Research Highlights

Highlights from the latest articles in nanomedicine

Drug delivery and titanium dioxide nanotubes

Evaluation of: Peng L, Mendelsohn AD, LaTempa TJ *et al.*: Long-term small molecule and protein elution from TiO₂ nanotubes. *Nano Lett.* doi: 10.1021/nl9001052 (2009) (Epub ahead of print).

Local delivery of drugs from an implant surface may be desirable as it can reduce the amount of drug required and may also lessen systemic side effects. Titanium dioxide (TiO₂) is a good candidate for a drug-eluting implant coating as it is highly biocompatible and previous studies have suggested that TiO₂ nanotubes may be able to control protein [1] and antibiotic [2] release.

In this proof-of-concept study, the authors demonstrate the prolonged drug-delivery capabilities of TiO₂ nanotubes. It was hypothesized that the nanotopography (tube height and diameter) of the nanotubes was directly responsible for the drug-elution behavior. The authors studied three sizes of TiO₂ nanotubes that were electrochemically deposited on titanium surfaces with a flat titanium surface as the control. It was found that total drug elution was most profoundly affected by nanotube length and that larger diameter nanotubes eluted less drug than 100 nm nanotubes of the same length. Nanotubes with a diameter of 100 nm and length of 5 µm provided the greatest total drug elution compared with those of other dimensions (diameter = 300 nm, length = 5 µm; diameter = 100 nm, length = 1 µm) and were therefore selected for further characterization. Elution curves were obtained for the small molecule drugs paclitaxel (1.3 kDa) and sirolimus (0.91 kDa) and for bovine serum albumin (66 kDa). It

was concluded that TiO₂ nanotubes can control small molecule delivery on the order of weeks and larger molecules on the order of months. However, it should be noted that in both cases the bulk of the elution occurred within a shorter time period.

This study also demonstrated *in vitro* growth rate inhibition of mouse vascular smooth muscle cells through the controlled release of sirolimus from the TiO₂ nanotubes. The possibility of utilizing TiO₂ nanotubes as a nano-depot for controlled drug release has been previously reported by Brammer *et al.*, where it was also suggested that the rate of release could be controllable by the diameter and aspect ratio of the nanotubes, and the current article confirms this observation [3]. Currently, drug-eluting implant coatings are widely used in coronary artery stents, however, the majority of coronary artery stents are not made of titanium. A large number of implants in dentistry and orthopedics are made from titanium alloys and the possibility of using TiO₂ nanotubes to deliver drugs and other compounds from these implants appears to be quite attractive.

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Titanium dioxide nano-network

Evaluation of: Chiang C-Y, Chiou S-H, Yang W-E *et al.*: Formation of TiO₂ nano-network on titanium surface increases the human cell growth. *Dent. Mater.* doi: 10.1016/j.dental.2009.03.001 (2009) (Epub ahead of print).

Titanium readily forms a protective oxide layer when it comes into contact with air. When a titanium implant is placed in the body, cells come into contact with the titanium oxide layer and cell behavior is influenced by the properties of this surface. Numerous surface modification techniques have been developed to improve the interaction between the cell layer and the implant surface [1–3]. Porous titanium surfaces are one approach that is currently being investigated [4,5]. Such a surface can be achieved by immersion, chemical or heat treatments, but these methods are generally complex and time consuming. Here, electrochemical techniques have been explored as an alternative surface-modification approach. This paper uses a straightforward electrochemical anodization treatment to create a structured nano-network titanium dioxide (TiO₂) surface and investigates the response of mesenchymal stem cells (MSCs), derived from human bone marrow, on this surface both *in vitro* and *in vivo*.

Commercially available pure titanium discs were polished. Anodic currents, I₁ and I₂ (I₁ < I₂), were applied to the discs in a 5 M NaOH solution at 25°C. The applied currents were reported to be under 0.2 A and the treatment time was less than 30 min. The specimens were designated I₁ and I₂ and untreated polished discs were designated I₀ where no current was applied. The anodized specimens had an average lateral pore size of 65 nm (I₁) and 94 nm (I₂). This nanoscaled surface layer was found on top of a 200–300 nm thick submicron-scale layer. Each of the 24 specimens per group was sterilized under UV light for 1 h before cell culture.

Adult human bone marrow MSCs (hMSCs) were used for the cell culture. The hMSCs were transfected with a gene

encoding green fluorescent protein (GFP) and this was used to evaluate the growth of the hMSCs. For *in vitro* cell growth, 5 × 10⁴ labeled hMSCs were seeded on each specimen and the fluorescence intensity was followed for 9 days. *In vivo* cell growth was evaluated by seeding the GFP labeled hMSCs onto the titanium specimens (I₀, I₁, I₂) and then inserting them subcutaneously under the back skin of 8-week-old nude mice. I₀ specimens without hMSCs were used as a negative control. The volume of cells expressing GFP was determined at the time of implantation. Over 28 days, GFP fluorescence was measured at various time points, calcium deposition was evaluated and avidin–biotin staining of the differentiated hMSCs was performed.

