Reduced Bacteria Colonization and Increased Bone Formation on Si3N4 Spinal Implants: An Evaluation of Bacterial Colonization on Existing Implant Materials

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Introduction

Bacterial infection of spinal implants is becoming increasingly problematic due to the resistance of bacteria to current antibiotics. For this reason, the objective of the present in vitro investigation was to determine bacterial functions on spinal implants made of various materials. We considered PEEK, Si3N4 (Silicon Nitride) and Titanium interbody cages for this study.

Learning Objectives

To develop a a greater understanding of the potential antibacterial surface properties of spinal implants, and the role of hydrophyllic properties and surface technologies.

Methods

Materials

Si3N4 materials (1 by 1 cm2 samples) were provided by Amedica Corp. Biomedical grade 4 Titanium (Fisher Scientific) and PEEK (Invibio, Inc.) were also used as controls. All samples were sterilized by UV light exposure for 24 hours on all sides and were qualitatively characterized for their roughness using standard scanning electron microscopy.

Bacteria Studies

Bacteria were inoculated (10 to the 5th power) on the surfaces and reviewed at 4, 24, 48 and 72 hours. Bacteria used included S. epidermidis, P. aeruginosa, and S. aureus all obtained from the American Type Culture Collection

Bacteria were cultured in a Luria broth supplemented with 10% Fetal Bovine Serum (Hyclone) solution. Bacteria number and functions were determined through standard live/dead and crystal violet staining. Lastly, enzyme-linked immuno- sorbent assays (ELISA) were used in the present study to determine initial protein adsorption events (specifically, fibronectin and vitronectin, proteins known to decrease the attachment and growth of bacteria) to the substrates of interest after 20 min, 1 and 4 hours.

Results

Results showed for the first time that without the use of antibiotics, the adhesion and growth of bacteria after all time points of interest to the present study were significantly lower on Si3N4 samples than currently implanted c.p. Ti and PEEK. Specifically, after 72 hours, crystal violet staining (a measure of bacteria number and activity) for Staph. epidermidis, Staph. aureus, and Pseudo. aeruginosa decreased by at least 4, 10, and 7 times on Si3N4 than PEEK. Lastly, we had significantly greater adsorption of vitronectin and fibronectin (proteins known to decrease bacteria function) on Si3N4 than c.p. Ti and PEEK after all time periods of interest, thus, providing a mechanism for the anti-bacterial properties of Si3N4.

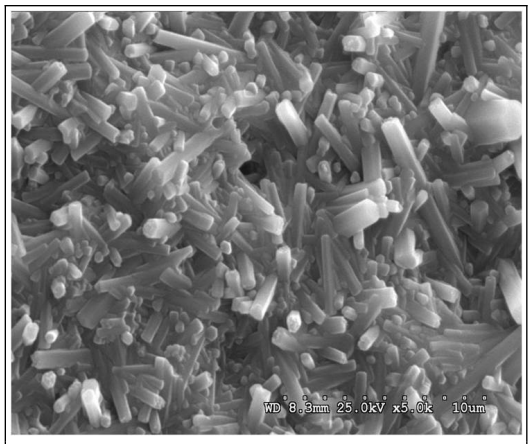


Figure 1, Si3N4 Scale Bar = 10 Micron

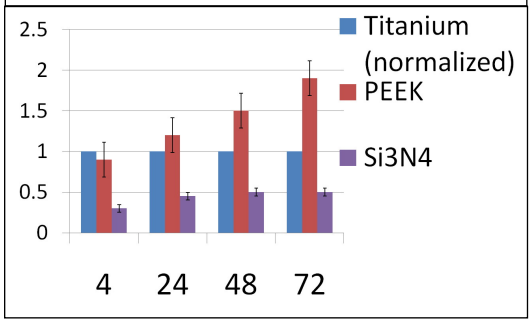


Figure 2: Decreased Staph epi. on Si3N4. y axis = crystal violet staining intensity and x axis = time in hrs. Data = mean +/- SEM; N = 3; * $p < 0.01$

Conclusions

This study demonstrated for the first time decreased bacteria functions on Si3N4 compared to titanium and PEEK and provided the first understanding why as Si3N4 increased vitronectin and fibronectin adsorption.

Acknowledgements:

The authors acknowledge the financial support of Amedica.

