Improved bone cell responses on Titanium integrated PEEK surfaces

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The success of bone fixation surgery relies on more than simply the insertion of a mechanical space-holder to replace damaged bone segments. The implanted wedge must promote the formation of a secure **biological fixation** with surrounding bone.[1,2] This biological fixation, or osseointegration, occurs via the attachment of osteoblasts (bone-forming cells) directly on the implant surface, followed by their subsequent production of mineralized bone matrix to bridge the peri-implant gap and establish bony union.[3,4]

The osseointegration process is significantly influenced by the properties of the implant. These properties include the mechanical, chemical, and topographical properties of the implant.[5–7]

Mechanical properties – The implant must be stiff enough to support the loads sustained at the site of insertion. However, materials that are too stiff can cause serious problems! For example, the significantly higher elastic (Young's) modulus of metals such as titanium alloys (110 GPa) and cobalt chromium alloys (210 GPa) compared to human cortical bone (17 GPa) or human cancellous bone (~2.3 GPa) often generates stress-shielding effects.[8,9] The high stiffness of the metallic implant reduces loads on surrounding bone tissue, thereby decreasing its metabolic activity, leading to tissue atrophy and eventual implant loosening.[10] In contrast, the thermoplastic polymer poly-ether-ether-ketone (PEEK) exhibits a Young's modulus (4 GPa) between that of cortical and cancellous bone (Fig. 1), making it an ideal bulk implant material.[11]

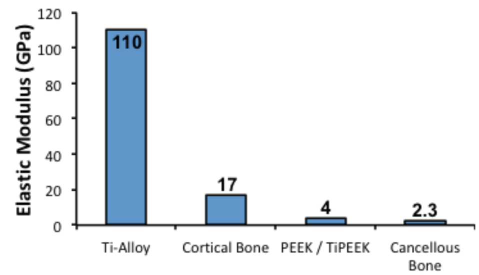


Figure 1. Elastic (Young's) modulus of titanium alloy (Ti-6Al-4V) and PEEK/TiPEEK compared to human cortical and cancellous bone.[11,12]

Chemical Properties – To stimulate osseointegration, an implant material must demonstrate bioactivity, providing a surface that actively promotes the attachment and growth of bone cells.[13] Although PEEK materials provide outstanding mechanical properties, their surface is bioinert and thus does not support the cellular adherence that more bioactive materials like titanium do.[14,15] To address this situation, implants sporting a titanium surface layer surrounding a PEEK core deliver bioactive surfaces in addition to mechanical excellence.[16,17] Then, strategically located surfaces of PEEK left exposed provide the benefit of reducing the adherence of soft tissues like tendons and skin that require a liberated range of motion.

Topographical Properties – The effectiveness of a bioactive surface chemistry is further enhanced by the incorporation of hierarchical surface features.[18,19] Surface topographies at various scales influence osseointegration in the following ways:

- Macro-scale roughness improves the friction fit between the implant and bone to provide primary implant stability.[1,2] This minimization of micromotion is necessary to allow subsequent biological fixation.[20]
- Micro-scale roughness provides more surface area for bone cells to proliferate and deposit newly formed bone matrix.[21,22] The greater area of cellular adhesion, the stronger the resulting bony bond.[23]
- Nano-scale roughness has been shown to influence cell-substrate communication and improve the cellular functions necessary for proper bone formation (monitored via calcium deposition).[24,25]

*These properties have been carefully considered in the design of the **TiPEEK integrated technology** and optimized to deliver superior osseointegration performance.*

The aim of the present study was to evaluate the effectiveness of the TiPEEK integrated technology compared to two industry standards, PEEK and Ti-6Al-4V, through *in vitro* testing with human osteoblasts (bone-forming cells). Specific attention was given to the surface properties of each material, and to their ability to support osteoblast adhesion, proliferation, and calcium deposition (bone matrix production).

TiPEEK provides more surface area and increased surface roughness

The surfaces of PEEK, Ti-6Al-4V (Ti), and TiPEEK samples were examined using scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Fig. 2). The pictures of each sample (bottom left of each panel) show the considerable visible roughness of TiPEEK samples compared to PEEK and Ti. This feature is designed to improve primary implant stability via interdigitation with bone. However, the roughness of the TiPEEK surface extends beyond visible limits into the micro- and nano-scales, where cells communicate. The surface features at this level are displayed via the inset boxes in the bottom panel, which show the TiPEEK surface at 100x, 1,000x, and 10,000x magnification. High magnification insets are not shown for PEEK or Ti because no additional surface features become visible at these scales. The micro-scale topography of each sample is represented three-dimensionally in the AFM reconstructions shown at the top left of each panel.

