A More Perfect Imitation of Nature

Literature Review

Copyright © 2015 Titan Spine, LLC

TITAN SPINE

STAND WITH THE FUTURE

A more perfect imitation of nature

Next generation nanoLOCK® implant surface is closest yet to ideal bone-growing environment for spinal fusion

Several *in-vitro* studies have confirmed the profound impact that the material composition and surface texture of an interbody fusion device have on the prospects for osteointegration and spinal fusion [1-4]. The research demonstrates that implants made of titanium alloy (Ti6Al4V) – specifically Titan Spine Endoskeleton® implants that have macro-, micro and nanoscale (MMN™) surface structures – provide a substantially enhanced osteogenic environment in comparison to polyetheretherketone (PEEK) and control surfaces. Borrowing principles from the growing field of biomimicry, the MMN™ micro and nanoarchitecture closely mimics the hierarchical structure of natural bone, and thus encourages cells to respond in a remarkable way:

- Boosting the production and differentiation of bone-forming osteoblasts
- Down-regulating excess production and activity of bone-resorbing osteoclasts
- Increasing factors that bring blood supply to support bone growth

The literature shows that complex, "rough" surfaces on titanium allow more natural cell signaling for bone remodeling than do smooth surfaces.[5] Several other studies suggest that implant surfaces with features geometrically analogous to osteoclastic pits promote better osteoblast response compared with smooth surfaces.[6-7]

Unsurprisingly, then, PEEK implants – with their smooth, virtually featureless surface textures – have been shown to produce an effect opposite that of MMN™ implants. Attachment-dependent stem cells encountering PEEK underproduce factors that promote osteoblastic differentiation and overproduce pro-inflammatory factors – resulting in the creation of a non-bony, fibrous layer around the implant that may lead to the need for revision.

The strong implication of these MMN™ versus PEEK comparisons is that – due mainly to each implant's respective surface texture and material composition – cells in the human body will react negatively to the PEEK implant and respond positively to the MMN™ Endoskeleton® device, the latter featuring a closer approximation of bone surface microstructure.

This paper will describe recent *in-vitro* research[8-10] related to Titan Spine's next generation surface, which – in comparison to the Titan Spine's first generation Endoskeleton surface – optimizes the shape and scale of nanoscale features, thus creating a more perfect imitation of nature. This new surface nanotopography – called nanoLOCK® – further amplifies the differentiation of osteoblasts and production of osteogenic and angiogenic factors.

A nanotextured surface for the "discriminating" cell

In the 2014 *in-vitro* study by Olivares-Navarrete, et al, researchers found that human mesenchymal stem cells (MSCs) and normal human osteoblasts responded differently to titanium implant surfaces depending on the characteristics of the surface's smallest (i.e., nanoscale) features.

Titan Spine Proprietary Information - Unauthorized use is prohibited

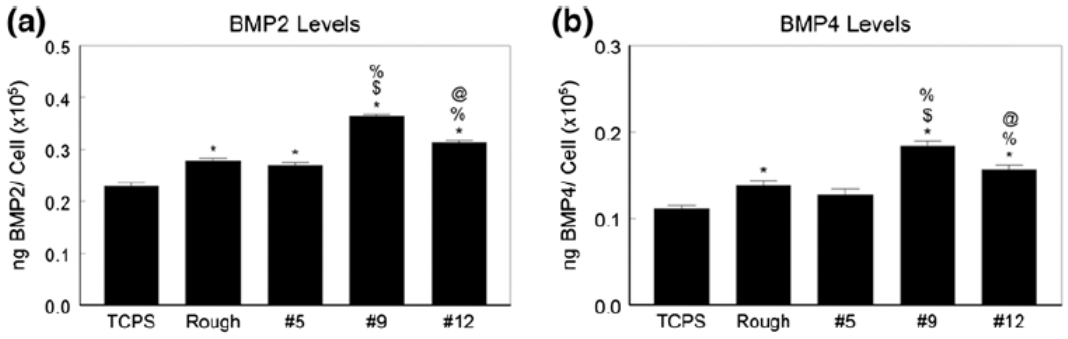
Investigators tested several implant surfaces, some of which had received additional secondary processing with variations of proprietary subtractive treatments (Titan Spine) [8]. This extra processing created finer and qualitatively different submicron and nanoscale features that differed in key parameters [8-9] (e.g, maximum peak height, maximum valley depth, skewness*, kurtosis**, etc.).

