to rupture and death of the cell. The external lipid bilayer in the microbes is ruptured by physical bombardment of the nanoparticles as well, causing the cytoplasmic material to be drained out.

Chitosans are complicated but useful non-aromatic semi-crystalline polysaccharides formed through incomplete ethanoylation of a ubiquitous natural polymer called chitin. The unique chemical properties of chitosan are rendered possible because of the occurrence of the protonable amino group in vicinity of the D-glucosamine residues. The chitosan, which has a net positive charge (Figure 12), is capable of electrostatic interaction with the negatively charged radicals and ions present on the microbial cell layers and due to this interaction the cell permeability is changed[45]. This causes unnatural exchange of material between the cytoplasm of the cell and the surrounding environment, especially of important genetic materials, ultimately leading to cell lysis. Also, the binding of cell DNA with the chitosan by virtue of the protonated amino groups causes the bacterial RNA synthesis to be negatively affected. The bactericidal nature of chitosan is through an amalgamation of these two methods.

Different mechanisms can be used for doping the nanotubes with chitosan to render the antimicrobial property. The most common method is that of electrospinning where a diluted solution of chitosan is dropped onto the substrate and the coating of chitosan is obtained on the surface by rotating the substrate at 500~100 rpm for a few seconds[42]. Upon drying the chitosan layer is firmly ensconced on top of the nanotube surface.
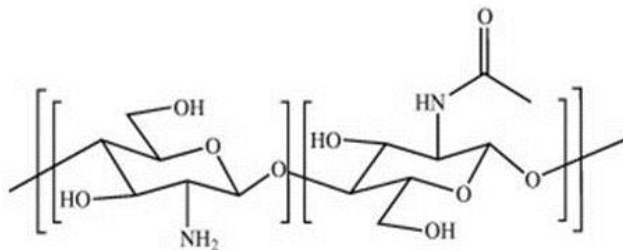

Figure 12: Chemical structure of chitosan showing the protonable groups that enables its unique chemical reactivity[42].

Another important way of using nanotubes for antimicrobial applications is through their use as nano-scale reservoirs for controlled and calculated drug release. Both carbon nanotubes and other metal-based nanotubes are exceptionally potent options for this purpose due to their increased aspect ratio, functionalizable surface, high pace of cellular integration and surface chemical reactivity. In case of carbon nanotubes a functionalizing procedure is carried out, usually with peptide base compounds, followed by drug loading through lyophilization[46]. In case of composite or metal nanotubes, drugs can be loaded simply by lyophilization or nano-pipetting[47]. The exact relation between the rates of release of these drugs is yet to be discovered but its dependence on the aspect ratio of the nanotube, the concentration of drug loading, and the time of release are known[48]. Also, since the chances of infection are highest immediately after the implant surgery, it is favorable that these nanotubes tend to release the drug in copious quantities initially and then gradually the release is reduced in amount over time. Commonly used drugs for such applications are Cefuroxime, Gentamicin, Curcumin, Indomethacin and Vancomycin.

Nanotubes for implant surfaces: The bio-mimicking effect for osseointegration

Orthopedic implant materials are usually of two types the implant surface made of metals, alloys or hard ceramics and the orthopedic scaffolds for tissue regeneration, which are important to ensure the implant is integrated into the body. This latter is often made of hydroxyapatite because of its chemical composition, which closely mimics that of naturally occurring apatite in the bones[28]. The biocompatibility and the high levels of osseointegration provided by HA make it an ideal surface to promote growth, proliferation and integration of bones. This however is offset by its punitive fracture resistance, minimal shear strength and insufficient wear resistance28. Thus, a second phase material is used as a reinforcing layer to HA. Ceramics and composite layers have been tested but these fall short on accounts of biocompatibility. However, the incorporation of a nanotube layer as a reinforcing sheath has been found to alleviate both the problems. Both carbon nanotubes and titania nanotubes have been extensively studied for this particular purpose and have both shown a lot of promise[49].

Potential Hazards of Using Nanotubes: Myths and Facts

Despite the obvious benefits of using nanotubular structures in orthopedic implants there have been persistent doubts and concerns raised by engineers, doctors and the general public. While many of these are invalidated, there are issues of medical significance that require addressing before large-scale incorporation of nanotubes in implants can be implemented.