Figure 3: Decreased Staph. aureus Production on Si3N4. y axis = crystal violet staining intensity and x axis = time in hrs. Data = mean +/- SEM; N = 3; * $p < 0.01$

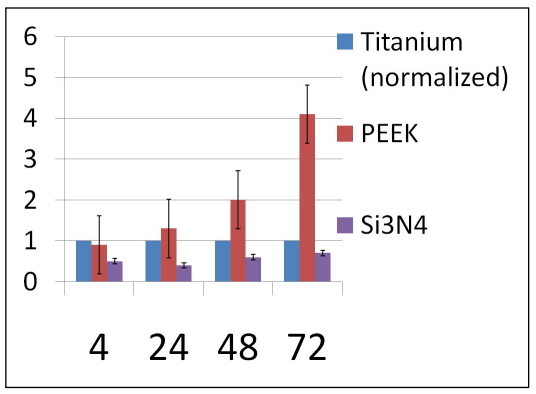


Figure 4: Decreased Pseudo. aeruginosa Biofilm Production on Si3N4. y axis = crystal violet staining intensity and x axis = time in hrs. * $p < 0.01$

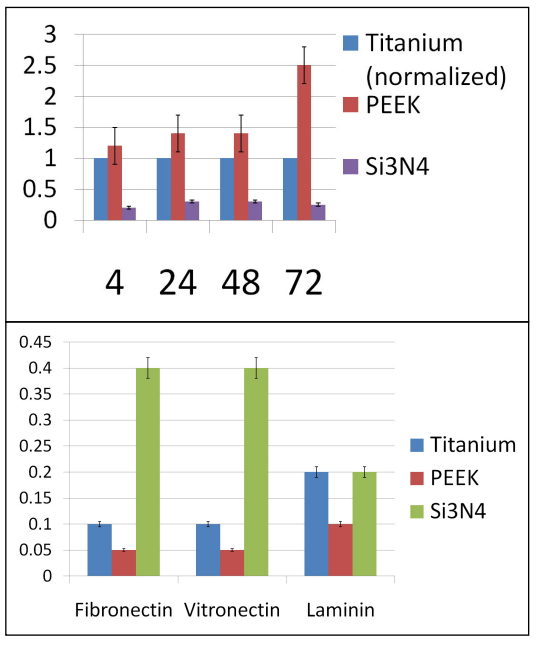
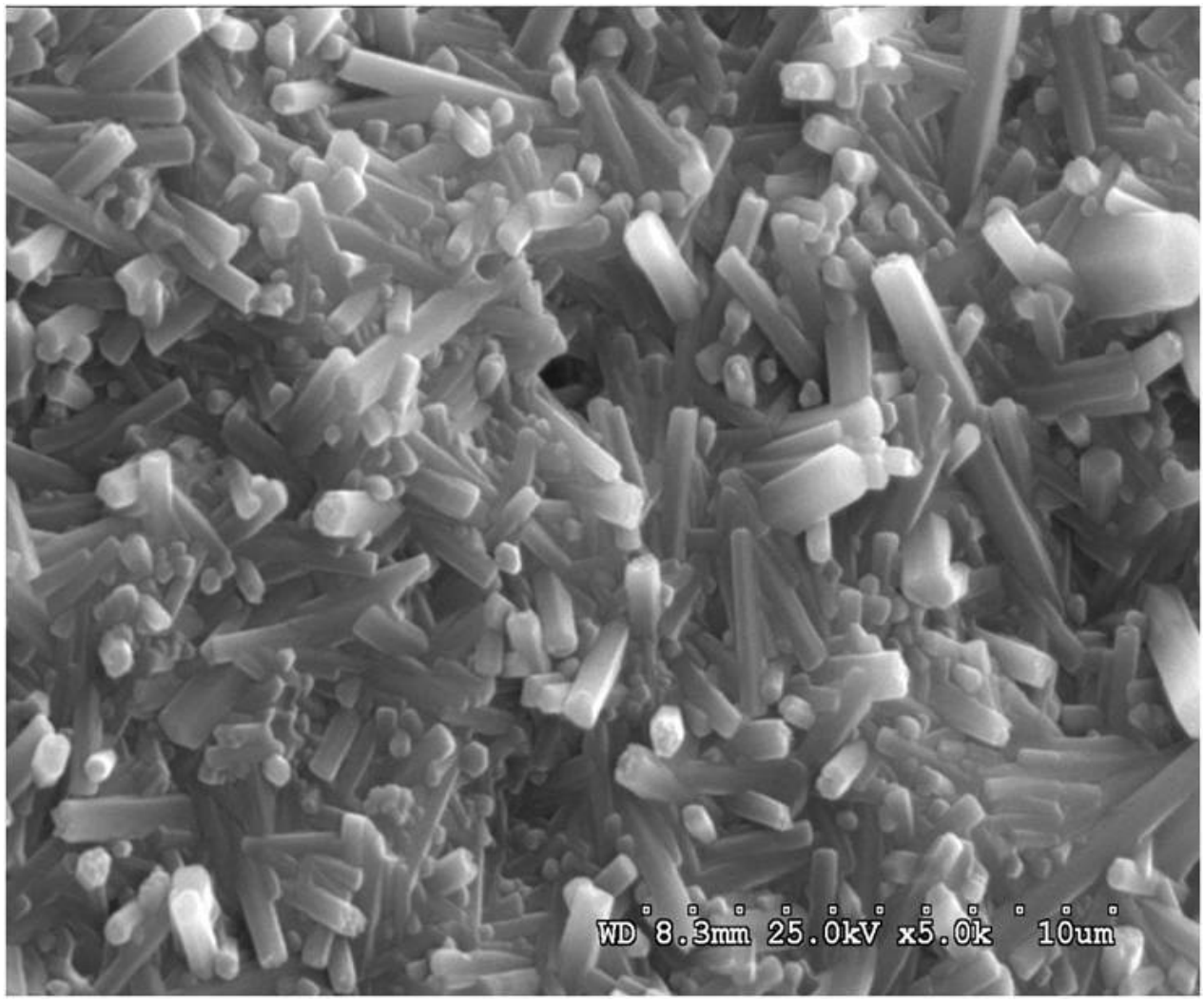