*Skewness refers to the symmetry of peaks to valleys. Positive skewness represents elevations from a relatively flat surface, whereas negative skewness represents wide plateaus eroded by deep valleys.

**Kurtosis is a parameter that describes the peakedness of a surface: kurtosis > 3 indicates sharp peaks, kurtosis = 3 indicates slightly rounded peaks, and kurtosis < 3 indicates wide, domed peaks.

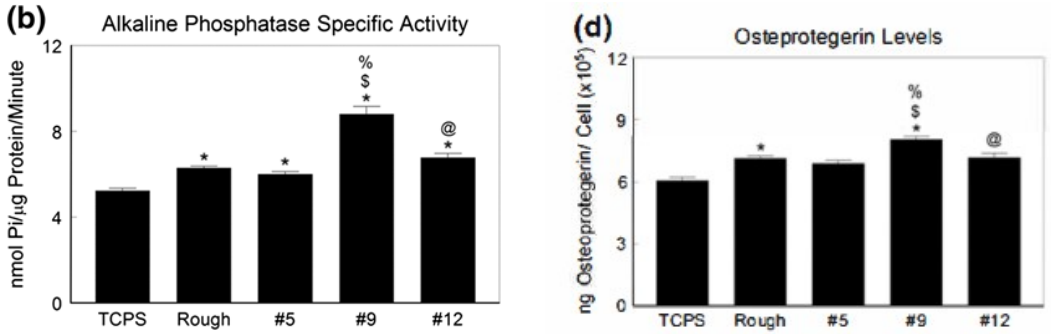
After extensive research and development to refine the micro and nanoscale features, three titanium alloy surfaces were selected for testing based on promising micro- and nanoscale roughness parameters. Using Titan Spine's original Endoskeleton® surface as a control (rTiAlV), HMSC's and osteoblasts were cultured on these three surfaces (No. 5, No. 9 and No. 12) for analysis of differentiation enzymes, osteogenic local factors and integrin subunit expression.

Of the four titanium alloy surfaces, No. 9 (Titan Spine's next-generation nanoLOCK® surface) ranked highest in numerous parameters related to osteoblast differentiation, production of osteogenic factors and integrin expression.



Local factor production by normal human osteoblasts on microstructured Ti6Al4V. Osteoblasts were cultured on TCPS, rTiAlV, #5, #9, or #12 surfaces and secretion of BMP2 (a), and BMP4 (b) measured in the conditioned media. * p< 0.05 vs. TCPS; $ p< 0.05 vs. rTiAlV; o; %p< 0.05 vs. #5; @p< 0.05 vs. #9.

Similarly, when assessing the MSC response, the No. 9 surface had the greatest alkaline phosphatase activity and highest levels of osteoprotegerin, in addition to the greatest amount of mRNA expression of ITGA1, ITGA2 and ITGAV integrins.