Severe and chronic disorders like granuloma, fibrosis of the lung and generation and accumulation of fluid in the lungs can be caused by long term deposition of nanoparticles within the human body[50]. This is of importance since nanotubular debris studies are limited and it can be logically assumed that over an extended period of time it is possible for them to undergo degradation and ultimately disintegrate into nanoparticulate matter. Factors such as physical dimensions, chemical activity and method of preparation are integral to the cytotoxicity considerations for nanotubes. Most of the toxicity studies conducted on nanotubes concentrate on CNTs, titania Nanotubes and boron nitride nanotubes.

Nanotubes of both single walled and multiwalled nature have been studied for their cytotoxic effect24. Multiwalled carbon nanotubes have been reported to form bigger clusters in biological tissues without transportation while SWCNTs tend to form tiny particles, which are ingested by phagocytes and then transferred to lymph nodes. Nanotubes can easily navigate through capillaries and adhere to blood vessels. SWCNTs in particular can potentially block the ionic transportation pathways for potassium due to its low diameter and tube-like structure. Cationic surfaces have the capability of effecting organism hemolysis and platelet aggregation and most NTs owing to their cationic charge are suspected to have similar effects, leading to accelerated vascular thrombosis[51]. Oxidative stress can result from activation of oxidative enzymatic channels by the nanotubes. As a result ROS generation is greatly enhanced. Long-chained unsaturated fatty acids within the cell lining undergo peroxidation when the ROS concentration is too high which causes a change in the permeability of the membrane of the mitochondria as well as the cell membrane causing rupture and release of cytoplasmic content. The dead or damaged cells give out toxins and debris which accumulate on the surface of blood vessels, RBC, organs like lungs, brain, heart and kidney and the GI tract. This is a potential cause of concern due to its carcinogenic nature. Also, nanotubes are known to be responsible for retardation of DNA reparation mechanisms owing to DNA strand breaks by suppressing proteins that are responsible for DNA repairs. In case of CNTs there have also been concerns over possible fibrosis being caused by individual carbon nanofibers, which remain intermingled with the nanotubes. In case of metal oxide nanotubes there are concerns regarding the release of heavy metal ions from the reactive species that remain on the substrate even after repeated cleaning. These ions have been known to potential carcinogens.

Apart from the concern over the nanotubes

themselves, there has been widespread fear in the medical community regarding the health hazards posed by the doping agents, which are proposed for use as antimicrobial surface treatment agents. Silver has been widely studied as a surface bactericidal agent, however, silver is known to be slightly toxic to humans in elemental form, the most dramatic effect being observed in patients with a condition called Argyria where the skin turns blue and patches appear on the conjunctiva[52]. However, silver in ionic state is a strong oxidizing agent and much like the mechanism in which it causes bacterial cell lysis it can cause irreparable damage to normal human cells[53]. The concentration level beyond which silver is toxic to normal human cells is still under debate and control of the silver bactericide release under this control value is of utmost importance.

The biggest challenge in assessing these risks when it comes to testing nanotubes for implants is that cytotoxicity assays carried out on implant material surfaces involve only cells of a certain type incubated in isolation in simulated body conditions on the implant surface[54]. While this can provide an idea about the level of cytocompatibility between the nanotubes and the cells being studied, it is a very crude approximation of how the implant containing the nanotubes behaves in the far more complicated and dynamic environment inside the host's body. In vivo studies are far more conclusive for such assessment and are currently being used to study long-term effects of nanotubes. But even then, the in vivo studies are limited in number due to ethical concerns, infrastructural and economic constraints. Also, human in vivo testing does not seem to be a possibility anywhere in the near future due to strict regulatory policies.
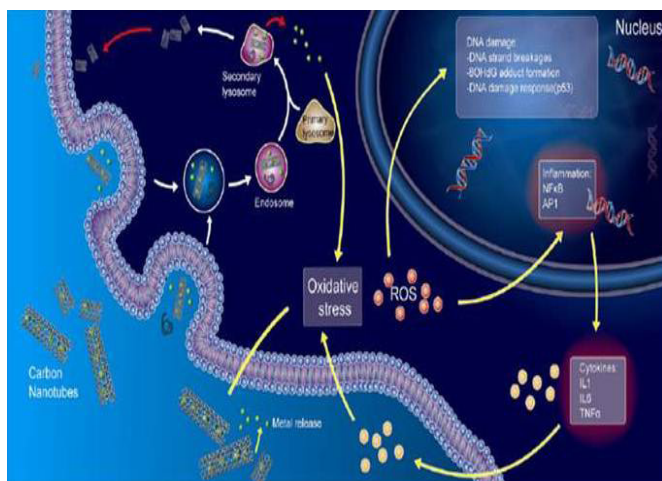

Figure13: Schematic showing different cytotoxicity mechanisms for CNTs[51].

