The background of the top half of the page is a scanning electron microscope (SEM) image of a porous titanium surface. The image shows a complex, interconnected network of fibers and pores, creating a highly textured, three-dimensional structure. The color palette is primarily grayscale with some brownish-orange highlights, suggesting a metallic material with a porous morphology. A dark gray, trapezoidal shape is overlaid on the right side of the image, containing the title text.

THE NANOSCALE WORLD OF SPINAL FUSION

Literature Review



STAND WITH THE FUTURE

The nanoscale world of spinal fusion

The case for optimized MMN™- textured Titan Spine titanium implants

Introduction

Recent research has demonstrated that the potential for successful spinal fusion following the implantation of an interbody device begins at a much earlier stage in the healing process and at a vastly different scale than previously thought. Rather than compressive biomechanical forces acting on the vertebral bodies between the implant over time (Wolff's law), the prospects for solid bony fusion are decided virtually as soon as the implant is in place and mediated through a complex *biochemical* chain of events that dictates – at submicron and nanoscopic levels – how the body responds to the implant at the cellular level.

In short, an important component of a successful fusion depends on the biochemical response to the interbody fusion device – specifically the material it's made of and the size and complexity of its surface features. The studies summarized in this paper tell the story of two different fusion processes from two distinct implant types. Depending on the implant and its surface texture, the body might respond by walling off the implant as a foreign body through fibrous encapsulation or integrating it through the formation of a robust osseous union.

Despite its pervasive use, polyetheretherketone (PEEK) implants have shown to be a suboptimal choice if the clinical goal is growing bone and accelerating fusion. The studies described in this paper demonstrate that PEEK implants, in fact, present an inhospitable environment for osteogenesis – complete with underproduction of factors that promote osteoblastic differentiation and overproduction of pro-inflammatory factors. This results in the proliferation of fibrous tissue on implant surfaces in the body's effort to protect itself from the material, which is counterproductive to fusion and may lead to the need for revision.

The research repeatedly suggests that PEEK implants – despite the high radiolucency and cortical bone-like modulus of elasticity that made them a common implant choice – serve mainly as spacers and actually create inflammation that inhibits bone growth.

On the other hand, many of these same investigations have shown that implants made of titanium alloy (Ti6Al4V), specifically Titan Spine implants that have macro-, micro- and nanoscale (MMN™) surface structures, present a substantially enhanced osteogenic environment in comparison to PEEK and control surfaces. A microtextured titanium alloy surface – created by Titan Spine's proprietary subtractive process – furnishes an environment that:

- Boosts the production and differentiation of osteoblasts that synthesize bone
- Down-regulates the excessive production and activity of osteoclasts (which resorb new bone, thereby reducing net new bone creation)
- Increases factors responsible for bringing blood supply to support bone growth

Not surprisingly, the development of optimized MMN™-textured titanium alloy implants was inspired by observing and mimicking the hierarchical structure of bone. Research has confirmed that specific microscale – and especially nanoscale – topographical features on bone or on an implant, trigger the cellular machinery to begin creating specialized cells for laying down and mineralizing new bone.