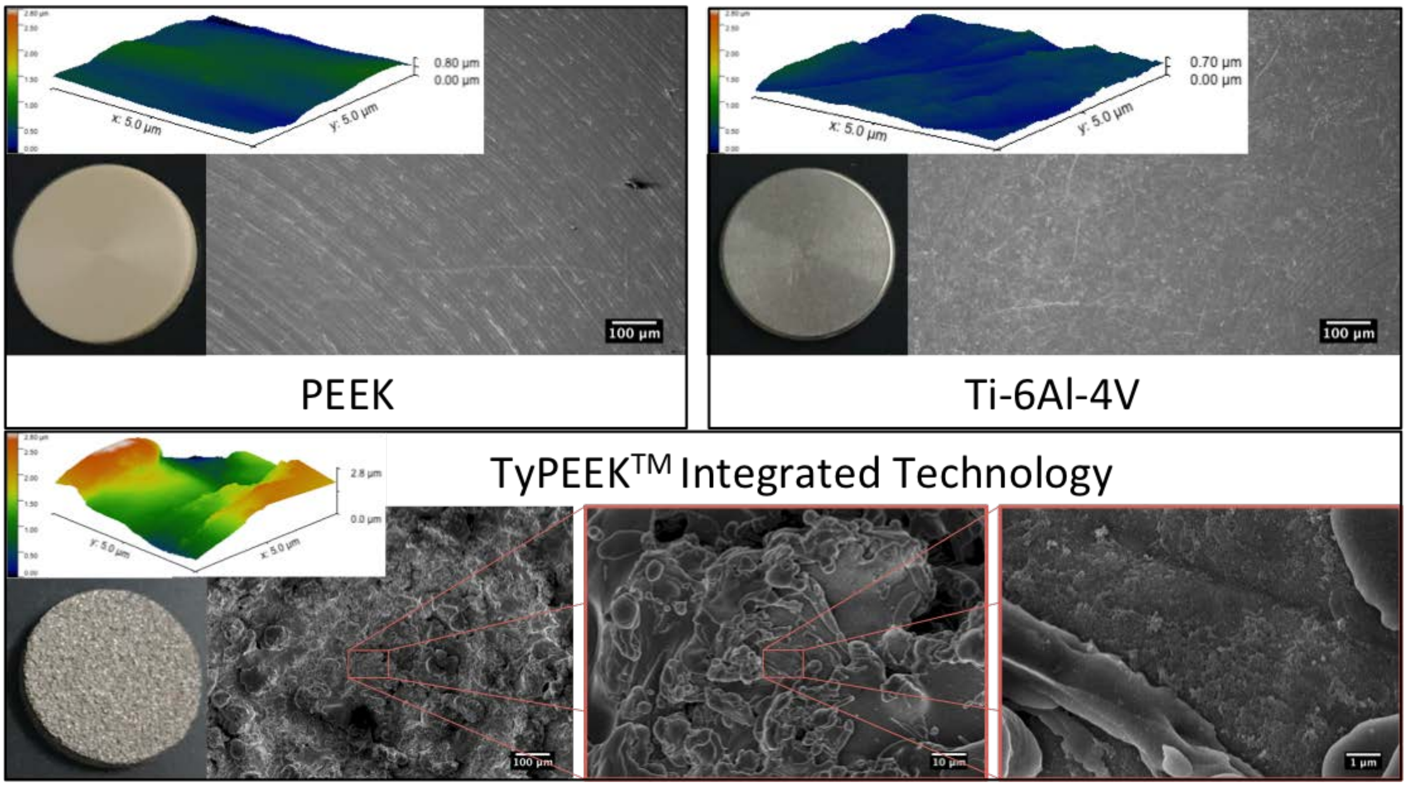


Figure 2. Study of the surface morphology of each sample. Pictures of the samples used in this study are shown in the bottom left of each panel. Representative atomic force microscopy (AFM) reconstructions are shown in the top left of each panel (5 x 5 µm scans; the same z-scale and color range is used for each sample; the data gathered via AFM is quantified below in Table 1). The scanning electron micrographs (SEMs) display the surfaces of PEEK and Ti at 100x magnification. The surface of the TiPEEK sample is presented at 100x, 1,000x, and 10,000x magnification using inset boxes to emphasize the surface roughness present at the macro-, micro-, and nano-scales. Zooming in on the PEEK and Ti surfaces does not reveal any features at smaller scales.

The AFM data were quantified to provide a tangible expression of the increase in roughness and surface area provided by TiPEEK surfaces (Table 1). TiPEEK samples exhibit a significant increase in root mean squared (RMS) surface roughness (>2x greater than PEEK, >5x greater than Ti). However, the more meaningful metric may be the increase in 'actual' surface area that this increased roughness creates. These AFM data indicate that TiPEEK surfaces provide ~30% more surface area per square micron for cells to adhere, proliferate, and fill in with newly produced bone matrix.

Table 1. Surface parameters gathered from 5x5 µm AFM scans of each sample surface.