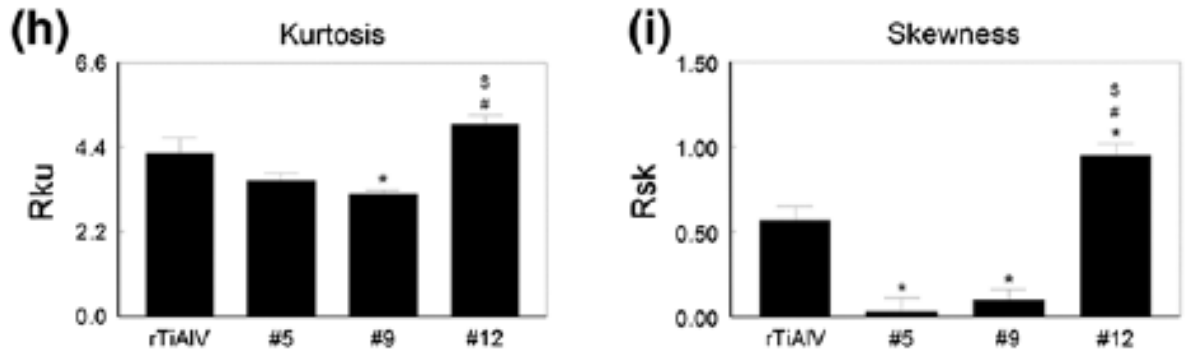


Osteoblastic maturation of normal human osteoblasts on microstructured Ti6Al4V. Osteoblasts were cultured on TCPS, rTiAlV, #5, #9, or #12 surfaces and osteoblast response measured by (b), alkaline phosphatase specific activity and (d), osteoprotegerin production measured.• p< 0.05 vs. TCPS; $p< 0.05 vs. rTiAlV; % p< 0.05 vs. #5; @ p< 0.05 vs. #9.

Titan Spine Proprietary Information - Unauthorized use is prohibited

The results confirm that osteoblasts on titanium alloy surfaces show a more differentiated phenotype and that both osteoblasts and MSCs are sensitive to specific topographical features of a nano-scale surface (i.e., peak height, kurtosis and skewness) and that the No. 9 surface (nanoLOCK®) produced a significantly greater amount of osteogenic and angiogenic factors than the Endoskeleton® surface.

No. 5 and No. 9 had lower maximum peak heights, lower kurtosis and lower skewness than the Endoskeleton® or No. 12 surfaces, with the lowest kurtosis on No. 9.



Measurement of roughness parameters. Disk roughness was characterized by kurtosis (h), and skewness (i) measured.*p<0.05 vs. rTIAlV; #p<0.05 vs. #5; $p<0.05 vs. #9

The researchers noted that:

> "Importantly, this study demonstrates that [normal human osteoblast] cells and MSC's are sensitive to specific topographical features of a microstructured surface. While the surfaces used for this study were similar in average roughness at the microscale, they differed in other topographical parameters, most notably peak height, kurtosis and skewness."

The ability of key cells in the healing process to discriminate nanoscale differences in surface topography clarifies the impact that implant surface nano-architecture has on spinal fusion.

The rougher the better at the nano level

In their *in-vitro* study that aimed to quantitatively differentiate Titan Spine's first and second generation surface technologies – Endoskeleton® and nanoLOCK®, respectively – Matteson et. al developed a method to quantify osteoclastic-like pits on each implant's surface.[9]

During natural bone remodeling – following an initial mineralization stage – osteoclasts resorb newly formed bone to repair microcracks and prepare the surface for new bone formation. The activity of osteoclasts creates microscale resorption pits (30µm diameter x 14µm deep) on the surface of the bone that possess even smaller nanoscale features within them. It's this unique, highly complex surface topography that is the signal that MSC's require when encountering a surface that requires new bone formation. The idea of imitating the hierarchical structure of natural bone on implant surfaces by incorporating osteoclast-like pits and nanoscale structures on commercial interbody implants comes from this observation.[3]

TITAN SPINE Titan Spine Proprietary Information - Unauthorized use is prohibited

The researchers compared disks of machined titanium alloy, in addition to disks with either the Endoskeleton® MM surface or next generation nanoLOCK® MMN™ surface.

SEM images at 250x: (a) machined Ti alloy surface (S), (b) etched Ti alloy surface (MM), and (c) etched Ti alloy surface with additional etching step (MMN)

Using an optical profilometer, they observed depressions on the Endoskeleton® and nanoLOCK® surfaces that had dimensions similar to osteoclastic pits. This observation guided the development of a methodology called a “valley count” analysis to assess the relative numbers of these pits on the MMN™ surfaces. The investigators found that the nanoLOCK® surface contained 11 times the number of osteoclastic-like pits compared to the Endoskeleton® surface.