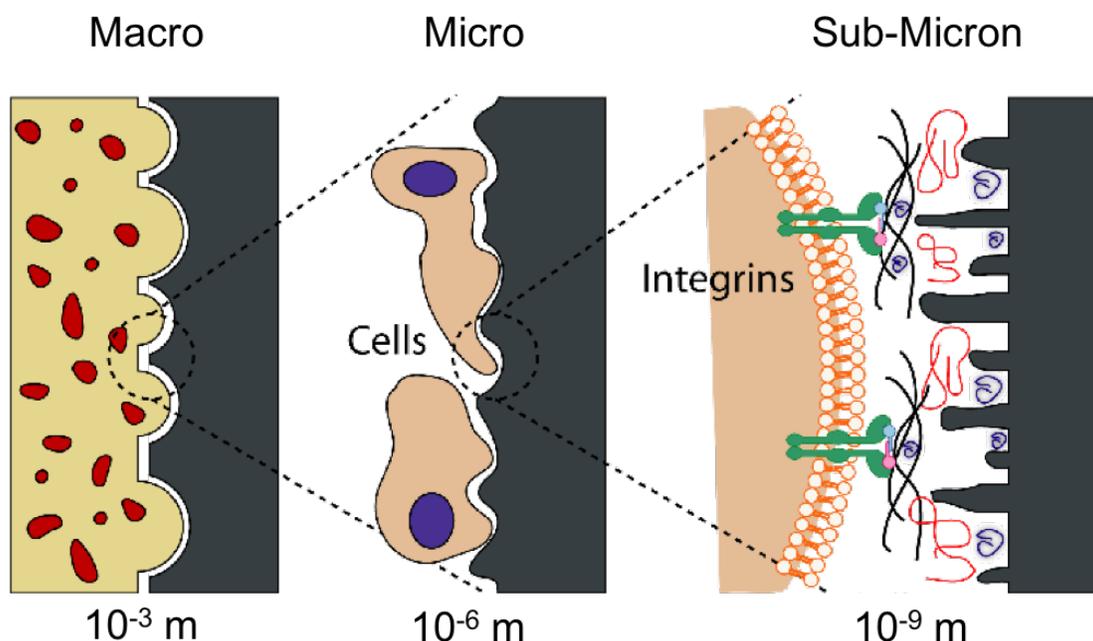
Simply put, far from serving merely as a spacer, the Titan Spine MMN™ implant actively participates in the fusion process by orchestrating the cellular behavior....etc. necessary for early bone growth and ultimately fusion.

The latest chapter in the story of Titan Spine's MMN™ titanium alloy implant involves a next generation, engineered surface that optimizes the shape and scale of nanoscale features – thus creating a unique and qualitatively enhanced nanoarchitecture. This new surface nanotopography – called nanoLOCK® – appears to further amplify the differentiation of osteoblasts and production of osteogenic and angiogenic factors.

The following is a summary of several *in-vitro* studies that extensively compared and contrasted PEEK, smooth titanium alloy, and microtextured Titan Spine MMN™ implants.