Future

Everyday new ideas are being generated for more directed and advanced uses of the unique properties of nanotubes for orthopedic implant. Many of these are aimed at improving the control over the behavior of nanotubes with respect to drug release. Nickel-titanium alloys, commonly known as Nitinol are known to exhibit strong shape memory effects[55]. Shape memory alloy (SMA) based implants made with nickel-titanium (Nitinol/NiTi) are under intense investigation as a means of providing the implant real time adaptability to the healing process. Dramatic advances in bone mending can be effected

through use of SMA as implant biomaterials which allow alteration of the stiffness of the implants at a particular time. Currently studies[56] have been conducted where Titania nanotubes have been formed on the surface of NiTi implants rendering it biocompatibility and also utilizing the shape memory effect of the 'smart material'. The primary concern in this context remains regarding the possible cytotoxicity and carcinogenic properties of Ni2+ ions that are potentially released from the implant over an extended period of time[57,58]. DNA/RNA nanotubes are being investigated for potential use as drug delivery vehicles[59]. The inherent biocompatibility of DNA/RNA strands makes them an effective means of delivering drugs into the body. Also, of particular interest are protein nanotubes, which can be grown on Alumina templates and then functionalized with aptamers. These nanotubes can be lyophilized with drugs of choice and the aptamers based on their selective binding with particular hormonal proteins can provide a basis for targeted drug release[60].

Investigations are being carried out to increase control of release rate of drugs/anti-inflammatory agents from the nanotubes by means of external stimuli such as ultrasound, UV light and laser radiation[61,62]. UV radiation in particular has been extensively studied for metal oxide nanotubes, which have photocatalytic properties whereby the radiation intensity can be used to regulate the number of electron/ion species available on the surface of the implant. This would, in turn, determine the selective chemical reactivity and adhesion property of the implant coating[61]. Since in-vivo testing of implants remains one of the most challenging aspects of nano-orthopedics, alternative ways of testing such as organ-on-chip are being looked into. Organs-on-chip are multichannel three dimensional microfluidic chips for culturing cells, which are made of respective organ cells and have tiny hydraulic or pneumatically actuated control mechanisms that simulate cellular motion in dynamic body conditions[63].

Conclusion

Nanotubes have revolutionized our approach to orthopedic implants in many ways. They drastically improve the osseointegration within the body, the mechanical strength of the implant, and adding infection resistance or drug delivery capabilities to the implants.Nanotube arrays allow for mimicking of the natural micro-nano hierarchical structure in the body thus stimulating greater levels of bio-compatibility. Essentially, this is a step towards the ultimate goal of self-regenerative medicine. Through targeted delivery of drugs infections can be resisted by orthopedic implants, thus eliminating the cause for failure of more than 40% of all medically installed implants.

Despite the positives there are still concerns in the medical community and among the greater masses about the safety aspect of nanotubes. Some of these concerns are legitimate and basically stem from the fact that any foreign element is resisted by the body and when of a nano-scale dimension can act as a carcinogen by initiating genetic mutation. There are, however, many fears surrounding the use of nanotubes, which are not based on any scientific evidence and must be dispelled. In summary, nanotubes can definitely be hailed as one of the most promising horizon on the front of orthopedic implants and in the years to come it is expected to

yield solutions to many problems that have plagued the field.

Acknowledgments

References

1) F. Witte (2010) The history of biodegradable magnesium implants: A review. Acta Biomaterialia 5: 1680-1692.

2) Kaplan EG, Kaplan GS, Kaplan DM, Kaplan RK (1984) History of implants. Clinics in Podiatry 1: 3-10.

3) Carter DR, Beaupre GS, Giori NJ, Helms JA (1998) Mechanobiology of skeletal regeneration. Clinical Orthopaedics and Related Research 355: 41-S55.

4) X. Liu, G.L. Niebur (2008) Bone ingrowth into a porous coated implant predicted by a mechano-regulatory tissue differentiation algorithm. Biomechanics and Modeling in Mechanobiology 7: 335–344.