	RMS surface roughness (nm)	Projected surface area (µm^2)	Actual surface area (µm^2)	Increase in surface area (%)
PEEK	179.3	25.0	27.9	11.5
Ti	70.2	25.0	27.6	10.3
TiPEEK	395.2	25.0	37.7	40.1

Osteoblasts populate TiPEEK surfaces in greater numbers

Figure 3 shows the relative number of viable osteoblasts measured on samples at time points between 4h and 21d. While cells attached onto all samples at similar levels after 4h, and proliferated most rapidly on Ti samples during the first 4 days, osteoblasts ultimately proliferated to their highest numbers on TiPEEK samples after two and three weeks of culture. Considering this overall trend, the observed dip in osteoblast proliferation on TiPEEK samples compared to Ti at 4d may be attributed to the increase in calcium deposition measured on TiPEEK samples at early time points, as shown below (Fig. 4). This is because decreases in cell division are often associated with increases in matrix production.[22] At time points longer than a week, osteoblasts proliferate to their greatest numbers on TiPEEK samples, most likely due to the increased surface area available to them. The bottom row of images in Figure 3 shows fluorescently labeled osteoblasts growing on each sample after 7 days of culture, and confirms the quantitative data in the chart.

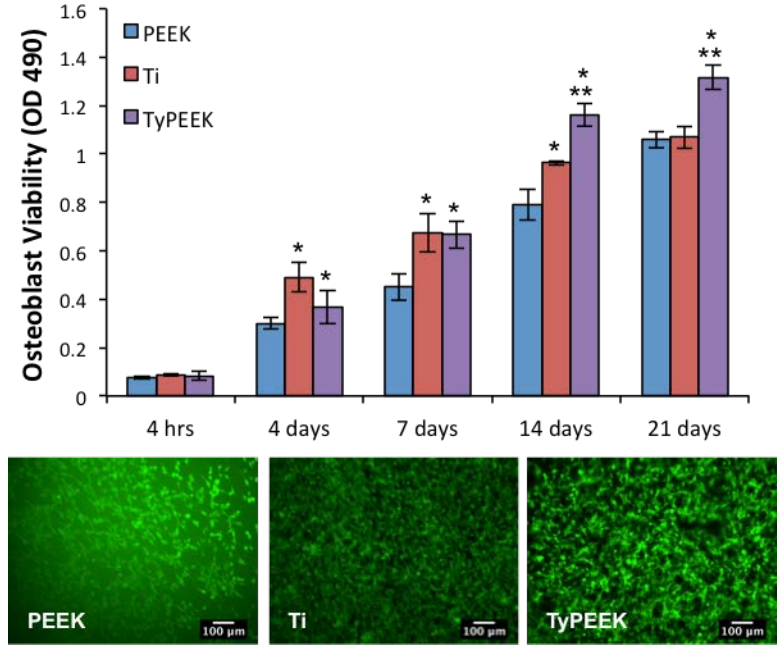


Figure 3. Top: The relative number of viable osteoblasts growing on each sample, as measured using an MTS assay. OD 490 = optical density at 490 nm. Data is avg +/- stdev. *p < 0.001 compared to PEEK at the same time point. **p < 0.001 compared to Ti at the same time point. **Bottom**: Fluorescently labeled osteoblasts growing on each sample after 7 days of culture. Viable osteoblasts are stained green by calcein.