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

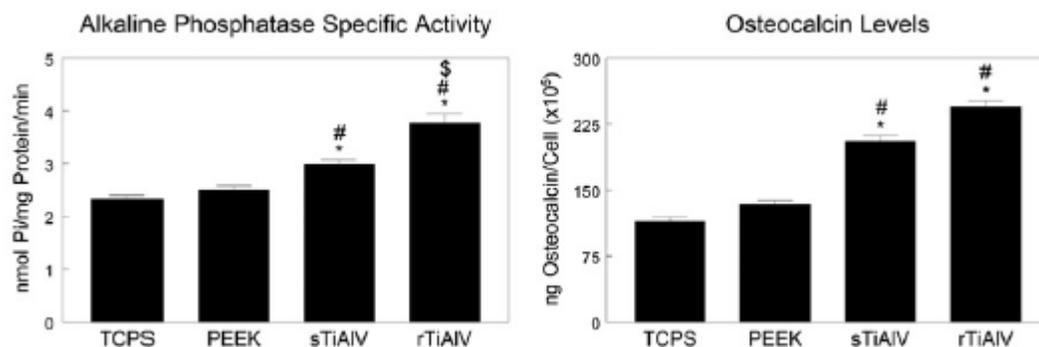


Creating an environment for osteogenesis

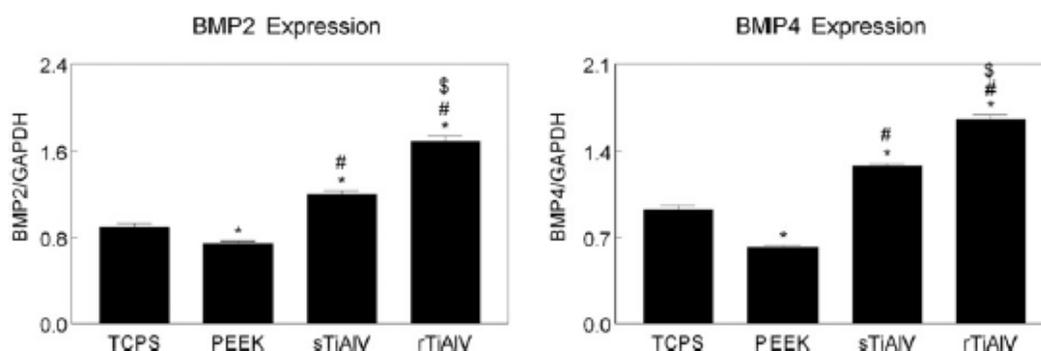
In a 2012 *in-vitro* study, Olivares-Navarrete, et al. compared the response of osteoblasts to smooth and microroughened titanium alloy (rTiAlV, Titan Spine Endoskeleton®) surfaces to that of PEEK[1]. They hypothesized that the microroughened titanium alloy acid-etched surface would generate a more differentiated osteoblast phenotype than cells cultured on PEEK or smooth titanium surfaces.

The researchers grew human immature osteoblast cells on the three different surfaces, harvested the cultures and measured markers for osteoblast maturation, as well as messenger RNA (mRNA) expression for bone growth factors, such as BMP2.

The results showed that differentiation markers (alkaline phosphatase and osteocalcin) were higher on the microroughened surface than on smooth titanium and PEEK. In addition, cells on smooth titanium had more BMP2 and BMP4 mRNA than PEEK, and the highest expression was found on the rTiAlV surface.



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 researchers concluded:

“rTiAlV surfaces increase osteoblast maturation and produce an osteogenic environment that contains BMP2, BMP4 and BMP7.”

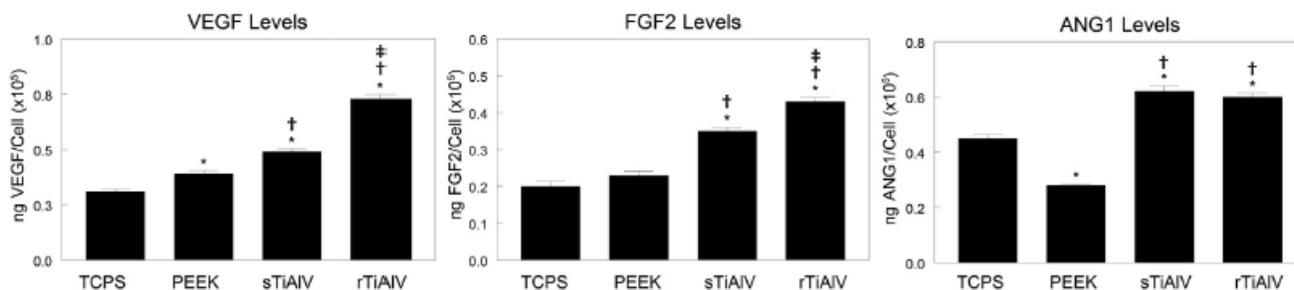
They added that modifying surface structure....etc. of titanium alloy implants to a *specific* roughened architecture (Titan Spine Endoskeleton®) is sufficient to create an osteogenic environment that could enhance bone formation and implant stability, without the need to add exogenous growth factors.

Growing the necessary blood supply

In a follow-up study, Olivares-Navarrete, et al. again explored how an implant's surface dictates the relative robustness of the bone-growing environment[2]. The researchers focused on how the degree of osteoblast differentiation might impact the evolution of the vasculature around the implant, as bone growth requires a healthy blood supply for nutrient delivery and waste removal.

They cultured human osteoblast cells on PEEK, smooth titanium alloy and microroughened titanium alloy (rTiAlV, Titan Spine Endoskeleton®) surfaces, then harvested the cell cultures and measured the relative quantities of secreted proteins and factors associated with an enhanced osteogenic and angiogenic environment. Angiogenic factors included vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor-2 (FGF-2) and angiopoietin-1 (ANG1). Important bone-forming factors included transforming growth factor- β 1 (TGF- β 1) and osteoprotegerin.