Figure 5: Increased Fibronectin and Vitronectin Adsorption on Si3N4 after 20 minutes. y axis = adsorbance. * $p < 0.01$ compared to all others for the same protein.



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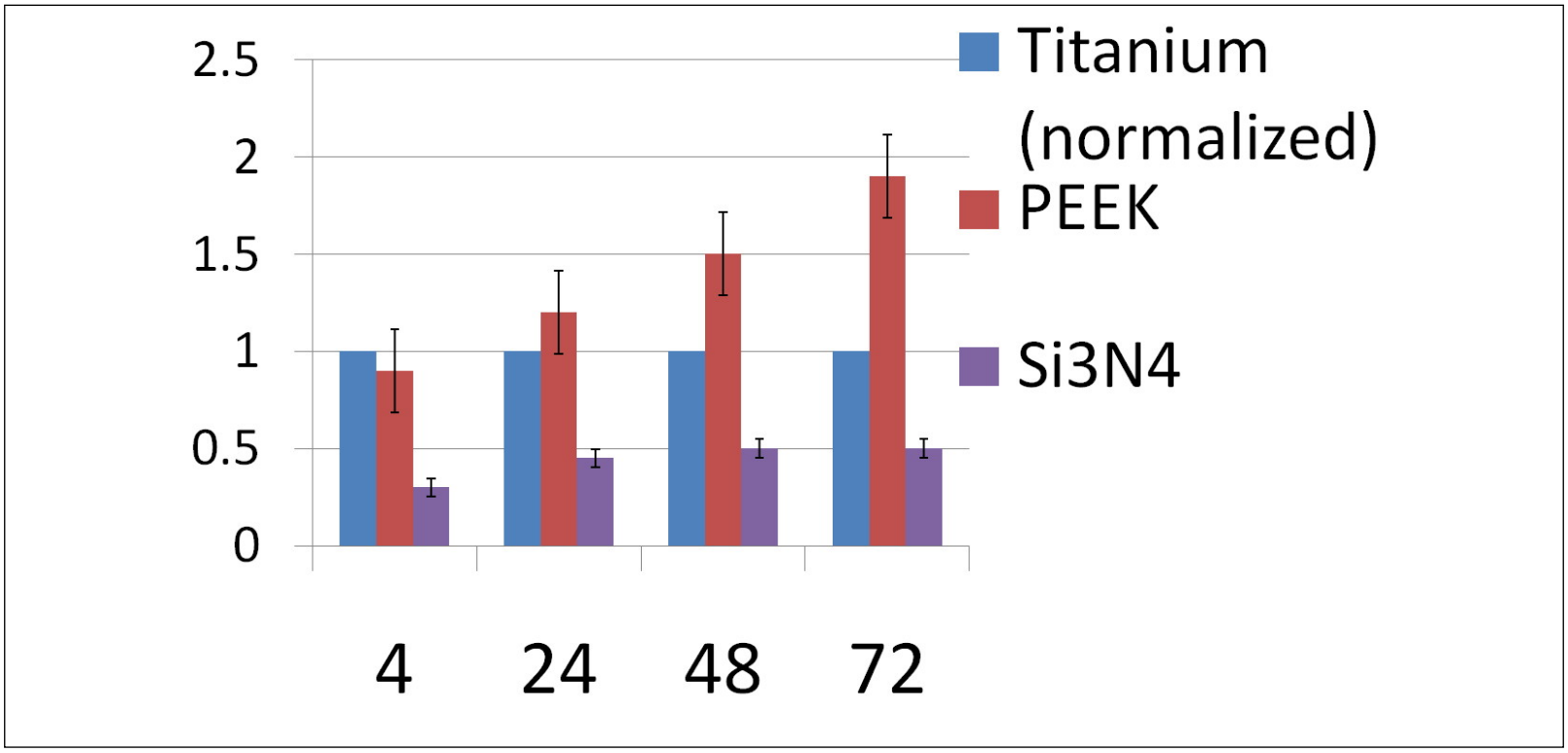
