

THE NANOSCALE WORLD OF SPINAL FUSION

Literature Review
CONDENSED VERSION



STAND WITH THE FUTURE

The nanoscale world of spinal fusion

The case for optimized MMN™- textured titanium implants

Introduction

Recent research has demonstrated that the potential for successful spinal fusion following implantation of an interbody device is mediated through a complex biochemical chain of events that dictates – at the submicron and nanoscopic levels – how the body responds to the implant. In scientific comparisons of specific titanium alloy implants (Titan Spine) and those made of polyetheretherketone (PEEK), the titanium implants with a unique, roughened surface architecture (macro- micro- nanoscale or MMN™) have been shown to provide an overwhelmingly more osteogenic, fusion-promoting environment than PEEK implants. In fact, research has demonstrated that PEEK implants present an inhospitable environment for osteogenesis, characterized by the proliferation of fibrous tissue on implant surfaces, which may lead to pseudoarthrosis and the need for revision.

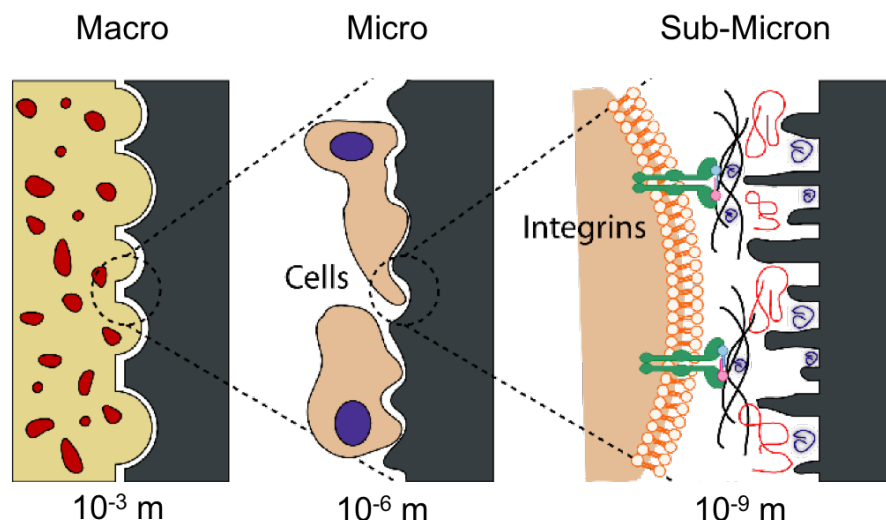
The most recent studies involved Titan Spine's latest generation MMN™ surface technology (nanoLOCK®), which provides not only more nano-scale surface features, but the features themselves are qualitatively enhanced. The effect has been to further amplify the osteogenic response.