The results demonstrated that cells on smooth titanium produced higher levels of VEGF than cells on PEEK, and the Titan Spine microroughened surface enhanced this effect. FGF-2 levels were 75% higher than PEEK on the smooth titanium surface and 100% higher on the microroughened surface. ANG1 levels were decreased by 50% on PEEK versus a culture plate control surface 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 authors noted:

“These results suggest that peri-implant osteoblasts may create an environment that modulates angiogenesis around the implant and in the adjacent tissues, indicating that the chemistry of the implant plays an important role in determining the nature of the angiogenic milieu.”

Investigators also found that osteoprotegerin production was markedly increased (100%) on the microroughened surface versus PEEK. Similarly, active TGF- β 1 was over 100% on both titanium surfaces compared to PEEK.

The researchers concluded that the microroughened Titan Spine surface stimulates an angiogenic-osteogenic environment with factors important in bone formation and remodeling, indicating that this environment “may enhance bone formation, implant stability and fusion.”

The effect of surface roughness on cell behavior

Research has shown that when the progenitors of bone-producing cells encounter a rough, micro- and nano-textured surface, they undergo a change that facilitates and hastens the manufacture of bone and bone-supporting factors. In a 2014 literature review, Gittens et al. describe the role of “microroughness” and nanostructures and their implications in the osseointegration of spinal implants[3].

The proteins and other biological factors – as well as implant surface characteristics – that mesenchymal stem cells (MSCs) encounter as they approach the implant surface are critical in determining the types of cells they will eventually become. The osteogenic environment that nano-textured implants provide prompts the differentiation of MSCs into osteoblasts, which mature rapidly and begin laying down bone-producing proteins. These proteins then promote further osteoblast recruitment and maturation.

In the next stage, osteoclasts remodel this newly formed bone and prime the surface for additional bone formation – creating microscale resorption pits that contain even smaller nanoscale features across their surface.

The researchers observed:

“This nanotopography, with its inherent biochemical information, could be the signal that osteoblasts require when looking for a surface that requires new bone formation. The concept of mimicking the hierarchical structure of bone on implant surfaces by including nanostructures on commercially available devices originates from this observation.”

Recent further manipulation of the implant surface to include additional, and more complex, nanoscale features has provided promising results in terms of osteoblast

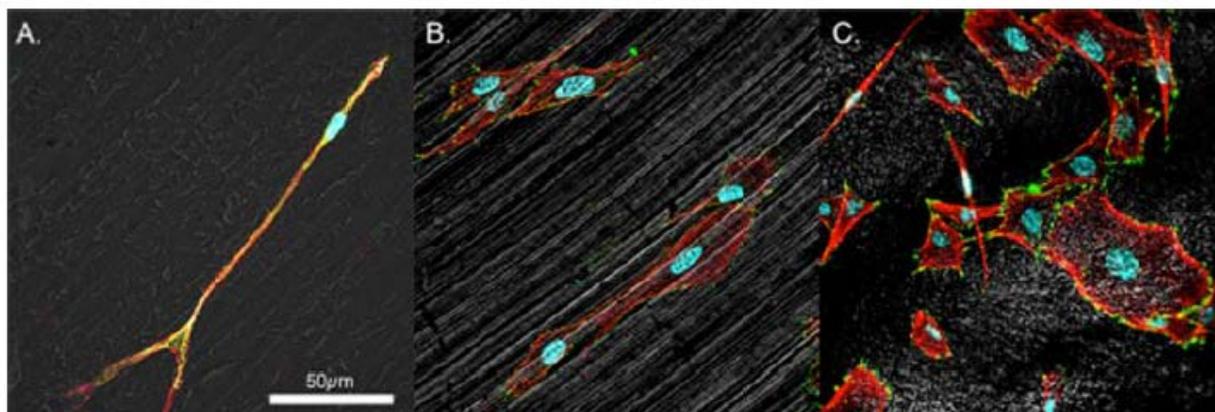
maturation and bone formation (see **The increasingly nanoscale world of spinal fusion** below).