Representative optical profilometry images (280 x 210 μm^2 field-of view): (a) S, (b) MM, and (c) MMN. The Z-range grayscale for a,b, and c are not equivalent. Features identified as “valleys” using the valley analysis protocol are identified with asterisks

Furthermore, using atomic force microscopy (AFM) to evaluate each of the three surface’s average roughness (Ra) at the nanoscale, the researchers found that the nanoLOCK® MMN™ surface was significantly rougher than the other two surfaces. The Ra of the nanoLOCK® surface was found to be nearly two-and-a-half times higher than the Ra of the Endoskeleton® surface.

nanoLOCK® may help explain the significant increase in nanoLOCK® osteogenic and angiogenic production reported by Olivares-Navarrete, et al.[8]

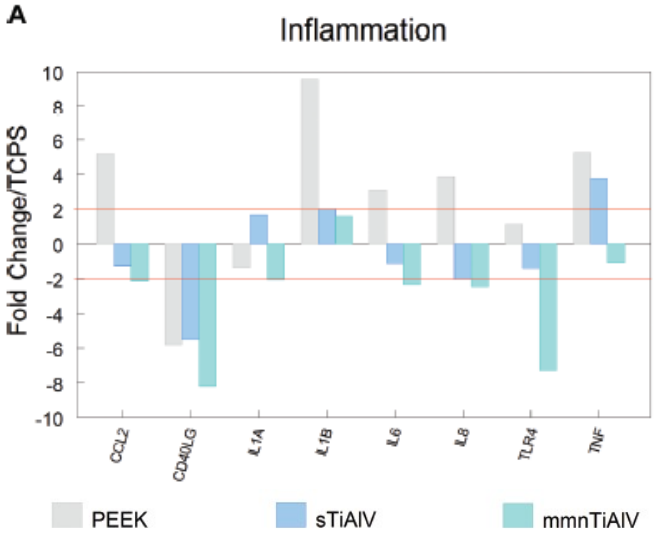
SIDEBAR

Pro-inflammatory PEEK

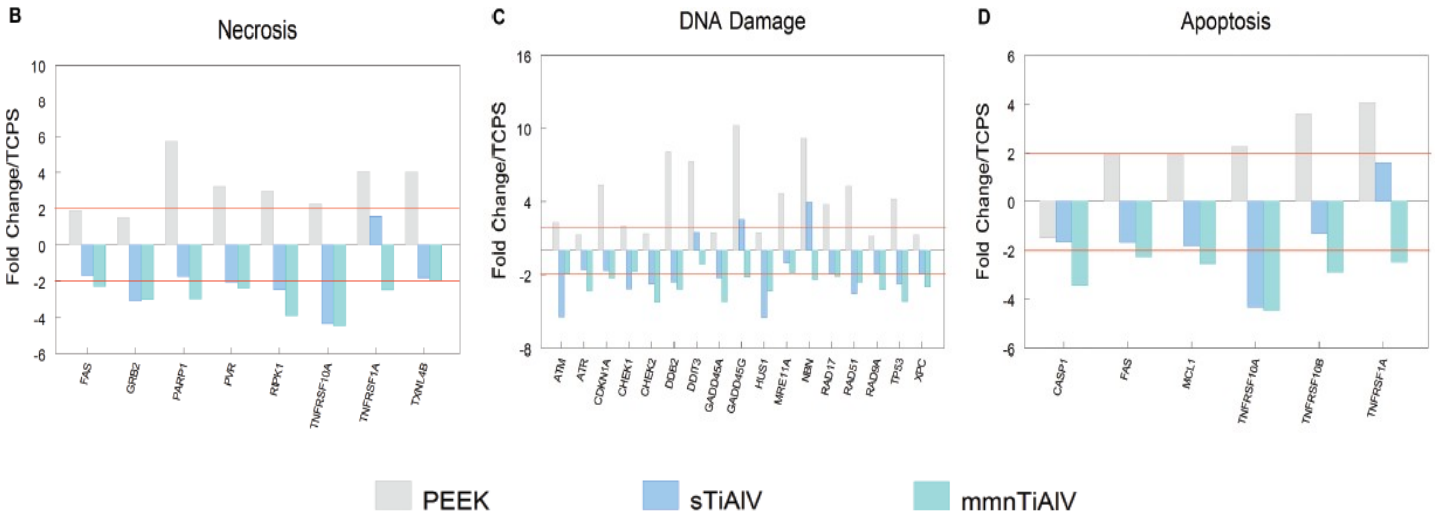
In a 2015 *in-vitro* study, Olivares-Navarrete, et al. suggested that the formation of fibrous tissue around a PEEK implant is most likely due to a combination of down-regulated osteoblast production and increased production of proinflammatory factors. The researchers compared smooth titanium alloy (TiAlV), Titan Spine's MMN™ implant surface (nanoLOCK®) and PEEK samples, analyzing the proteins and factors produced in each surface's cellular microenvironment.[10]

Mesenchymal stem cells (MSCs) were grown on each surface for seven days.

