5-1-2014

Nanotechnology: current concepts in orthopaedic surgery and future directions.

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■ INSTRUCTIONAL REVIEW: GENERAL ORTHOPAEDICS

Nanotechnology

CURRENT CONCEPTS IN ORTHOPAEDIC SURGERY AND FUTURE DIRECTIONS

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Nanotechnology is the study, production and controlled manipulation of materials with a grain size < 100 nm. At this level, the laws of classical mechanics fall away and those of quantum mechanics take over, resulting in unique behaviour of matter in terms of melting point, conductivity and reactivity. Additionally, and likely more significant, as grain size decreases, the ratio of surface area to volume drastically increases, allowing for greater interaction between implants and the surrounding cellular environment. This favourable increase in surface area plays an important role in mesenchymal cell differentiation and ultimately bone–implant interactions.

Basic science and translational research have revealed important potential applications for nanotechnology in orthopaedic surgery, particularly with regard to improving the interaction between implants and host bone. Nanophase materials more closely match the architecture of native trabecular bone, thereby greatly improving the osseo-integration of orthopaedic implants. Nanophase-coated prostheses can also reduce bacterial adhesion more than conventionally surfaced prostheses. Nanophase selenium has shown great promise when used for tumour reconstructions, as has nanophase silver in the management of traumatic wounds. Nanophase silver may significantly improve healing of peripheral nerve injuries, and nanophase gold has powerful anti-inflammatory effects on tendon inflammation.

Considerable advances must be made in our understanding of the potential health risks of production, implantation and wear patterns of nanophase devices before they are approved for clinical use. Their potential, however, is considerable, and is likely to benefit us all in the future.

Cite this article: *Bone Joint J* 2014; 96-B:569–73.

The chemical reactions and molecular interactions that take place in the human body involve matter composed of 4 to 400 atoms that are measured in nanometres (nm).[1,2]

Nanotechnology is the production and manipulation of materials on a scale of < 100 nm and the integration of these nanoscopic materials into microscopic and macroscopic systems.[1,3] In 1959, Richard Feynman first introduced the concept of nanotechnology, describing "a field in which little has been done, but in which an enormous amount can be done in principle".[4] Since then, nanotechnology has seen major advances and real-life applications in the fields of electronics, water and air filtration, metallic surface technology, cosmetics, homeware, medicine and much more.[5] Furthermore, market research performed by BCC Research predicts that annual global nanotechnology sales will reach $48.9 billion by 2017.[6]

The classical laws of physics do not apply at the nano-level, at which nanomaterials exhibit novel and markedly different properties from those of materials composed of larger particles.[1,3,7] Nanophase materials are composed of matter with a grain size much smaller than that of their conventional counterparts, but with the same basic atomic structure. There are two fundamental characteristics that distinguish one from the other. The first is that the behaviour of nanophase materials is explained by quantum, rather than classical, mechanics. Particles with a grain size < 100 nm behave in a markedly different way from larger particles in terms of melting point, conductivity, combustibility and reactivity.[7] The second is the concept that as grain size decreases, surface area increases for a given volume. For example, a volume of 1 cm^3 filled with cubes of 1 μm^3 has a total surface area of 6 m^2, whereas the same volume filled with cubes of 1 nm^3 has a total surface area of 6000 m^2. On a more practical level, reducing the grain size of an orthopaedic implant surface coating from

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doi:10.1302/0301-620X.96B5.33606 $2.00

Bone Joint J 2014;96-B:569–73.

micrometres to nanometres increases its surface area by a factor of thousands.[8] This fundamental principle is what gives nanotechnology the potential to be "the transformational technology of this century" in the orthopaedic device industry.[9] Basic science and translational research have revealed important potential applications in orthopaedics. In this review, we discuss the current and future roles of nanotechnology as they relate to total joint replacement (TJR), orthopaedic trauma surgery, oncology, the prevention and treatment of orthopaedic-related infections, soft-tissue regeneration and orthopaedic drug delivery systems.

Nanotechnology and bone cell function

When biomaterials are introduced into the human body, interactions between the surface of the biomaterial and the surrounding bone and soft tissues are critical to cellular differentiation and osseo-integration (bony adherence to the implant surface). Mesenchymal stromal cells appear to be one of the first cell types involved when a nanophase biomaterial is introduced into a cellular environment.[10] By mimicking the nanoscopic, three-dimensional (3D) extracellular and cell surface topography, nanophase implant surfaces and scaffolds may improve osseo-integration by promoting both differentiation of these mesenchymal stromal cells and the adsorption of the extracellular adhesion molecules essential to osteoblast function.[11,12] Multiple *in vitro* studies have shown their ability to control and enhance osteoblast differentiation and cellular adhesion by the introduction of uniquely shaped nanophase scaffolds without the use of additional osteogenic chemicals.[10,11,13,14] One promising area of future research is the development of specific osteogenic, extracellular nano-topographical surfaces that mimic known biological configurations. For example, type X collagen is thought to induce endochondral ossification through its known nano-topographical structure.