Winning the “race to the surface”

A 2016 *in-vitro* study by Banik et al. suggests that the first 24 hours post-implant are crucial not only in setting the stage and driving fusion at the biochemical level, but also in preventing biomaterial-associated infection (BAI)[4]. Tissue cells must win the “race to the surface,” spreading out and proliferating on the implant surface before harmful microbes can gain a foothold and cause a BAI.

The researchers measured the early responses of mesenchymal stem cells (MSCs) to MMN™ titanium alloy (Endoskeleton®, Titan Spine), smooth titanium and PEEK surfaces, gathering data on MSC morphology and migration in the first 2 to 24 hours after stem cells are exposed to these surfaces. They also examined differentiation markers for osteoblasts and long-term MSC morphology.

Results for early morphology showed that, over 24h, MSCs on the Endoskeleton® surface spread at a significantly higher rate than on either the PEEK or titanium....etc. smooth surfaces. Over time, MSCs on the smooth surfaces also became more spindle-shaped, a typical morphology of fibroblastic tissue, whereas the MSCs on the Endoskeleton® surface showed more star-shaped or cuboidal morphologies....etc. at 24h. These shapes on the Endoskeleton® surface are indicative of stem cells undergoing osteoblastic differentiation.



Surface morphology of PEEK and titanium samples. (A) PEEK. (B) smooth titanium. And (C) rough, acid-etched endoskeleton surface.

Regarding the rate and direction of early MSC migration (6-12h), the MSCs on both smooth surfaces showed migration mainly along one axis. In contrast, the stem cells on the Endoskeleton® surface revealed multi-directional, random migration. Migration velocity also was highest on the titanium samples, with PEEK coming in last.

At 10 days, MSCs on Endoskeleton® were differentiating toward the osteoblast lineage as indicated by increased levels of differentiation markers compared to PEEK. The long-term morphology at 7 days indicated that the MSC shapes observed at 24h persisted on the surfaces; spindle-shaped on the smooth samples and star-shaped and cuboidal on the Endoskeleton® surface.

The investigators concluded that:

“[T]he acid etched endoskeleton surface [is] the best option for randomly and uniformly distributing the [MSC] population, in addition to covering the available area when compared to either of the smooth surfaces.”

Essentially, MSCs that encounter a properly microtextured implant (MMN™) would win the race to the surface.

The increasingly nanoscale world of spinal fusion

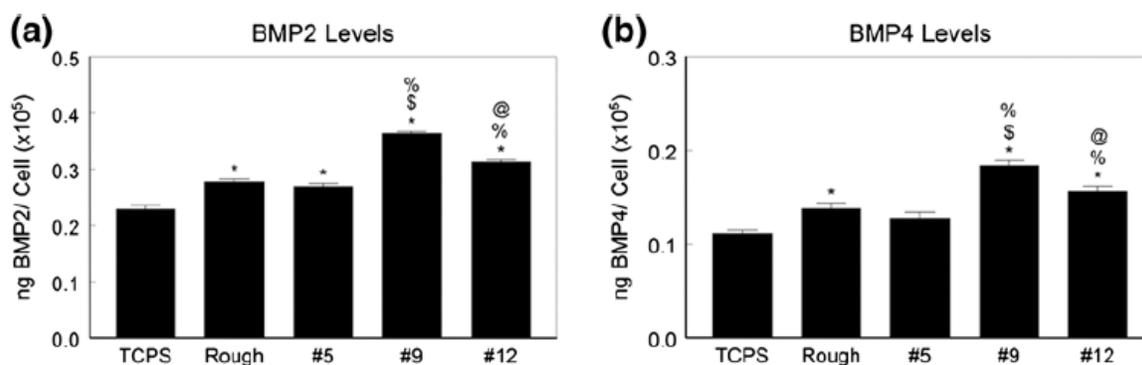
By 2014, Olivares-Navarrete, et al. and other research groups had comprehensively established the importance of micro- and nanoscale implant surface features in driving bone-implant osseointegration. Titanium alloy surfaces, particularly those with specific microroughness (Titan Spine Endoskeleton®), presented cells with a more suitable osteogenic environment than smooth-surfaced implants.