Hierarchy of implant surface topography

***Macro** level (10^{-3} m) - implant stability

***Micro** level (10^{-6} m) - cellular attachment

***Nano** level (10^{-9} m) - cell activation via surface interface with integrin receptors

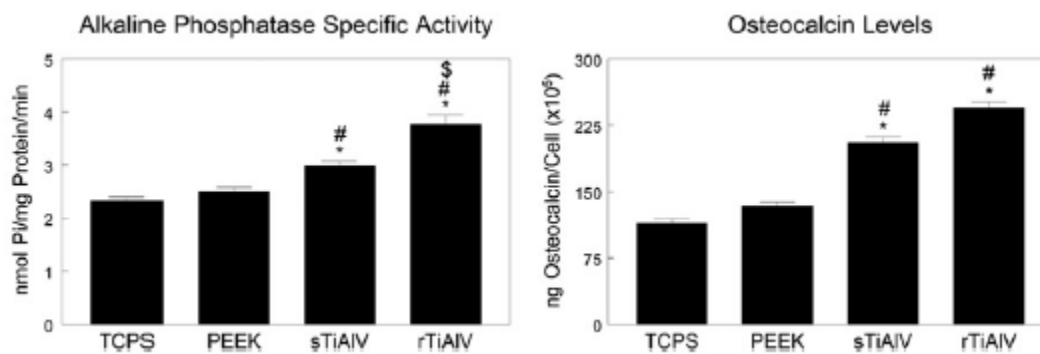


Creating an environment for osteogenesis

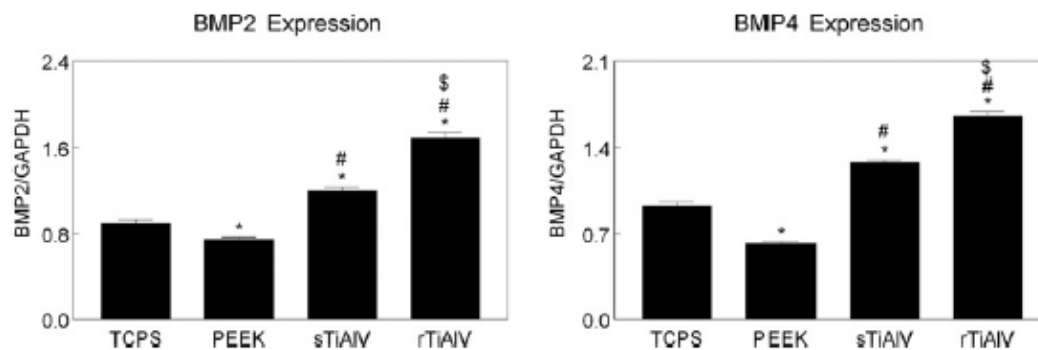
Both smooth and rough titanium alloy surfaces encourage greater osteoblast differentiation and production of factors associated with osteogenesis than do PEEK surfaces – with the greatest effect observed on rough titanium (MMN™), according to a 2012 *in-vitro* study by Olivares-Navarrete, et al.[1]

The researchers cultured osteoblasts on the three different surfaces and measured markers for osteoblast maturation and messenger RNA expression for bone morphogenetic proteins (BMPs).

The results showed that production of differentiation markers and endogenous BMP was significantly higher on MMN™ than on smooth titanium and PEEK surfaces.



Human MG63 osteoblast-like cells were harvested 24 hours after confluence on TCPS. (Left) alkaline phosphatase-specific activity in cell lysates, and (Right) levels of osteocalcin in the conditioned media were measured. * $p < .05$ versus TCPS; # $p < .05$ versus PEEK; \$ $p < .05$ versus sTiAlV. TCPS, tissue culture polystyrene; PEEK, poly-ether-ether-ketone; sTiAlV, smooth Ti6Al4V; rTiAlV, roughened Ti6Al4V.



Human MG63 osteoblast-like cells were harvested 12 hours after confluence on TCPS. Levels of messenger RNA for (Left) BMP2 and (Right) BMP4 were measured by real-time qPCR and normalized to GAPDH. * $p < .05$ versus TCPS; # $p < .05$ versus PEEK; \$ $p < .05$ versus sTiAlV. TCPS, tissue culture polystyrene; BMP, bone morphogenetic protein; qPCR, quantitative polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PEEK, poly-ether-ether-ketone; sTiAlV, smooth Ti6Al4V; rTiAlV, roughened Ti6Al4V.