5) Kienapfel H, Sprey C, Wilke A, Griss P (1999) Implant fixation by bone ingrowth. Journal of Arthroplasty 14: 355-368.

6) Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW (2012) Assessment of compromised fracture healing. Journal of the American Academy of Orthopaedic Surgeons 20: 273-282.

7) Aro HT, Alm JJ, Moritz N, Makinen TJ, Lankinen P (2012) Low BMD affects initial stability and delays stem osseointegration in cementless total hip arthroplasty in women: a 2-year RSA stuady of 39 patients. ActaOrthopaedica 2: 107-114.

8) Luthringer BJ, Ali FF, Akaichi H, Feyerabend F, Ebel T, et al. (2013) Production, characterisation, and cytocompatibility of porous titanium-based particulate scaffolds. Journal of Material Science. Materials in Medicine 24: 2337-2358.

9) Del Pozo JL and R Patel (2009) Infection associated with prosthetic joints. The New England Journal of Medicine. 361: 787–794.

10) Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW (2012) Assessment of compromised fracture healing. Journal of the American Academy of Orthopaedic Surgeons 20: 273-282.

11) Chye KhoonPoh, YanliCai, Xiao Wei Tan, HarkChuan Tan, Wilson Wang (2013) An in vitro assessment of surface modification strategies for orthopedic applications. Thin Solid Films 544: 254-259.

12) VivianaMourino, Juan P Cattalini, JudithARoether, PrachiDubey, Ipsita Roy, et al. (2013) Composite polymer-bioceramic scaffolds with drug delivery capability for bone tissue engineering. Expert opinion on drug delivery 10: 1353-1365.

13) Neil Cobelli, Brian Scharf, Giovanna M Crisi, John Hardin, Laura Santambrogio(2011) Mediators of the inflammatory response to joint replacement devices. Nature Reviews Rheumatology 7: 600-608.

14) Arens S, Kraft C, Schlegel U, Printzen G, Perren SM, and Hansis M (1999) Susceptibility to local infection in biological internal fixation. Experimental study of open vs. minimally invasive plate osteosynthesis in rabbits. Archives of Orthopaedic and Trauma Surgery 119: 82–85.

15) Schmidmaier G, Lucke M, Wildemann B, Haas NP, Raschke M (2006) Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. Injury 37: 105-112.

16) Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, et al. (2008) Infection burden for hip and knee arthroplasty in the United States. Journal of Arthroplasty 23: 984–991.

17) Moriarty TF, Schlegel U, Perren S, Richards RG (2010) Infection in fracture fixation: can we influence infection rates through implant design? Journal of Materials Science: Materials in Medicine 21: 1031-1035.

18) Namba RS, Inacio MC, Paxton EW (2012) Risk factors associated with surgical site infection in 30,491 primary total hip replacements. Journal of Bone and Joint Surgery - Series B 94: 1330-1338.

19) Raju Vaishya, Mayank Chauhan, Abhishek Vaish (2013) Bone cement Journal of Clinical Orthopaedics and Trauma 4: 157-163.

20) Bruno Dutra Roos, Milton ValdomiroRoos, Antero CamisaJúnior (2012) Circumferential proximal femoral allografts in revision surgery on total hip arthroplasty: case reports with a minimum follow-up of 20 years. RevistaBrasileira de Ortopedia 47: 384-388.

21) Bernd Fink (2009) Revision of late periprosthetic infections of total hip endoprostheses: pros and cons of different concepts. International Journal of Medical Science6: 287-295.

22) Wang X, Li Q, Xie J, Jin Z, Wang J, et al. (2009) Fabrication of Ultralong and Electrically Uniform Single-Walled Carbon Nanotubes on Clean Substrates Nano Letters 9: 3137–3141.

23) Shuilin Wu, Xiangmei Liu, Kelvin W.K. Yeung, Huan Guo, et al. (2013) Surface nano-architectures and their effects on the mechanical properties and corrosion behavior of Ti-based orthopedic implants. Surface and Coatings Technology 233: 13-26.

24) Raymond M. Reily (2007) Carbon Nanotubes: Potential Benefits and Risks of Nanotechnology in Nuclear Medicine. The Journal of Nuclear Medicine 48: 1039-1042.