What is less appreciated is whether osteoblasts and their stem cell progenitors would respond differently to subtle differences in nanoscale roughness on implant surfaces that had otherwise comparable macro- and microscale roughness.

In a 2014 *in-vitro* study, Olivares-Navarrete et al. tested several implant surfaces, some of which had received additional secondary processing with variations of proprietary subtractive treatments (Titan Spine)[5]. This extra processing created finer and qualitatively different submicron and nanoscale features that differed in key parameters [5-6].

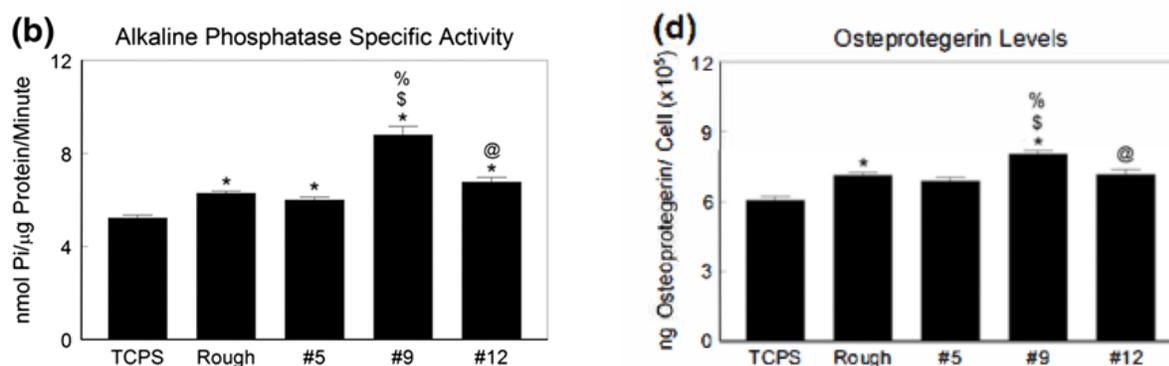
After extensive research and development to refine the micron and nanoscale features, three titanium alloy surfaces were selected for testing based on promising micro- and nanoscale roughness parameters. Using Titan Spine's original Endoskeleton® surface as a control (rTiAlV), MSCs and osteoblasts were 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; % p< 0.05 vs. #5; @p< 0.05 vs. #9.

Similarly, when assessing the MSC response, No.9 had the greatest alkaline phosphatase activity and highest levels of osteoprotegerin, in addition to the greatest amount of mRNA expression of ITGA1, 2 and V integrins.



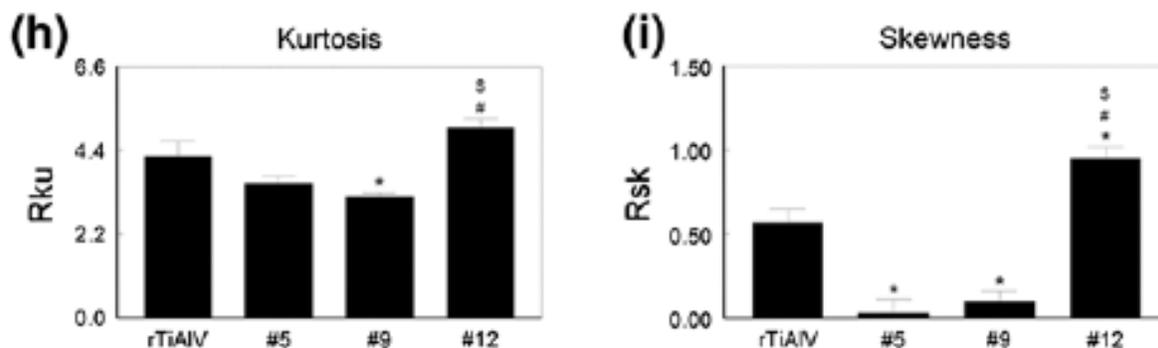
Osteoblastic maturation of normal human osteoblasts on microstructured Ti6Al4V. Osteoblasts were cultured on TCPS, rTiAlV, #5, #9, or #12 surfaces and osteoblast response measured by (b), alkaline phosphatase specific activity and (d), osteoprotegerin production measured. * p< 0.05 vs. TCPS; \$p< 0.05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

