Enhancing Osseointegration of Orthopaedic Implants with Titania Nanotube Surfaces

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Disclosure

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- All author disclosures are located in the AOFAS program.
Introduction

• The number of orthopaedic surgical procedures, especially joint replacements, continues to increase annually.\(^1\)
  – In 2011, there were nearly 1 million total joint replacements in the United States, with total hip and knee joint replacement procedures most common.\(^2,3\)

• Arthroplasty components require long-term biologic fixation between the bone and implant.
  – Metal components in contact with bone are often coated with macroscale surface features to encourage osseointegration.
Postoperative Complications

• One long-term complication is osteolysis, defined as bone resorption at the bone-implant interface.\textsuperscript{4}
  – Wear debris generation is often implicated.
  – Leads to cascade of events, including implant loosening and eventually revision.

• New methods and technologies to improve osseointegration and reduce production of wear debris are needed.
  – Enhancing implant fixation and wear performance could lead to increased term of implantation thereby improving patient outcomes.
  – Increased osseointegration rates could decrease postoperative recovery time as well as encourage fixation in challenging anatomic locations and environments.
Novel Surface Treatment

• In an electrochemical process refined and characterized previously by the Multi-scale Technologies Institute at Michigan Technological University, titania nanotubes (TiNT) are etched from titanium (both CP and alloys).

• Etching is not limited by geometry, and processing parameters can be modified to obtain different nanotube characteristics and morphologies.
Study Hypotheses

Compared to unmodified controls, titania nanotube surfaces will:

• Show greater potential for osseointegration at the cellular level.

• Demonstrate increased bone formation in a clinically-relevant animal model of osseointegration.

Study Design

**In Vitro Experiment:**
- Cell Response
  - Timepoints: 30 min, 2 hr, 4 hr, 3 d, 7 d, 14 d, 21 d
  - Total Population: n=40,000 cells/coupon
  - Protein Expression (Alkaline Phosphatase and Osteocalcin Assays)
  - Gene Expression (qPCR; IGF-1, Col1a1, osteonectin)

**In Vivo Experiment:**
- Osseointegration
  - Endpoints: 4 & 12 wk
  - Total Population: n=32
  - Bone-Implant Contact (μCT)
  - Backscatter SEM
  - Histology (Longitudinal sections stained with Stevenel’s Blue and van Gieson picrofuchsin)
In Vitro Cell Differentiation via Protein Expression Assays

- Cells were isolated from long bones of Sprague Dawley rats and drop-seeded onto titanium alloy (Ti-6Al-4V) coupons. These cells approximate the same environment in contact with implants and same strain as in vivo experiment.

- Osteocalcin and ALP expressed throughout the experiment, demonstrating the differentiation of the BM cells to osteoblastic phenotype.

- OC Statistics: *Aligned TiNT vs. Control @ 3 d (p=0.005), **Aligned TiNT vs. Control and #Trabecular TiNT vs. Control @ Day 14 (p=0.003 and p=0.039, respectively), ***Aligned TiNT vs. Control and ##Trabecular TiNT vs. Control @ 3 wk (p=0.009 and p<0.001, respectively).

- ALP Statistics: *Aligned TiNT vs. Control @ 3 d (p=0.014), **Aligned TiNT vs. Control and #Trabecular TiNT vs. Control @ 2 wk (p=0.004 and p=0.037, respectively), ***Aligned TiNT vs. Control and ##Trabecular TiNT vs. Control @ 3 wk (p=0.001 and p<0.001, respectively).
**In Vitro Gene Expression via qPCR**

**Collagen, type 1, alpha-helix 1 (Col1a1):**
- Protein that strengthens and supports bone tissue; commonly assayed to quantify Type I Collagen, the primary constituent of bone.
- Trabecular TiNT vs. Control @ 3 d and 1 wk (p=0.004) and 1 wk and 3 wk (p=0.004).

**Insulin-like growth factor-1 (IGF-1):**
- Protein molecularly-similar to insulin that stimulates cell growth and proliferation of many cell types, including osteoblasts, while inhibiting apoptosis (cell death)
- Statistics: No differences between either TiNT surface and Control.

**Osteonectin (ON):**
- Osteonectin production is a phenotypic characteristic of osteoblasts. This protein binds calcium and is secreted by osteoblasts during matrix mineralization.
- Trabecular TiNT vs. Control @ 3 d and 1 wk (p=0.046).
**In Vivo Osseointegration of TiNT Surfaces**

- Kirschner wires were bilaterally implanted into Sprague Dawley rat (outbred strain) femora via retrograde insertion, using a lateral parapatellar approach. Animals were randomized to treatment group, testing group, and endpoint.\(^7-9\) [K-wire specifications: Ti-6Al-4V ELI hard, 1.25 mm OD, trocar tip, 6” length (~1” implanted)]

- **Backscatter electron imaging** was used to assess presence/absence of bone at each pixel along the line profile. Percentage of BIC was calculated. In the Distal ROI, BIC was greater than Control for both treatment groups at both endpoints. In the Midshaft ROI, the results were highly variable.

- **Undecalcified histology** showed increased bone-implant contact in TiNT-implanted femora.

<table>
<thead>
<tr>
<th>Group</th>
<th>Endpoint (wk)</th>
<th>Bone-Implant Contact Ratio (TiNT/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aligned TiNT</td>
<td>4</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.58</td>
</tr>
<tr>
<td>Trabecular TiNT</td>
<td>4</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.50</td>
</tr>
</tbody>
</table>
µCT: Bone-Implant Contact

- Volume fraction increased from the 4 wk to 12 wk endpoint for both TiNT groups vs. Control.
- Comparable trend was observed for total bone volume.
Conclusions

- *In vitro* studies demonstrated improved support for osteogenic functions of cultured marrow-derived cells on both the Aligned and Trabecular morphology titania nanotube surfaces compared to unmodified controls.

- μCT, backscatter electron imaging, and histologic analyses, performed in conjunction with the *in vivo* study, demonstrated increased bone formation in the titania nanotube-implanted femora (both morphologies) at both the 4- and 12-week endpoints and within the specified “distal” and “midshaft” volumes of interest.
References