Reproducing this may allow the controlled enhancement of the endochondral ossification of secondary bone healing.[14] A variety of nanostructured materials have been shown to enhance osteoblast function. These include the nanophase ceramics, aluminium oxide and titanium dioxide; carbon; selenium; titanium alloy (Ti6AlV); cobalt–chrome alloys; and nanocrystalline diamond.[15-21] Furthermore, multiple *in vitro* studies have shown more osteoid mineralisation on nano surfaces than on micro-roughened surfaces.[22-24]

Extracellular adhesion proteins, such as fibronectin and vitronectin, also play an important role in mediating the recognition, activation and adhesion of the osteoblast to a biomaterial, ultimately leading to osseo-integration.[25,26] Fibronectin and vitronectin interact more effectively with nanophase implant surfaces than with conventional surfaces.[7,15] In addition to the increased quantity of biomolecules adsorbed onto the nanosurface, their conformation is altered by interaction with the nanosurface to increase the availability of specific cell-adhesive epitopes within each biomolecule.[16,25] The resulting increased adsorption and favourable conformational changes of fibronectin and vitronectin, optimise the environment for osteoblast adhesion.[7,15] Overall, these findings suggest that nanomaterials have considerable potential use in surfacing orthopaedic implants by virtue of their capacity for improved osseo-integration and osteoid mineralisation.

Orthopaedic applications

Aseptic loosening is the leading cause of TJR failure. It may be the result of loosening after initial integration of the implant, or a failure to integrate from the outset.[27,28]

The application of a nanotextured material may reduce the risk of implant failure by improving osseo-integration.[7,15,25,26,29] Mature bone has an inorganic mineral size of roughly 50 nm × 25 nm × 4 nm, which represents a coarse surface in nanometric terms.[8,30] In contrast, modern orthopaedic implant surfaces are smooth at the nanometric level.[7] Smooth surfaces preferentially induce the growth of fibrous tissue rather than bone, whereas a nanotextured surface may enhance the function of osteoblasts and reduce that of fibroblasts. This differential cellular activity is seen on nanotextured hydroxyapatite-coated surfaces, as well as on many other nanostructured surfaces, and is thought to be a direct result of their decreased grain size.[7,15,17] In addition to the favourable properties of nanophase hydroxyapatite, nano-engineered titanium and cobalt–chromium–molybdenum (CoCrMo) encourage osteoblast adhesion more than their conventional counterparts.[15]

Nanocrystalline hydroxyapatite (HA) paste has been used as a filler of bone defects, with encouraging results. A series of fractures of the distal radius showed this to be an acceptable substitute for bone graft in metaphyseal defects.[31] A further series by the same group showed similarly encouraging results when it was used to treat metaphyseal defects in fractures of the tibial plateau.[32]

Nanocomposite scaffolds composed of type I collagen and nanostructured HA are currently being used in the treatment of osteochondral defects of the knee. Kon et al[33] have shown encouraging short-term clinical and radiological results with a nanocomposite biological implant for the treatment of focal osteochondral lesions. They used a trilayered biological implant consisting of a cartilage layer (100% type I collagen), a transition region (Nano-HA 40% and type I collagen 60%), and a bone region (Nano-HA 70% and type I collagen 30%). This type of implant may be an easier, less morbid and cell-free 'off-the-shelf' solution to focal defects of articular cartilage than either two-stage autologous chondrocyte engineering procedures or single-stage autograft mosaicplasty.[33,34]

Recent research in oncology has shown selenium to be a powerful potentiator of chemotherapeutic agents.[35] When manufactured on the nanometric scale and applied to titanium orthopaedic implants, nanophase selenium appears to inhibit the growth of malignant osteoblasts at the implant–tissue interface.[36] Similarly, nanophase HA causes *in vitro* inhibition and apoptosis of osteosarcoma cells.[37]

Nanophase silver is proving a significant source of interest for orthopaedic traumatologists. Silver has been used on wounds for centuries as an antibacterial agent. Over the past decade, nanophase silver dressings have reached the market and proved to be better at preventing wound infections and stimulating healing than traditional silver-based or plain dressings.[38,39] Similarly, nanophase silver incorporated onto the surface of titanium orthopaedic implants in the form of titanium nanotubes, has immediate powerful bactericidal and anti-adhesive effects, which last up to 30 days.[40] This could eventually prove to be of benefit in the prevention of the acute post-operative infection of TJRs and trauma implants.