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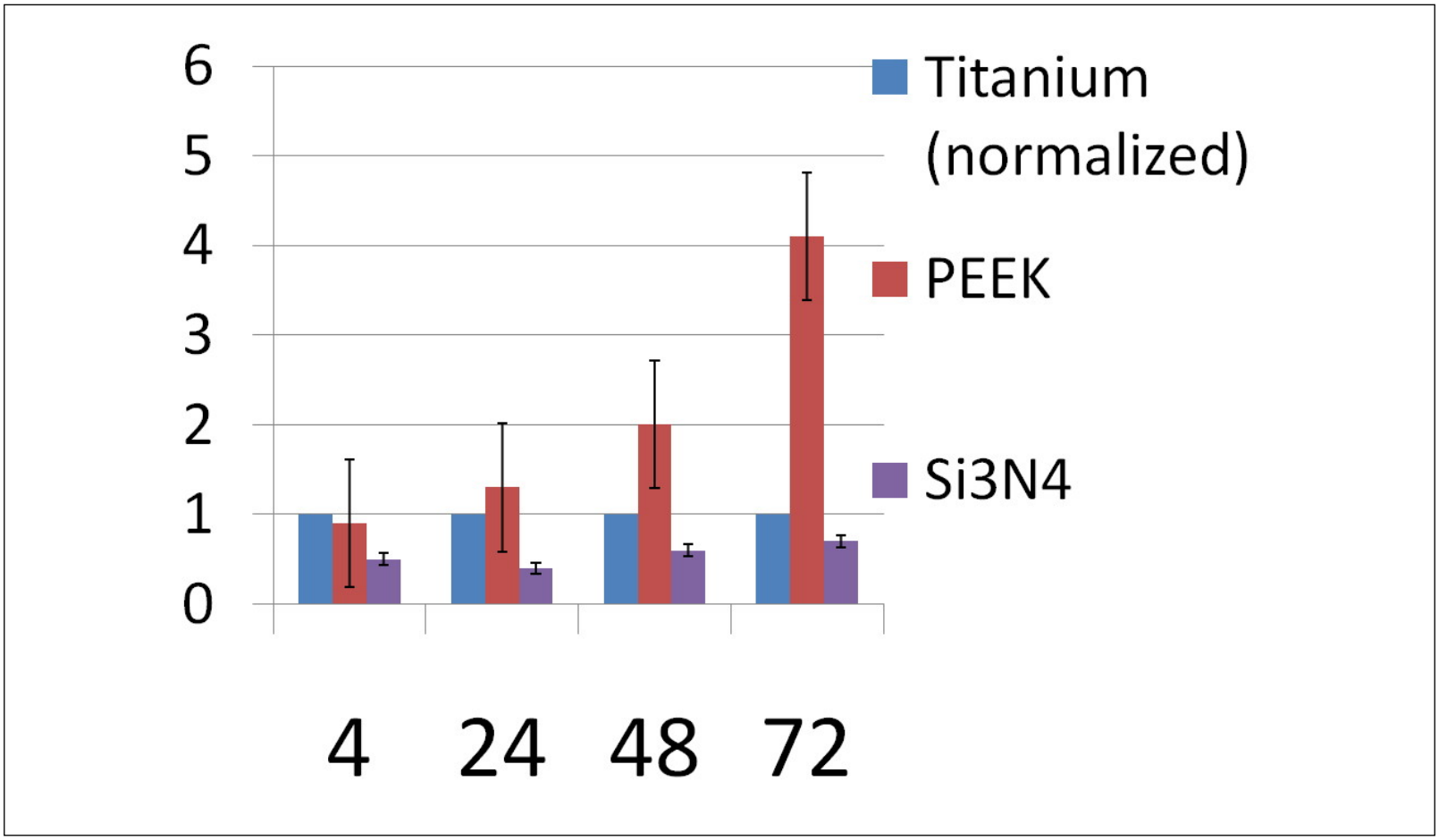
1
0.5
0
4
24
48
72
Titanium (normalized)
PEEK
Si3N4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Titanium (normalized)