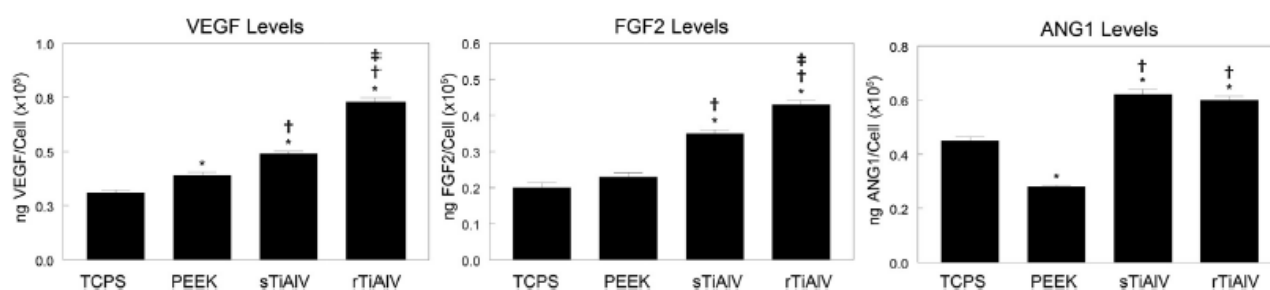
The investigators concluded that modifying the surface of titanium alloy implants with a specific roughened architecture (i.e., MMN™) creates an osteogenic environment that

could enhance bone formation and implant stability, without the need to add exogenous growth factors.

Growing the necessary blood supply

Rough titanium surfaces (MMN™) provide an optimum environment for growing a healthy blood supply to support new bone growth, concluded Olivares-Navarrete, et al., in a follow up *in-vitro* study.[2] The researchers compared smooth titanium, PEEK and MMN™ surfaces, measuring angiogenic factors (VEGF-A, FGF-2, ANG1) and bone-forming factors (e.g., TGF-β1).

They found that cells on smooth titanium produced higher VEGF levels than PEEK, with MMN™ significantly enhancing this effect. FGF-2 levels were 75% higher than PEEK on the smooth titanium surface and 100% higher on MMN™. ANG1 levels were decreased by 50% on PEEK versus a TCPS control and significantly higher on both titanium surfaces.



Secreted VEGF-A (Left), FGF-2 (Middle), and ANG-1 (Right) were measured in the conditioned media of cells cultured on TCPS, PEEK, smooth titanium alloy (sTiAlV), or rough titanium alloy (rTiAlV). Levels were normalized to total cell number. *p<.05, versus TCPS; †p<.05, versus PEEK; ‡p<.05, versus sTiAlV. VEGF-A, vascular endothelial growth factor A; FGF-2, fibroblast growth factor 2; ANG-1, angiopoietin-1; TCPS, tissue culture polystyrene; PEEK, polyether-ether-ketone; sTiAlV, smooth Ti6Al4V; rTiAlV, rough Ti6Al4V.

The researchers also measured markedly higher (100%) osteoprotegerin (which inhibits osteoclasts) production on MMN™ versus PEEK, and that active bone-producing TGF-β1 was over 100% higher on titanium surfaces compared to PEEK.

They concluded that MMN™ surfaces stimulate an angiogenic-osteogenic environment with factors important in bone formation and remodeling, an environment that “may enhance bone formation, implant stability and fusion.”

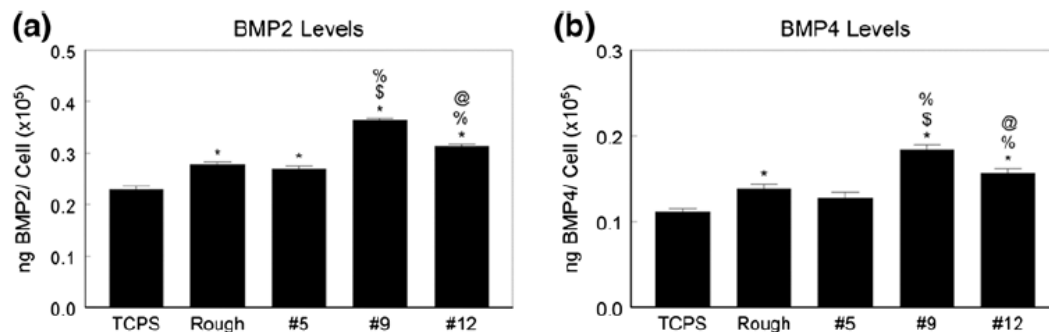
The increasingly nanoscale world of spinal fusion

In a 2014 *in-vitro* study, Olivares-Navarrete, et al. explored whether osteoblasts and MSCs would respond differently to subtle differences in nanoscale roughness on implant surfaces that had otherwise comparable macro- and microscale roughness.[3]

The researchers tested several MMN™ titanium alloy surfaces, some of which had received additional subtractive processing (Titan Spine) that created finer and qualitatively different submicron and nanoscale features that differed in key parameters.

