Effect of Titania Nanotubes on the Differentiation of Adipose Derived Stem Cells

Kari Cowden[a], Marcela Ferreira Dias-Netipanyj[a,b] and Ketul C. Popat[a,c]

[a] Department of Mechanical Engineering, Colorado State University, Fort Collins, Colorado, USA
[b] Pós-graduação em Ciências da Saúde, Escola de Medicina, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil
[c] School of Biomedical Engineering, Colorado State University, Fort Collins, Colorado, USA

Background and Introduction

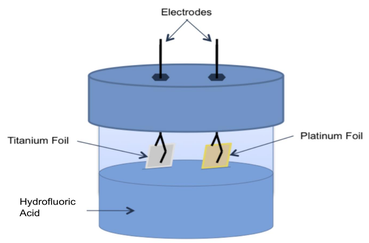
- Titanium implants experience a high level of failure at the implant site due to fibrous capsule formation, inflammation, and infection.
- There are many microscale surface modifications to titanium being explored. Since bone tissue is composed of various nanoscale components an implant surface that mimics this structural hierarchy could enhance integration of the implant to the surrounding bone.
- Titanium implants with a nanotube (NT) surface topography have been shown to improve cellular function and implant fixation.
- Adipose Derived Stem Cells (ADSC) are obtained from subcutaneous adipose tissue, are available in large numbers, easily accessible, and attach and proliferate rapidly in culture, making them an attractive cell source for tissue engineering. Additionally, recent studies demonstrated ADSC to have a substantial *in vitro* bone formation capacity, similar to that of mesenchymal stem cells.

Objective

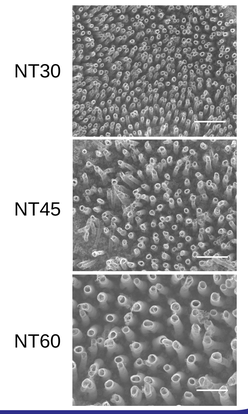
Few studies have investigated the proliferation and differentiation of ADSC on titania (TiO_2) nanotube surfaces. Thus, ADSC were cultured on three different nanotube surfaces and untreated titanium (Ti) as a control. The goal of this study is to determine the effect of nanotube size on proliferation and differentiation of ADSC.

Fabrication of Titania Nanotube Surfaces

- A simple anodization process was used to fabricate highly uniform titania nanotubes (NT) at three different sizes using anodization at 30 volts, 45 volts, and 60 volts.
- The surface morphology and nanotube size was evaluated through scanning electron microscopy (SEM).



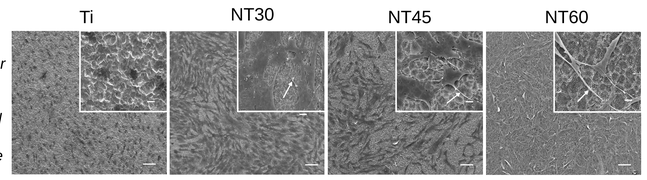
Schematic of anodization setup for fabrication of titania nanotube surfaces.



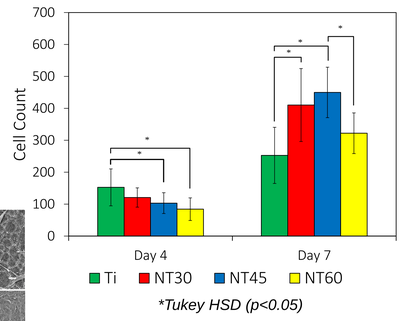
SEM images of nanotube surfaces at 25,000x magnification (scale bar 1μm) .

ADSC Adhesion and Proliferation on Nanotube Surfaces

- SEM images and fluorescent staining were performed to visualize ADSC morphology and quantify cell proliferation.
- Cell count is greater on nanotubes than titanium by day 7.
- ADSC on nanotube surfaces are elongated with long filopodia extending across the surfaces. This may be due to the dispersion of protein which is influenced by the nanotube surface area.



SEM images of nanotube surfaces after 7 days of ADSC proliferation at 100x (scale bar 100μm) and 1,000x (scale bar 10μm). Arrows indicate filopodia.



