Local Delivery of SDF-1β via Titania Nanotube Surfaces to Promote Osseointegration

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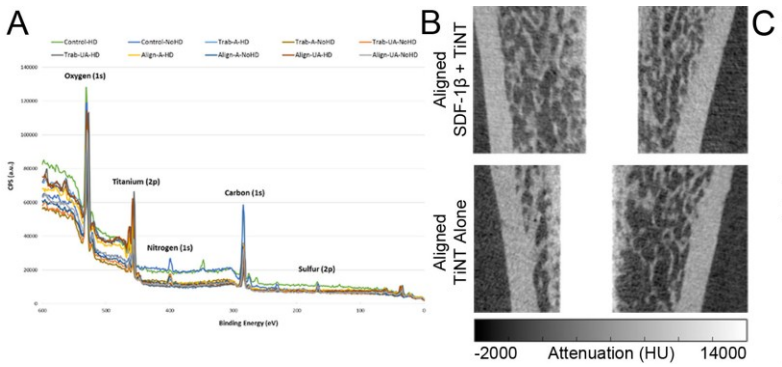
INTRODUCTION: During primary or revision orthopaedic surgical procedures, the periprosthetic environment is exposed and primed for local delivery of pharmacologic agents and small molecules, which may modulate various biologic responses, including inflammation, foreign body response, and osteogenesis. The objective of this study is to evaluate SDF-1β delivery potential via functionalization of heparin bound titania nanotubes (TiNT) surfaces, which have been previously shown to increase osseointegration. SDF-1β is highly chemotactic for mesenchymal stem cells. We hypothesize that SDF-1β delivery will result in enhanced recruitment of stem cells and subsequent new bone formation by recruited cells.

METHODS: TiNT surfaces were etched from Ti-6Al-4V alloy via electrochemical anodization. Samples were sonicated to produce Aligned TiNT (aligned hollow tubular structures), or unsonicated for Trabecular TiNT (disorganized structure mimicking bone). Annealed and unannealed surfaces were evaluated with unetched titanium alloy as the Control. To promote the attachment of SDF-1β to the implant, materials were functionalized with a heparin-dopamine (Hep-DOPA). Confirmation of Hep-DOPA conjugation was confirmed via toluidine blue staining and X-Ray photoelectron spectroscopy (XPS). Attachment of SDF-1β was confirmed by quantifying the unbound SDF-1β left in the soak solution. A pilot release experiment was conducted with three SDF-1β dosages (50, 100, 500 ng). Samples were immersed in a phosphate buffered saline + bovine serum albumin solution, and the supernatant was analyzed at four timepoints (0, 24, 48, 72, 144 hrs). After IACUC approval, Sprague-Dawley rats received bilateral femoral intramedullary implants—Ti alloy Kirschner-wires (1.25 mm diameter) with either Aligned or Trabecular TiNT surfaces (n=8/group)—and were allowed *ad libitum* activity until the 12-week study endpoint. One limb received a TiNT-etched (either Aligned or Trabecular) implant loaded with 100ng SDF-1β, while the other limb received a TiNT-etched implant (same morphology) without SDF-1β. Periprosthetic bone formation was characterized via longitudinal μCT imaging (Viva80-CT, Scanco USA) at 4- and 12-week postoperative timepoints (n=8/group). Characterization was performed in a metaphyseal and diaphyseal volumes-of-interest (VOIs) to determine bone-implant contact (BIC) as well as standard bone morphometry and densitometry. Endpoint backscattered electron imaging (BEI) and undecalcified histology (n=3/group) will also be performed. Strength of the bone-implant interface will be characterized via biomechanical pull-out testing (n=5/group), performed on an electromechanically-actuated uni-axial materials loading frame (Insight 150, MTS Systems). Animals were randomized to both TiNT group as well as characterization group (BEI/histology vs. biomechanics).