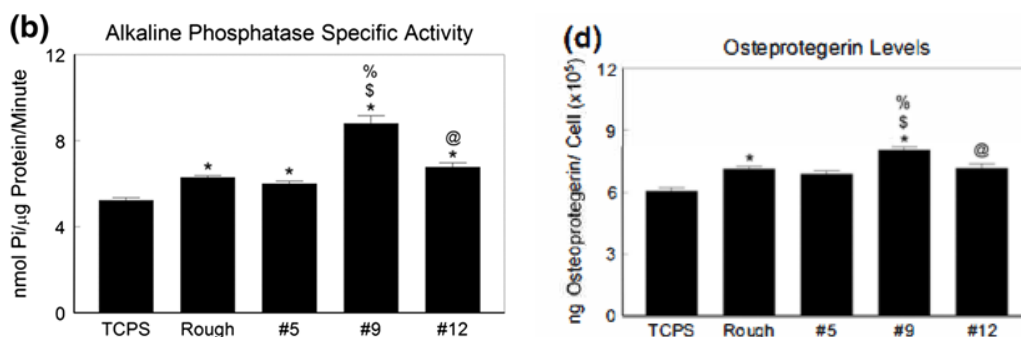
From 35 different iterations, researchers selected three Ti6Al4V surfaces for testing based on promising micro- and nanoscale roughness parameters. With the original Titan Spine Endoskeleton® surface as a control, MSCs and osteoblasts were cultured on the three surfaces (No's 5, 9, 12) and analyzed for differentiation enzymes, osteogenic local factors and integrin subunit expression.

No. 9 – Titan Spine's next-generation nanoLOCK® surface – ranked highest in osteoblast differentiation, production of osteogenic factors and integrin subunit 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; % $p < 0.05$ vs. #5; @ $p < 0.05$ vs. #9.

Regarding MSC response, No. 9 showed the greatest alkaline phosphatase activity and the highest osteoprotegerin levels, in addition to the greatest amount of mRNA expression of integrin subunits.



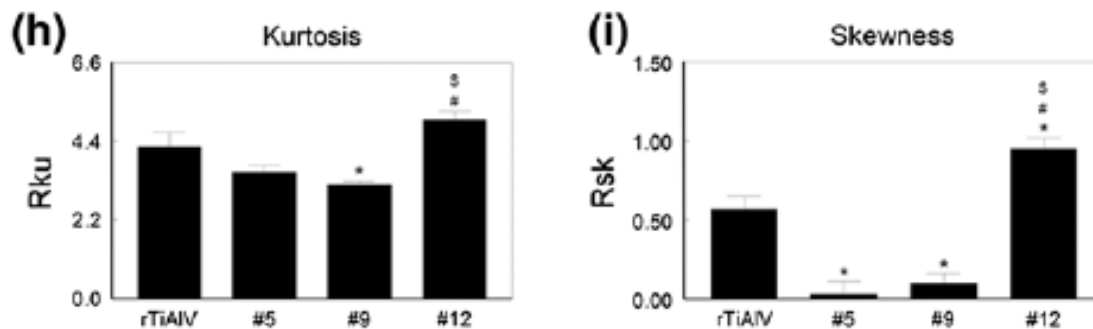
Osteoblastic maturation of normal human osteoblasts on microstructured Ti6Al4V. Osteoblasts were cultured on TCPS, rTiAlV, #5, #9, or #12 surfaces and osteoblast response measured by (b), alkaline phosphatase specific activity and (d), osteoprotegerin production measured. * $p < 0.05$ vs. TCPS; \$ $p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

The results confirm that both osteoblasts and MSCs are sensitive to specific topographical features of a nanostructured surface (i.e., peak height, kurtosis, skewness) and that No. 9 (nanoLOCK®) produced a significantly greater amount of osteogenic and angiogenic factors than the Endoskeleton® surface.