PEEK
Si3N4
4
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48
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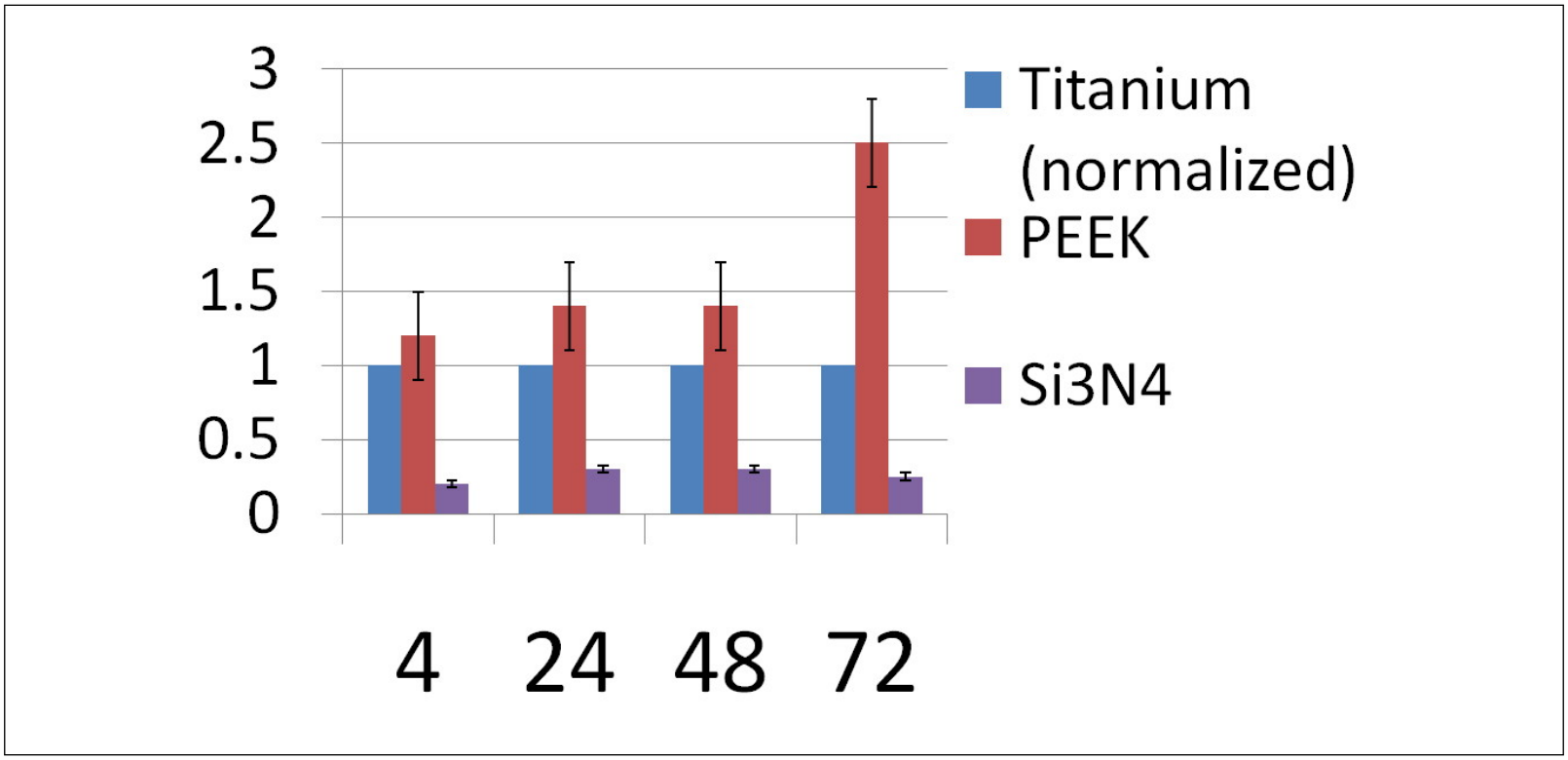
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Titanium (normalized)