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 nanoscale surface (i.e., peak height, kurtosis and 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.

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:

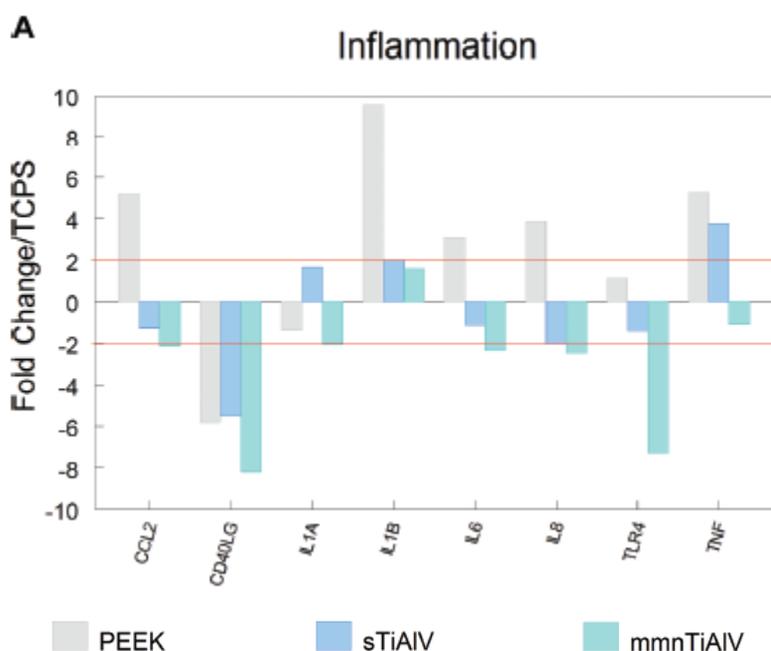
“Importantly, this study demonstrates that [normal human osteoblast] cells and MSC’s are sensitive to specific topographical features of a microstructured surface. While the surfaces used for this study were similar in average roughness at the microscale, they differed in other topographical parameters,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