*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.

The investigators concluded that committed osteoblasts and MSCs can discriminate surface features at the nanoscale and are particularly sensitive to the kurtosis and skewness of nanoLOCK®.

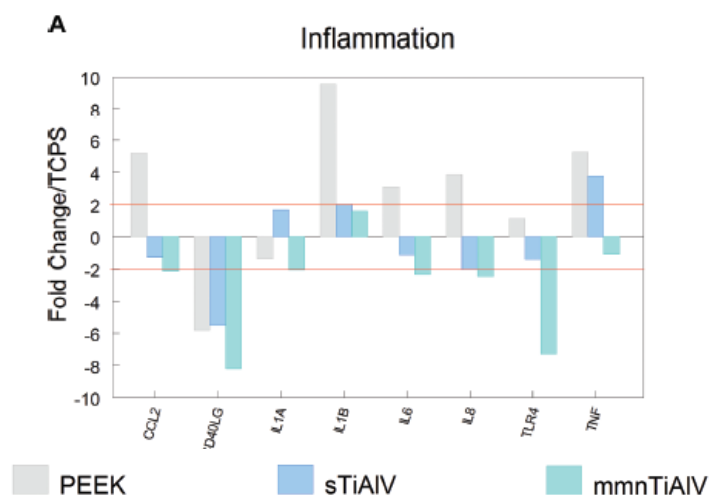


Measurement of roughness parameters. Disk roughness was characterized by kurtosis (h), and skewness (i) measured. *p<0.05 vs. rTiAlV; #p<0.05 vs. #5; \$p<0.05 vs. #9

Pro-inflammatory PEEK

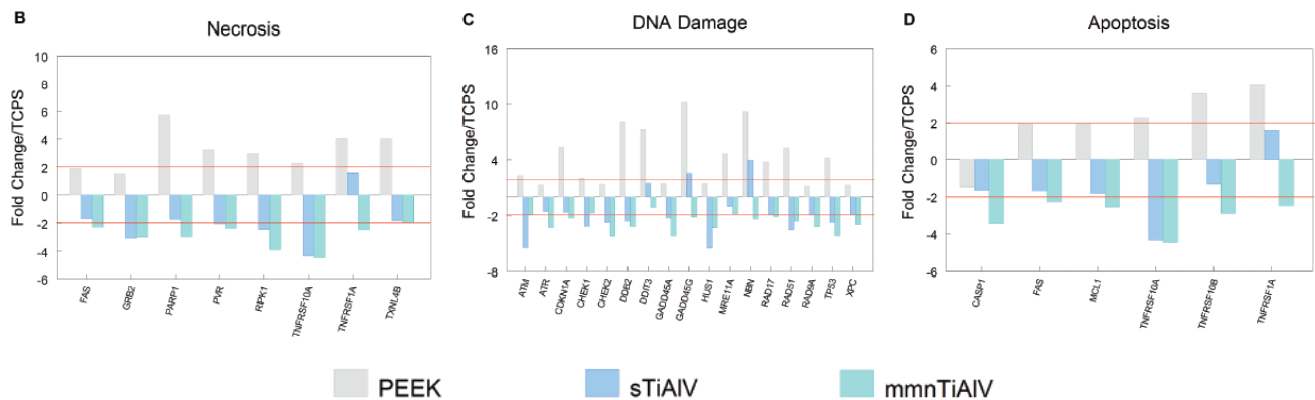
Olivares-Navarrete, et al. compared Titan Spine's nanoLOCK® surface to PEEK and smooth titanium in an *in-vitro* study to compare each surface's inflammatory microenvironment. The researchers suggested that the formation of fibrous tissue around a PEEK implant is likely the result of limited osteoblast production and increased production of pro-inflammatory factors.[4]

MSCs were grown on each surface for seven days. The results showed that production of pro-inflammatory factors was highest on PEEK compared to the other surfaces, whereas production was lowest on nanoLOCK® – even lower than the TCPS control.