PEEK
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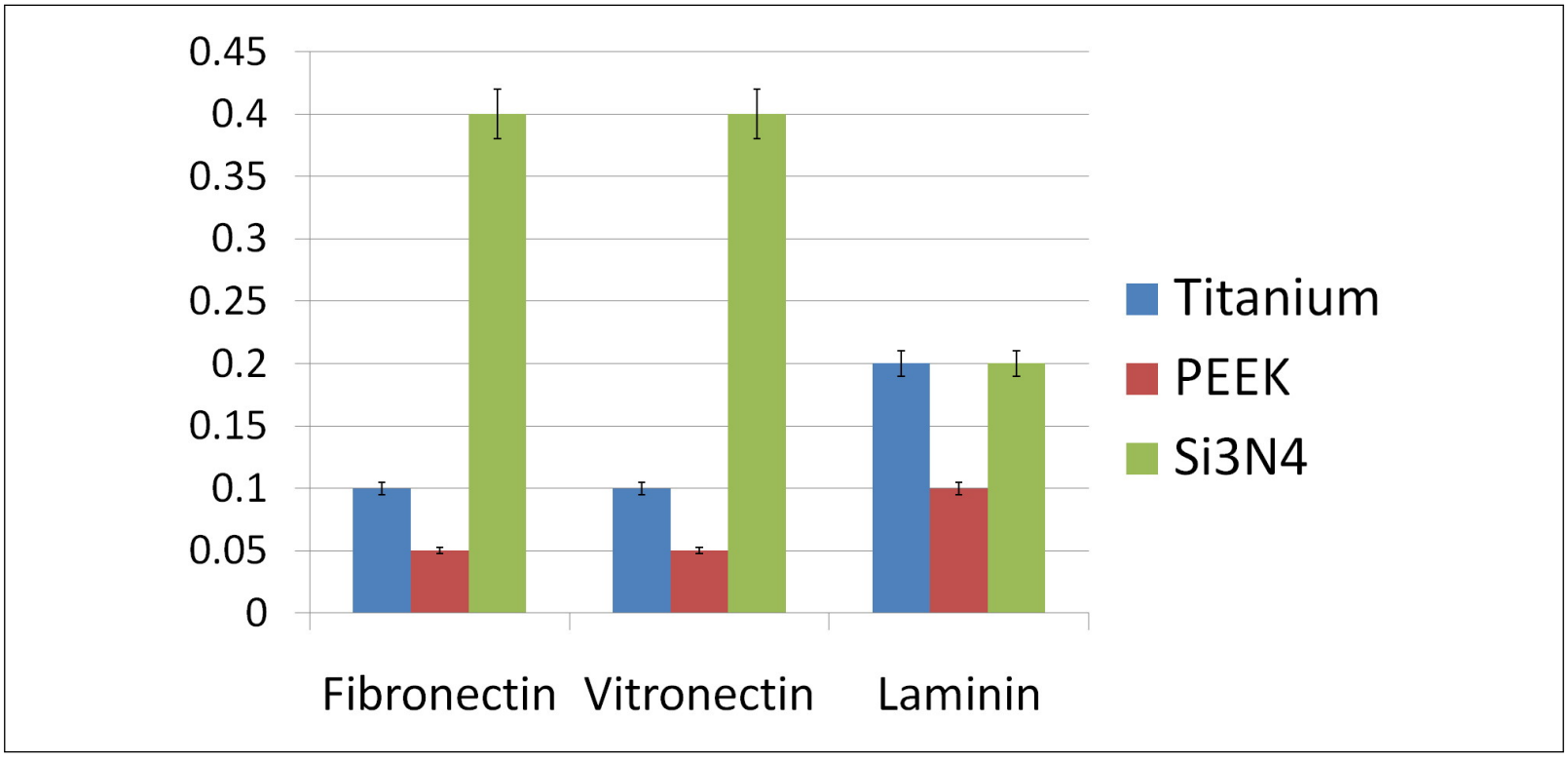
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0.15
0.1
0.05
0
Fibronectin
Vitronectin
Laminin
Titanium
PEEK
Si3N4