Production of pro-inflammatory factors by MSCs was highest on PEEK compared with all other materials. Conversely, production was significantly lower on the nanoLOCK™ surface and was even lower than on the control sample. Levels of anti-inflammatory interleukin 10 (IL-10) cytokines were significantly greater in cultures grown on both titanium alloy substrates than on PEEK, with the greatest amount found on the nanoLOCK™ surface.



An analysis of the MSCs for pro-inflammatory proteins and those associated with necrosis showed that the cells on nanoLOCK® exhibited the lowest mRNA levels. The nanoLOCK® surface also demonstrated the least DNA damage and apoptosis. All of these parameters were highest on PEEK.



The researchers noted that an increase in pro-inflammatory factors is associated with fibrous tissue formation. The lowest levels of these factors were observed in the MMN™ cell cultures, whereas the highest levels were on PEEK.

The investigators commented:

> "Taken together, our results showed that [nanoLOCK®] reduced the local inflammatory environment, decreasing the proinflammatory cytokines, but also increasing [anti-inflammatory IL-10]."

The researchers concluded that the nanoLOCK® surface presents MSCs with an osteogenic microenvironment characterized by reduced production of inflammatory factors, while at the same time up-regulated production of anti-inflammatory mediators compared with PEEK.

Titan Spine Proprietary Information - Unauthorized use is prohibited

References

1. Olivares-Navarrete R, Gittens RA, Schneider JM, et al. Osteoblasts exhibit a more differentiated phenotype and increased bone morphogenetic protein production on titanium alloy substrates than on poly-ether-ether-ketone. *Spine J* 2012;12:265-72. DOI: 10.1016/j.spinee.2012.02.002

2. Olivares-Navarrete R, Hyzy SL, Gittens RA, et al. Rough titanium alloys regulate osteoblast production of angiogenic factors. *Spine J* 2013;13:1563-70. DOI:10.1016/j.spinee.2013.03.047

3. Gittens RA, Olivares-Navarrete R, Schwartz Z, et al. Implant osseointegration and the role of microroughness and nanostructures: Lessons for spine implants. *Acta Biomater*2014;10:363-71. DOI: 10.1016/j.actbio.2014.03.037

4. Banik BL, Riley TR, Platt CJ, et al. Human mesenchymal stem cell morphology and migration on microtextured titanium. *Front Bioeng Biotechnol* 2016;4, 41.http://doi.org/10.3389/fbioe.2016.00041

5. Brinkmann J, Hefti T, Chlottig F, et al. Response of osteoclasts to titanium surfaces with increasing surface roughness: An in vitro study. *Biointerphases* 2012;7:34-42.

6. Marchisio M, Di Carmine M, Pagone R, et al. Implant surface roughness influences osteoclast proliferation and differentiation. *J Biomed Mater Res* Part B: *Appl Biomat* 2005; 75B:251-256.

7. Marks SC, Hermey DC. The structure and development of Bone. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of Bone Biology. San Diego, California: Academic Press, Inc; 1996. Pp 3-14.

8. Olivares-Navarrete R, Hyzy SL, Berg ME, et al. Osteoblast lineage cells can discriminate microscale topographic features on titanium-aluminum-vanadium surfaces. Ann Biomed Eng. 2014 Dec;42(12):2551-61. doi: 10.1007/s 10439-014-1108-3. Epub 2014 Sep
17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4239824/

9. Matteson JL, Greenspan DC, Tighe TB, et al. Assessing the hierarchical structure of titanium implant surfaces. *Journal of Biomedical Materials Research* Part B Applied Biomaterials 104(6). May 2015. DOI:10.1002/jbm.b.33462.

10. Olivares-Navarrete R, Hyzy SL, Slosar PJ, et al. Implant materials generate different peri-implant inflammatory factors. *Spine* 40.6 (2015):399-
404. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4363266/

TITAN SPINE Titan Spine Proprietary Information - Unauthorized use is prohibited