RESULTS: Unannealed coupons contained a greater amount of bound heparin than annealed or control coupons, based on toluidine blue staining. XPS demonstrated decreased nitrogen and sulfur contents on surfaces with bound heparin, further confirmation of effective Hep-DOPA functionalization (Figure 1A). Based on these results, the release and *in vivo* experiments were conducted with unannealed TiNT. The pilot release experiment demonstrated greater than 99% attachment of SDF-1β on all surfaces (TiNT and Control) and all dosages at time zero. All samples showed similar release profiles through the 144-hour timepoint, with total released quantity of SDF-1β ranging from 0.0365 to 0.0487 pg for all groups and dosages. *In vivo* μCT (Figure 1B, C) demonstrated no significant differences in BIC between groups, though bone tissue mineral density (TMD) was significantly greater in Aligned TiNT + SDF-1β implanted femora vs. Aligned TiNT alone (p=0.02). Overall mean BICs of the metaphysis and diaphysis, respectively, were 0.49 ± 0.14 and 0.32 ± 0.12. Morphometric analysis in the metaphysis demonstrated significantly greater trabecular number (Tb.N, p=0.02) and significantly lower trabecular spacing (Tb.Sp, p=0.01) in Aligned TiNT + SDF-1β implanted femora vs. Aligned TiNT alone. In the diaphyseal VOI, there were trending increases in bone-implant contact, bone volume/total volume (BV/TV), and bone mineral density (BMD) in both TiNT + SDF-1β implanted femora vs. TiNT alone at 4- and 12-week timepoints, although not significant. When normalizing TiNT + SDF-1β implanted femora to TiNT alone for each animal, Tb.N was both significantly greater in Trabecular TiNT-implanted femora vs. Aligned TiNT at 4 weeks (p=0.05), demonstrating a more profound positive effect of SDF-1β in the Trabecular TiNT group compared to the Aligned TiNT group, and significantly greater in Aligned TiNT + SDF-1β vs. Aligned TiNT alone at 4 weeks vs. 12 weeks (p=0.01), indicating a more profound positive effect of SDF-1β at an earlier timepoint.

DISCUSSION: We have developed a method for functionalizing TiNT with Hep-DOPA in order to attach SDF-1β and demonstrated proof-of-concept via XPS, toluidine blue staining, and drug release kinetics. *In vivo* μCT imaging demonstrated that SDF-1β exhibits some osteogenic effects. In the metaphysis, higher TMD, greater Tb.N and lower Tb.Sp. were observed in the SDF-1β + TiNT group as 12 weeks, suggesting better peri-implant bone growth in this group. Diaphyseal analysis reveals minimal effect of SDF-1β administration, but considerable bone growth within the medullary canal was observed in all groups. Biomechanical, BEI, histologic analyses as well as ELISA testing on an extended release study (12 timepoints; range, 24 hrs – 91 d) are currently underway to confirm and further elucidate these results.

SIGNIFICANCE: For implants requiring bony fixation, efficient osseointegration as well as a stable bone-implant interface are early indicators of clinical success. TiNT surfaces have been shown to enhance osseointegration as a result of increased *in vitro* osteoblast attachment and *in vivo* bone mineralization, and the addition of SDF-1β may recruit additional stem cells to the site further promoting bone-implant fixation.



C

Metaphysis:

	Study Group		BIC	TMD (HU)	Tb.N	Tb.Sp. (mm)
Aligned	4-week	No SDF-1β	0.49 ± 0.18	5029 ± 268	2.94 ± 0.39	0.19 ± 0.03
		SDF-1β	0.48 ± 0.11	4967 ± 142	2.85 ± 0.35	0.19 ± 0.02
	12-week	No SDF-1β	0.49 ± 0.10	5398 ± 235	2.34 ± 0.40	0.26 ± 0.05
		SDF-1β	0.53 ± 0.08	5128 ± 161	2.69 ± 0.45	0.21 ± 0.04
Trabecular	4-week	No SDF-1β	0.54 ± 0.16	5079 ± 192	2.92 ± 0.48	0.20 ± 0.03
		SDF-1β	0.50 ± 0.18	5201 ± 535	2.83 ± 0.66	0.23 ± 0.07
	12-week	No SDF-1β	0.41 ± 0.18	5173 ± 393	2.41 ± 0.70	0.24 ± 0.06
		SDF-1β	0.47 ± 0.10	5064 ± 292	2.44 ± 0.56	0.25 ± 0.07

Diaphysis:

	Study Group		BIC	TMD (HU)	Tb.N	Tb.Sp. (mm)
Aligned	4-week	No SDF-1β	0.30 ± 0.06	4480 ± 204	2.05 ± 0.45	0.37 ± 0.05
		SDF-1β	0.31 ± 0.07	4576 ± 144	1.83 ± 0.54	0.42 ± 0.14
	12-week	No SDF-1β	0.34 ± 0.17	4950 ± 489	1.53 ± 0.63	0.41 ± 0.09
		SDF-1β	0.39 ± 0.10	4888 ± 221	1.97 ± 0.90	0.43 ± 0.23
Trabecular	4-week	No SDF-1β	0.26 ± 0.09	4537 ± 103	1.52 ± 0.74	0.46 ± 0.14
		SDF-1β	0.30 ± 0.07	4538 ± 189	1.92 ± 0.64	0.37 ± 0.11
	12-week	No SDF-1β	0.36 ± 0.13	4930 ± 282	1.59 ± 0.56	0.45 ± 0.13
		SDF-1β	0.36 ± 0.11	4865 ± 418	1.77 ± 0.38	0.39 ± 0.10

Figure 1. *(A) XPS analysis of TiNT materials. (B) Representative μCT images of bone growth in the metaphysis at 12 weeks. (C) Bone morphometric and densitometric data for the metaphyseal and diaphyseal VOIs.*