The results of the *in vitro* tests showed a higher GFP signal, indicating better cell growth on the anodized specimens compared with the control after 6 days, with I₁ showing stronger GFP fluorescence than I₂. There was also significantly faster cell growth on the test specimens compared with the control, with I₁ growing faster than I₂.

The *in vivo* tests revealed that the I₁ and I₂ specimens had higher GFP signals than the control at 28 days, with I₁ having the highest GFP signal. This implies that the size of the network has a significant influence on the *in vivo* cell growth. The I₁ and I₂ specimens contained osteocyte-like cells and osteopontin was expressed in the hMSC-derived tissues, thus indicating osteogenic differentiation.

This study confirms that a TiO₂ nanoscale topography can significantly enhance the growth of hMSCs both *in vitro* and *in vivo* and may also induce hMSCs to differentiate into osteogenic cells *in vivo*. However, the influence of the submicron-scale network layer on osseointegration is still unknown and warrants further investigation.

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Titanium dioxide nanotubes

Evaluation of: Bjursten LM, Rasmusson L, Oh S *et al.*: Titanium dioxide nanotubes enhance bone bonding *in vivo*. *J. Biomed. Mater. Res. A* (2009) (Epub ahead of print).

Commercially pure titanium and titanium alloys (Ti6Al4V) are currently used to make orthopedic and dental implants. Implant failure due to a lack or loss of osseointegration can be problematic for the patient. To optimize osseointegration, implant prostheses have traditionally undergone surface modifications in terms of surface chemistry or surface topography [1,2], and micron rough surfaces have been shown to have greater bone–implant contact and bone–implant bond strength compared with smooth surfaces [3,4]. Surface-modification techniques have traditionally included grit blasting, acid etching and coatings to produce micron rough surfaces. However, there is increasing evidence in the literature to suggest that nanotextured surfaces further enhance *in vitro* cell behavior and may also provide enhanced osseointegration [5,6].

Previous work from Oh *et al.* found that titanium dioxide (TiO₂) nanotubes enhanced osteoblast adhesion and mineralization *in vitro* compared with unmodified TiO₂ [7]. Here, Bjursten *et al.* investigated whether or not the increased *in vitro* bone response to TiO₂ nanotubes translated to an increase in *in vivo* bone bonding.

Commercially pure titanium disks were manufactured and implanted into rabbit tibia with four implant sites per animal. A total of 11 nanotube surfaces and ten grit-blasted surfaces were fabricated, of which seven nanotube and six grit-blasted samples

were used for pullout testing. From the pullout samples, one from each group was used to measure calcium phosphorus levels. The remaining four nanotube and four grit-blasted samples were used for histological evaluation.

The TiO₂ nanotubes were fabricated by electrochemical anodization and the structure of the nanotubes was verified by scanning-electron microscopy and transmission-electron microscopy. The titanium control surface was manufactured by grit blasting with size 22 mesh titanium powders with a pore size of 0.8 microns. The micron rough surface was verified by scanning-electron microscopy.

Following anesthesia, the titanium plates were implanted onto planar surfaces prepared on the rabbit tibia. Teflon caps were placed over the implant surfaces not in contact with the bone to prevent bone overgrowth and a bracket was used to keep the implants and caps in place. The rabbits were sacrificed 4 weeks after implantation. Pullout testing was carried out using a tensile test machine with a test speed of 0.1 mm/s. The maximum fracture force for each sample was recorded.

The force required to remove the nanotube-coated disk (10.8 ± 3.1 N) was significantly greater than the grit-blasted control (1.2 ± 2.7 N). The bone–implant contact was 78.3 ± 33.3% for the nanotube-coated surface versus 21.7 ± 24.7% for the grit-blasted surface. It is worthwhile noting that the interface bonding with TiO₂ nanotubes was so strong that the fracture occurred within the growing bone rather than at the implant–bone interface.

This paper establishes the enhanced osseointegration capabilities of TiO₂

nanotubes in an *in vivo* rabbit model. TiO₂ nanotubes have, in earlier studies, shown accelerated osteoblast adhesion, and increased alkaline phosphate and mineralization levels *in vitro*, and here the translation of these results *in vivo* has been demonstrated. This approach therefore appears to hold some promise for translation to the clinical setting where osseointegration is a prerequisite for the success of an implant.

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