Levels of anti-inflammatory interleukin-10 cytokines (IL10) were significantly greater on both titanium surfaces than on PEEK, with the greatest amount found on the nanoLOCK® surface.

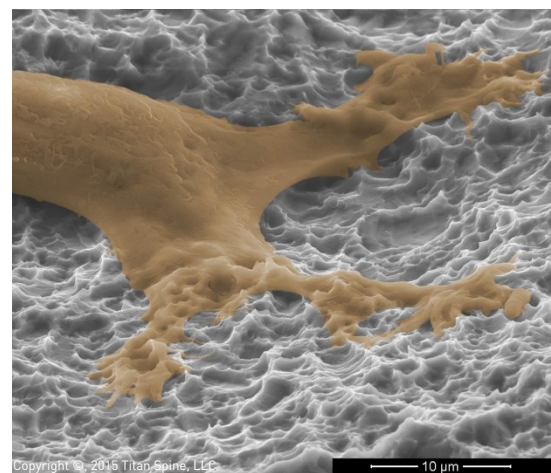
Analyzing MSCs for pro-inflammatory proteins and those associated with necrosis, researchers found that cells on nanoLOCK® had the lowest mRNA levels. nanoLOCK® also showed the least DNA damage and apoptosis. All of these were significantly higher on PEEK.



The investigators concluded that nanoLOCK® presents MSCs with an osteogenic microenvironment featuring reduced production of inflammatory factors and up-regulated production of anti-inflammatory mediators compared with PEEK.

Conclusion

Titanium alloy interbody implants with optimized macro- micro- and nanoscale (MMN™) surface features present an osteogenic environment that promotes bone growth virtually as soon as the device is implanted. Via a complex biochemical process of cellular signaling, the MMN™ surface triggers heightened manufacture of bone-producing osteoblasts and osteoinductive factors – including those for bone mineralization and angiogenesis. In addition, MMN™ surfaces decrease factors that inhibit bone growth, such as pro-inflammatory factors and osteoclasts.



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 Pictured: An SEM of a human mesenchymal stem cell on the nanoLOCK® surface at 24 hours.

PEEK, on the other hand, has been shown to present a hostile environment for growing bone, the result of overproduction of pro-inflammatory factors. The resulting

inflammatory response causes fibrous tissue to surround the PEEK implant, which is counterproductive to bone growth and fusion, which may lead to nonunion and revision surgery.

The growing body of scientific inquiry on surface engineering of spinal fusion implants presents a compelling case for macro- micro- and nanoscale (MMN™) textured titanium interbody devices. If a fusion is absolutely necessary to correct spine pathology, the surgeon's dual concerns are how well the operation corrects the problem and how quickly patients can return to their daily routine.

MMN™ titanium interbody devices from Titan Spine address these concerns by mimicking as closely as possible the cellular environment and characteristics of normal human tissues, particularly the rough hierarchical texture of bone. When an MMN™ device is implanted, it is "sensed" by individual cells at the implant site to drive osteoblastic differentiation. This process, in turn, leads to rapid bone formation and osseous integration of the implant – not the propagation of fibrous tissue around the implant as in PEEK device implantations.

If the clinical goal is to grow bone around an implant and return patients to normal function as quickly as possible, the published studies summarized here indicate that Titan Spine MMN™ implants are uniquely designed for this purpose.

References

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3. 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 10439-14-1108-3. Epub 2014 Sep 17. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4239824/>
4. 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/>