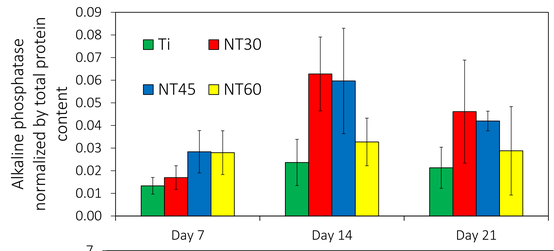
**Tukey HSD ($p<0.05$)*

ADSC Differentiation on Nanotube Surfaces

To confirm osteogenic differentiation and to determine the level of activity of the differentiated ADSC, the following investigations were performed.

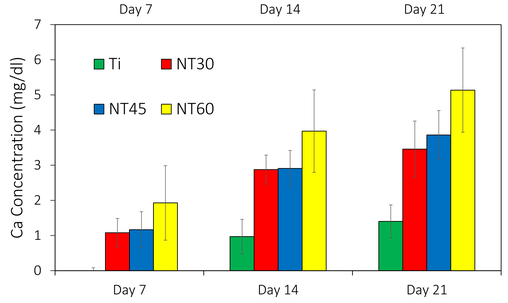
ALP Activity

- ALP is an enzyme whose increased activity is an early marker of osteoblast differentiation and leads to increased levels of phosphate, a component of the mineral phase of bone or hydroxyapatite.
- Nanotubes surfaces stimulate greater ALP activity than titanium surfaces.
- As nanotube size increased ALP activity decreased, suggesting slower osteoblast enzyme function on larger nanotubes.



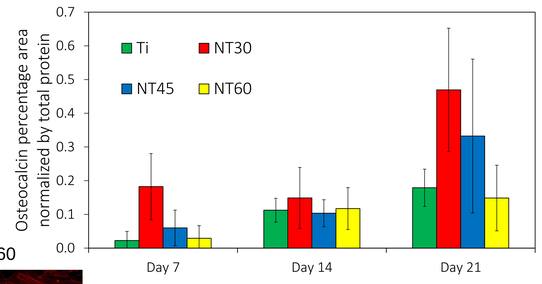
Calcium Concentration

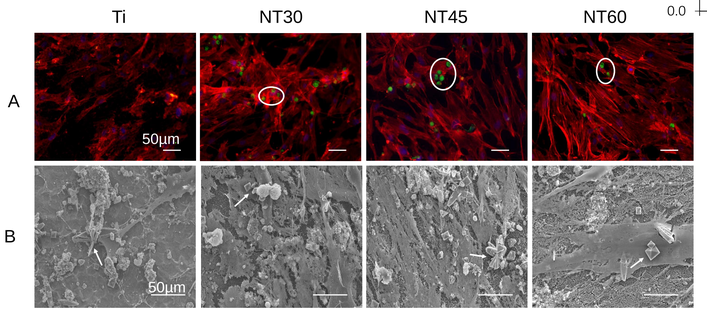
- Differentiated ADSC will deposit bone matrix minerals on the surface, mainly calcium and phosphorous.
- After 21 days, all nanotube surfaces have significantly larger concentrations of calcium than the titanium surfaces suggesting that nanotube surfaces stimulate ADSC to perform greater bone matrix deposition than titanium surfaces.



Osteocalcin

- Osteocalcin is a small molecular weight protein produced by osteoblasts that aids in mineralization and is considered a late marker of differentiation.
- Immunofluorescent staining was used to visualize ADSC differentiation and quantify the osteocalcin distributed on the nanotube and titanium surfaces.
- Only smaller nanotubes displayed higher average osteocalcin than titanium after 21 days.





(A) Immunofluorescent images after 7 days of ADSC differentiation with circles indicating osteocalcin. (B) SEM images of nanotube surfaces after 21 days of ADSC differentiation with arrows indicating mineral deposits.

Mineral Deposition

- The morphology of ADSC on surfaces was investigated using SEM images of nanotube and titanium surfaces.
- After 21 days, the surfaces were almost completely covered by differentiated ADSC and mineralized matrix components were prevalent on both nanotube and titanium surfaces.

Conclusions

- This study confirms that nanotube surfaces promote osteogenic differentiation of human ADSC and may improve implant stability.
- Further studies are now directed toward understanding the nanoscale mechanisms that affect ADSC cell function.
- Additional research on the optimal nanoscale of titanium implants *in vivo* could lead to nanostructured implants that are tailored to improve fixation to the surrounding bone tissues and reduce implant failures.