25) Shokuhfar T, Gao Q, Ashtana A, Walzack K, Heiden P, et al. (2010) Structural instabilities in TiO2 nanotubes. Journal of Applied Physics 108: 104310.

26) Yan Hu, Kaiyong Cai, Zhong Luo, Dawei Xu, Daichao Xie, et al. (2012) TiO2 nanotubes as drug nanoreservoirs for the regulation of mobility and differentiation of mesenchymal stem cells Acta Biomaterialia 8: 439-448

27) Xiliag Luo, Christopher Matranga, Susheng Tan, Nicolas Alba, Xinyan T. Cui (2011) Carbon nanotube nanoreservoir for controlled release of anti-inflammatory dexamethasone. Biomaterials 32: 6316-6323.

28) DebrupaLahiri,SanatGhosh, ArvindAgarwal (2012) Carbon nanotube reinforced hydroxyapatite composite for orthopedic application: A review. Materials Science and Engineering 32: 1727–1758.

29) Peter Newman, Andrew Minett, Rutledge Ellis-Behnke, Hala Zreiqat (2013) Carbon nanotubes: Their potential and pitfalls for bone tissue regeneration and engineering. Nanomedicine: Nanotechnology, Biology and Medicine 9: 1139-1158.

30) Barry M. Gumbiner (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis Cell 9: 345-57.

31) Feng Wanga, Liang Shic, Wen-Xi Hea, Dong Hand, Yan Yan, et al. (2013) Bioinspired micro/nano fabrication on dental implant–bone interface. Applied Surface Science 265: 480– 488.

32) Noreen J. Hickok, Irving M. Shapiro (2012) Immobilized antibiotics to prevent orthopaedic implant infections. Advanced Drug Delivery Reviews 64: 1165-1176.

33) Aslan S, Loebick CZ, Kang S, Elimelech M, Pfefferle LD (2010) Antimicrobial biomaterials based on carbon nanotubes dispersed in poly(lactic-co-glycolic acid). Nanoscale 2: 1789-1794.

34) Zhou J, Qi X (2011) Multi-walled carbon nanotubes/epilson-polylysine nanocomposite with enhanced antibacterial activity. Letters in Applied Microbiology 52: 76-83.

35) Yu-Fu Young, Hui-Ju Lee, Yi-Shan Shen, Shih-Hao Tseng, Chi-Young Lee, et al (2012) Toxicity mechanism of carbon nanotubes on Escherichia coli. Materials Chemistry and Physics 134: 279-286.

36) Xiaobao Qi, Poernomo Gunawan, Rong Xu, Matthew Wook Chang (2012) Cefalexin-immobilized multi-walled carbon nanotubes show strong antimicrobial and anti-adhesion properties. Chemical Engineering Science 84: 552-556.

37) Sabrina D Puckett, Erik Taylor, Theresa Raimondo, Thomas J Webster (2010) The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials 31: 706–713.

38) Tomas Fiedler, Achim Salamon, Stefanie Adam, Nicole Herzmann, Jan Taubenheim, Kirsten Peters (2013) Impact of bacteria and bacterial components on osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Experimental Cell Research 319: 2883-2892.

39)Samar Basu, Karl Michaëlsson, Helena Olofsson, Sara Johansson, Håkan Melhus (2001) Association between oxidative stress and bone mineral density. Biochemical and Biophysical Research Communications 288: 275-279.

40) Lee YH, Lee NH, Bhattarai G, Oh YT, Yu MK, et al. (2010) Enhancement of osteoblast biocompatibility on titanium surface with terrein treatment. Cell Biochemistry and Function 288: 678-685.

41) Eckhardt A, Gerstmayr N, Hiller KA, Bolay C, Waha C, et al. (2009) TEGDMA-induced oxidative DNA damage and activation of ATM and MAP kinases. Biomaterials 30: 2006–2014.

42) Florence Croisier, Christine Jérôme (2013) Chitosan-based biomaterials for tissue engineering. European Polymer Journal 49: 780-792.

43) Michał Moritz, Małgorzata Geszke-Moritz (2013) The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. Chemical Engineering Journal, 228: 596-613.

44) Sara Tavassoli Hojati, Homayoon Alaghemand, Faeze Hamze, Fateme Ahmadian Babaki, Ramazan Rajab-Nia, et al. (2013) Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. Dental Materials 29: 495-505.