Peripheral nerve injuries may also benefit from nanotechnology. Ding et al[41] have shown that nanophase silver-impregnated type I collagen scaffolds significantly increase the quantity of adsorbed proteins critical to nerve healing and significantly reduce the time to nerve regeneration. In a study that compared nanosilver-impregnated type I collagen scaffolds to control type I collagen scaffolds in rabbits with an experimentally induced 10 mm sciatic nerve defect, the nanosilver-impregnated group showed thicker myelin sheaths, improved nerve conduction and higher rates of laminin adsorption.[41]

A significant amount of energy is currently being directed at precision delivery of drugs. Gold has the potential to be an effective transcutaneous drug delivery system for iontophoresis in the treatment of tendinopathy. Dohnert et al[42] used a rat Achilles tendinopathy model to show that diclofenac administered with 30 nm gold nanoparticles by iontophoresis significantly lowered levels of the inflammatory cytokines interleukin 1 (IL1)-β and tumour necrosis factor (TNF)-α in tendinopathic tissue compared with both untreated controls and diclofenac-only groups. This suggests that nanophase gold may enhance the effectiveness of diclofenac as a transcutaneous anti-inflammatory agent.[42] In addition to nanophase gold, nanofibre poly-L-lactic acid (PLLA) appears to be an excellent nanoscopic drug delivery system. Large calvarial bony defects close rapidly with increased expression of osteoblastic lineage cells when nanofibre PLLA is used as the delivery system for bone morphogenetic protein (BMP)-2.[12]

Nanophase drug delivery systems are also being studied for their application in TJR. Li et al[43] used a biodegradable polypeptide nanofilm coating on total joint prostheses for the delivery of cefazolin into a simulated TJR environment and observed a reduction of bacterial load and improved osteoblastic response. The adhesion of *Staphylococcus aureus (S. aureus)* onto a bare nanofilm implant surface was substantially less than on a conventional prosthesis. Furthermore, when the same polypeptide nanofilm was loaded with cefazolin and used as a drug delivery system, it produced a dose-related reduction in the *S. aureus* population. This system also has the ability to tightly control the pharmacokinetics of cefazolin release. Polypeptide nanofilms allow for the targeted release of cefazolin therapy during the critical post-implantation period (first two hours). Additionally, compared with a bare implant surface, nanofilm-coated surfaces showed significantly greater osteoblast adherence, proliferation and viability, whether loaded with cefazolin or not, making them potentially an ideal surface for osseo-integration.[43]

The safety of nanotechnology and areas of future research

The principal unanswered questions in nanotechnology are related to its clinical safety. At present, the health effects of nanomaterials are essentially unknown. Nanoparticles may be released into the body over time as a result of the degradation of implanted nanomaterials. In addition, nanoparticles could potentially be harmful to those manufacturing or disposing of nanocomposite orthopaedic implants.[44] The metabolism of nanoparticles involves various organ systems, including the blood, liver and kidneys, and may result in inflammation and oxidative stress.[6,45,46] Some research suggests that nanoparticulate material is associated with increased cytotoxicity in the brain and lungs,[47,48] whereas others argue that nanodebris may actually facilitate cell viability in bone and lung tissue.[47,49] Since 2008, the United States government has sponsored $1.4 billion of nanotechnology research, only 3% of which is devoted to its safety and effects on health.[3] Owing to the uncertainty about safety, rigorous studies must be conducted to evaluate the toxicity of nanophase materials before using them on the population at large.

Our understanding of the health effects of nanoscopic wear debris is also limited. Early research on bearing surfaces showed that > 90% of polyethylene wear debris is < 1000 nm in diameter; the particle size generally being approximately 500 nm.[28] When various immune cells are exposed to nanoscopic ultra-high molecular weight polyethylene wear debris *in vitro*, several important patterns arise. Macrophages are unable to phagocytose debris < 200 nm in size. On the other hand, dendritic cells, which are policing cells critical to the immune response, are capable of initiating a potent immune response to polyethylene wear debris as small as 50 nm. When dendritic cells encounter polyethylene wear on this scale, they release the inflammatory cytokines IL1-β and IL6. These cytokines may then activate the osteoclastic cells responsible for osteolysis.[50]

A topic currently at the forefront of joint replacement is the clinical effect of metal-on-metal (MoM) wear debris. Nanoscopic metal wear debris, with a mean particle size of 25 nm to 36 nm, is thought to be the driving force behind the toxicity associated with MoM hip replacement.[46,51] Dramatically elevated local and systemic cobalt and chromium ion levels have important local and systemic effects. At the level of the prosthesis itself, a destructive inflammatory response to nanoscopic metal ion wear debris may occur, resulting in soft tissue damage and pseudotumour formation.[52,53] The clinical significance of elevated systemic cobalt and chromium ion levels remains largely

unknown; however, there is concern about their effects on peripheral and central nervous tissue, as well as on the cardiovascular and endocrine systems.[52,53]

Although still in its infancy, nanotechnology has gained a firm foothold in the basic science and preclinical realm of orthopaedic research. Its vast potential is now being realised as we see the early positive results of applied nanotechnology in clinical trials. Extensive basic-science research suggests that many of the promising theoretical benefits of the application of nanotechnology lie in orthopaedic surgery. A few of the potential applications with excellent *in vitro* data include nano-coated joint replacement implants, with the potential for improved osseo-integration and countering infection; rapidly incorporating fillers for osteochondral defects; anti-tumour selenium-coated endoprostheses; and powerful targeted drug delivery systems for the prevention of infection and the treatment of chronic overuse injuries.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

This article was primary edited by A. Ross and first proof edited by G. Scott.

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