Osteoblasts produce significantly more bone matrix on TiPEEK surfaces

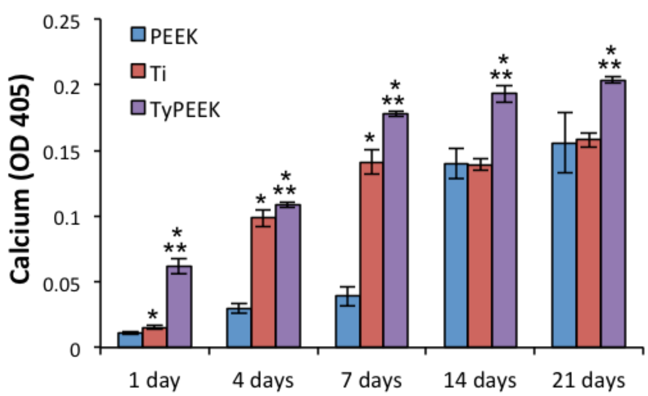
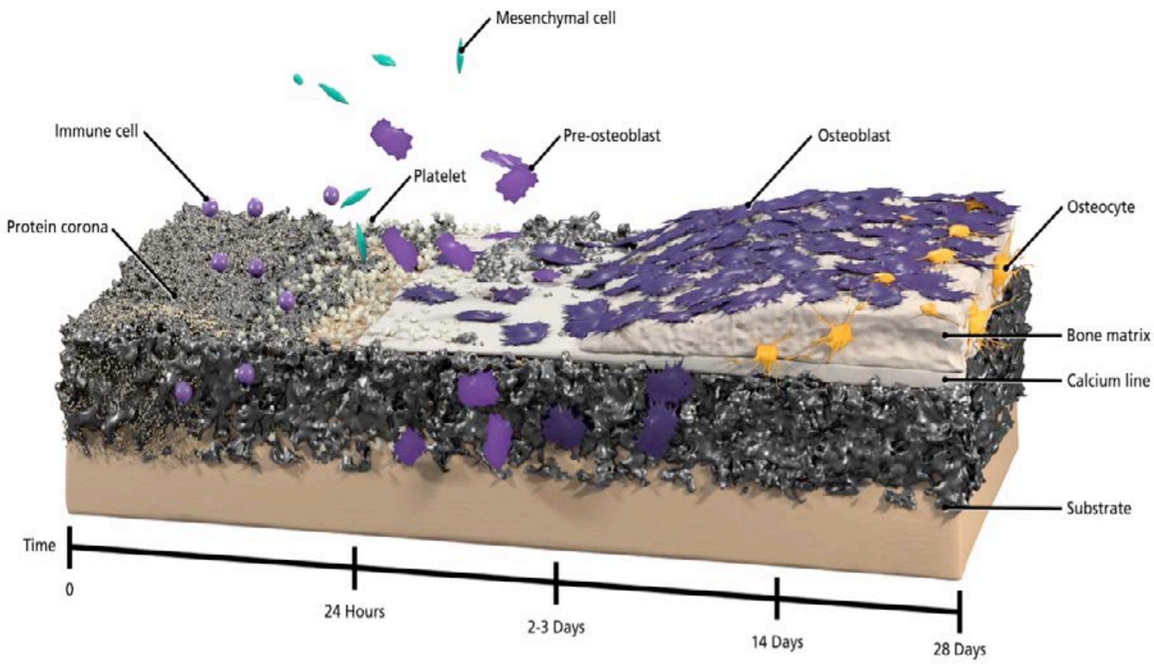


Figure 4. The relative amount of calcium deposited from osteoblasts grown on each sample as measured by elution of Alizarin Red S staining. OD 405 = optical density at 405 nm. Data is avg +/- stdev. *p < 0.001 compared to PEEK at the same time point. **p < 0.001 compared to Ti at the same time point.

The ultimate assessment of osteoblast performance is their deposition of mineralized bone matrix. On successful orthopedic implants, osteoprogenitor cells populate the surface and lay down a non-collagenous interfacial calcified layer within 24 h after implantation, analogous to the cement line of developing osteon structures.[26] In agreement with this timeline, TiPEEK samples induced high calcium deposition from osteoblasts after 1 day of culture (Fig. 4). Additionally, the amount of calcium measured on TiPEEK samples remained significantly higher than on both PEEK and Ti samples at all time points. It is hypothesized that the increased matrix production on TiPEEK samples is due to the presence of micro- and nano-features, which create a surface that better resembles the topography of bone, and enhances cell-substrate communication. This may increase the speed at which osteoblasts assimilate to their substrate, causing them to mature exceptionally fast and maintain high matrix production compared to that observed on the smooth surfaces of PEEK and Ti.

Conclusions: It is well documented that implant surfaces exhibiting hierarchical roughness at the macro-, micro-, and nano-scale influence protein adsorption, enhance cell-substrate communication, and can lead to improved osseointegration. This *in vitro* study demonstrated that the TiPEEK integrated technology increased available surface area, enhanced osteoblast proliferation, and most importantly, induced significantly higher levels of bone matrix production (measured as calcium deposition). Ultimately, these results indicate that TiPEEK materials provide a more favorable environment for bone growth.

Key Takeaways



TiPEEK integrated technology is engineered to provide immediate mechanical stability and participate in the healing process to promote rapid and long-lasting biological fixation with supporting bone. This is accomplished through a number of unique design considerations:

- PEEK core with mechanical properties similar to bone provides mechanical stability and effectively disperses dynamic loads to minimize stress shielding effects
- Titanium integrated surface treatment creates a bioactive surface that promotes the attachment and growth of bone forming cells
- Osteoblasts ultimately populate TiPEEK surfaces in numbers more than 23% greater than on PEEK or Ti-6Al-4V surfaces after 21 days of culture
- Incorporation of roughened surface features at the micro- and nano-scales increases the *in vitro* formation of mineralized bone matrix from osteoblasts by more than 28% compared to Ti after 21d
- After only 1d, calcium deposition increased by 470% compared to PEEK, and 305% compared to Ti

Combined, the TiPEEK integrated technology provides an ideal structure for bone fixation procedures by ensuring sustained mechanical and biological stability throughout the bone remodeling process.

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