45) Sutha S, Karunakaran G, Rajendran V (2013) Enhancement of antimicrobial and long-term biostability of the zinc-incorporated hydroxyapatite coated 316L stainless steel implant for biomedical application. Ceramics International 39: 5205-5212.

46) Ahmad Amiri, Hadi Zare Zardini, Mehdi Shanbedi, Morteza Maghrebi, Majid Baniadam, et al. (2012) Efficient method for functionalization of carbon nanotubes by lysine and improved antimicrobial activity and water-dispersion. Materials Letters 72: 153-156.

47) Maho A, Linden S, Arnould C, Detriche S, Delhalle J, Mekhalif Z (2012) Tantalum oxide/carbon nanotubes composite coatings on titanium, and their functionalization with organophosphonic molecular films: A high quality scaffold for hydroxyapatite growth. Journal of Colloid and Interface Science 371: 150-158.

48) Sławomir Boncel, Piotr Zając, Krzysztof Koziol KK (2013) Liberation of drugs from multi-wall carbon nanotube carriers. Journal of Controlled Release 169: 126-140.

49) Kodama A, Bauer S, Komatsu A, Asoh H, Ono S, et al. (2009) Bioactivation of titanium surfaces using coatings of TiO2 nanotubes rapidly preloaded with synthetic hydroxyapatite. Acta Biomaterialia 5: 2322-2330.

50) Song Y, Li X, Du X (2009) Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. European Respiratory Journal 34: 559-567.

51) Liu Y, Zhoa Y, Sun B, Chen C (2012) Understanding the Toxicity of Carbon Nanotubes. Accounts of Chemical Research 46: 702-713.

52) Niels Hadrup, Henrik R Lam (2014) Oral toxicity of silver ions, silver nanoparticles and colloidal silver – A review. Regulatory Toxicology and Pharmacology 68: 1-7.

53) Porntipa Chairuangkitti, Somsong Lawanprasert, Sittiruk Roytrakul, Sasitorn Aueviriyavit, Duangkamol Phummiratch, et al. (2013) Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways. Toxicology in Vitro 27: 330-338.

54) Wadhwa S, Rea C, O'Hare P, Mathur A, Roy SS, et al. (2011) Comparative in vitro cytotoxicity study of carbon nanotubes and titania nanostructures on human lung epithelial cells. Journal of Hazardous Materials 191: 56-61.

55) Huang WM, Song CL, Fu YQ, Wang CC, Zhao Y, et al. (2013) Shaping tissue with shape memory materials. Advanced Drug Delivery Reviews 65: 515-535.

56) Ruiqiang Hang, Xiaobo Huang, Linhai Tian, Zhiyong He, Bin Tang (2012) Preparation, characterization, corrosion behavior and bioactivity of Ni2O3-doped TiO2 nanotubes on NiTi alloy. Electrochimica Act 70: 382-393.

57) Yeong-Joon Park, Yo-Han Song, Ji-Hae An, Ho-Jun Song, Kenneth J Anusavice (2013) Cytocompatibility of pure metals and experimental binary titanium alloys for implant materials. Journal of Dentistry 41: 1251-1258.

58) Shuying Gu, Beibei Yan, Lingling Liu, JieRen (2013) Carbon nanotube–polyurethane shape memory nanocomposites with low trigger temperature. European Polymer Journal 49: 3867-3877.

59) William Cheung, Francesco Pontoriero, Oleh Taratula, Alex Chen M, Huixin He (2010) DNA and carbon nanotubes as medicine. Advanced Drug Delivery Reviews 62: 633-649.

60) Hou S, Wang J, Martin CR (2005) Template-Synthesized Protein Nanotubes. Nano Letters 5: 231-234.

61) Moom Sinn Aw, Dusan Losic (2013) Ultrasound enhanced release of therapeutics from drug-releasing implants based on titania nanotube arrays. International Journal of Pharmaceutics 443: 154-162.

62) Zoran Markovic M, Ljubica Harhaji-Trajkovic M, Biljana Todorovic-Markovic M, Dejan Kepić P, Katarina Arsikin M, (2011) In vitro comparison of the photothermal anticancer activity of graphene nanoparticles and carbon nanotubes. Biomaterials 32: 1121-1129.

63) Šeila Selimović, Mehmet R Dokmeci, Ali Khademhosseini (2013) Organs-on-a-chip for drug discovery. Current Opinion in Pharmacology 13: 829-833.