Pro-inflammatory PEEK

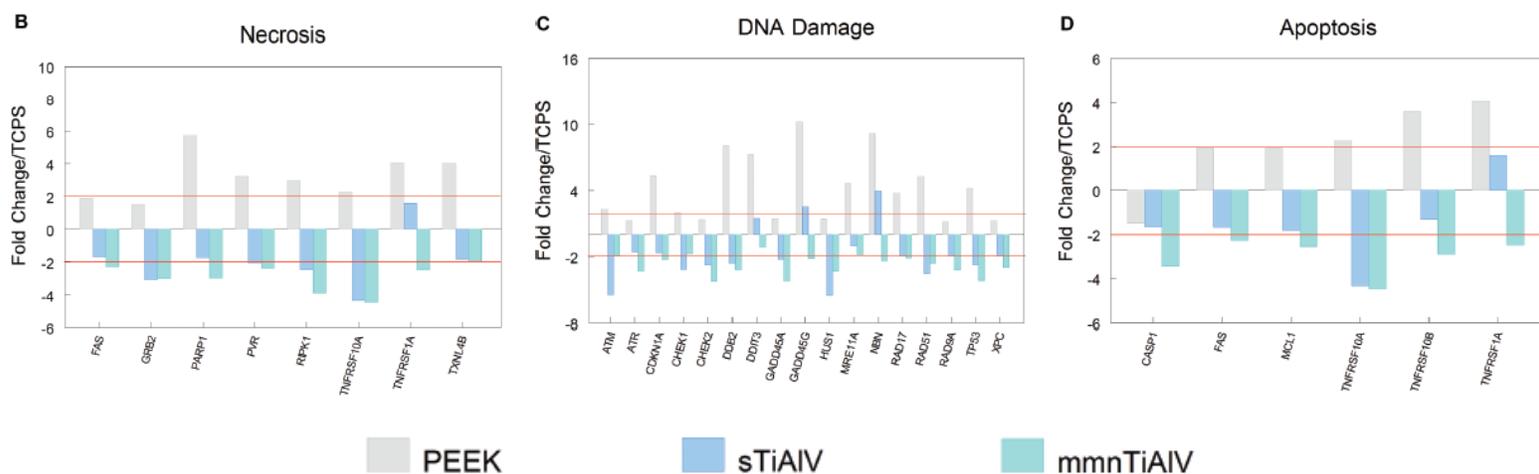
In a 2015 *in-vitro* study, Olivares-Navarrete, et al. suggested that the formation of fibrous tissue around a PEEK implant is most likely due to a combination of down-regulated osteoblast production and increased production of pro-inflammatory factors. The researchers compared smooth titanium alloy (TiAlV), Titan Spine’s MMN™ implant surface (nanoLOCK®) and PEEK samples, analyzing the proteins and factors produced in each surface’s cellular microenvironment.[7]

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 proinflammatory 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 significantly higher on PEEK.



The researchers noted that an increase in proinflammatory 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.

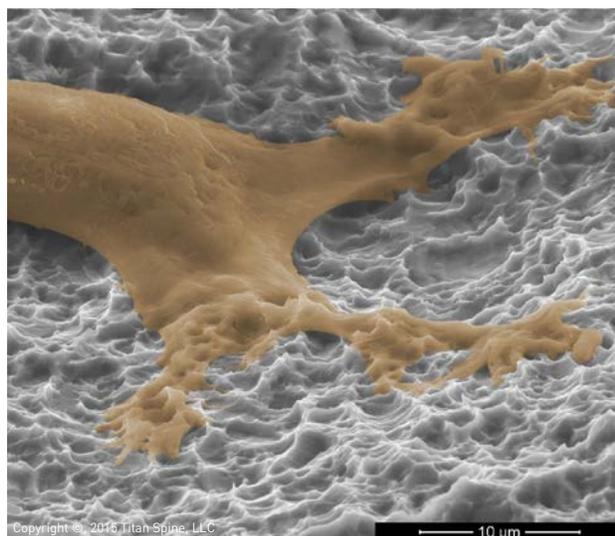
The investigators commented:

“Taken together, our results showed that [nanoLOCK®] reduced the local inflammatory environment, decreasing the pro-inflammatory 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.

Conclusion

Titanium alloy interbody implants with optimized macro- micro- and nanoscale (MMN™) surface features have been shown in numerous studies to present an osteogenic environment that promotes bone growth. This effect begins as soon as the device is implanted and is mediated by a complex biochemical cascade of cellular signaling that results in heightened production of bone-producing osteoblasts and osteoinductive factors – including those for bone mineralization and angiogenesis. At the same time, micro- and nanotextured surfaces down-regulate the production of factors that inhibit bone growth, such as pro-inflammatory cytokines and bone-resorbing osteoclasts. Conversely, research has demonstrated that PEEK presents, at best, an inhospitable environment for growing bone due to overproduction of pro-inflammatory factors that results in the formation of fibrous tissue growth surrounding the implant. This is counterproductive to bone growth and fusion and may lead to nonunion and revision surgery.



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

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.

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