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# Comparing Biocompatibility of Nanocrystalline Titanium and Titanium-Oxide with Microcrystalline Titanium

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## INTRODUCTION

➢Kurtz, et. al.[1], predict that by 2010 hip and knee replacements will number over one million, and by 2030 we will see 4.5 million annually.

➢Primary concerns of orthopaedic devices are wear failure of biointegration [2,3].

➢Thus, there is a need to accommodate such overload by the development of biocompatible surfaces with superior cell adhesion, survival, growth and proliferation, which is the overall objective of our lab.

➢It was shown that the biocompatibility of titanium (Ti) metal is due to the presence of a thin native sub-stoichiometric titanium-oxide ($TiO_2$) layer that enhances the adsorption of mediating proteins on the surface [4].

➢The present study evaluates the adhesion, survival and growth of cells on the engineered nanocrystalline $TiO_2$ and Ti produced by ion-beam-assisted-deposition (IBAD) technique, and compares them with microcrystalline Ti (orthopaedic grade).

## METHODS

Method of Nano-fabrication (IBAD Technique):
The process combines physical vapor deposition with concurrent ion beam bombardment in a high vacuum environment. The nano-structure films produced by IBAD (with 1 to 70nm grain size) possess combined properties of super hardness and complete wetting behavior.

**Fig. 1: IBAD System**

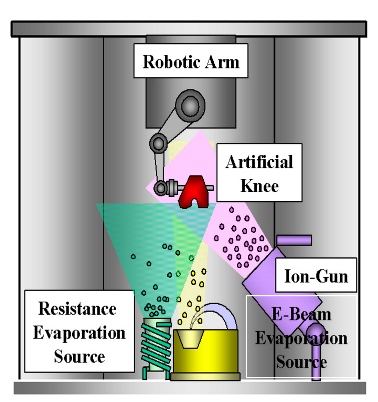



**Fig. 2: Schematic of IBAD Processor**

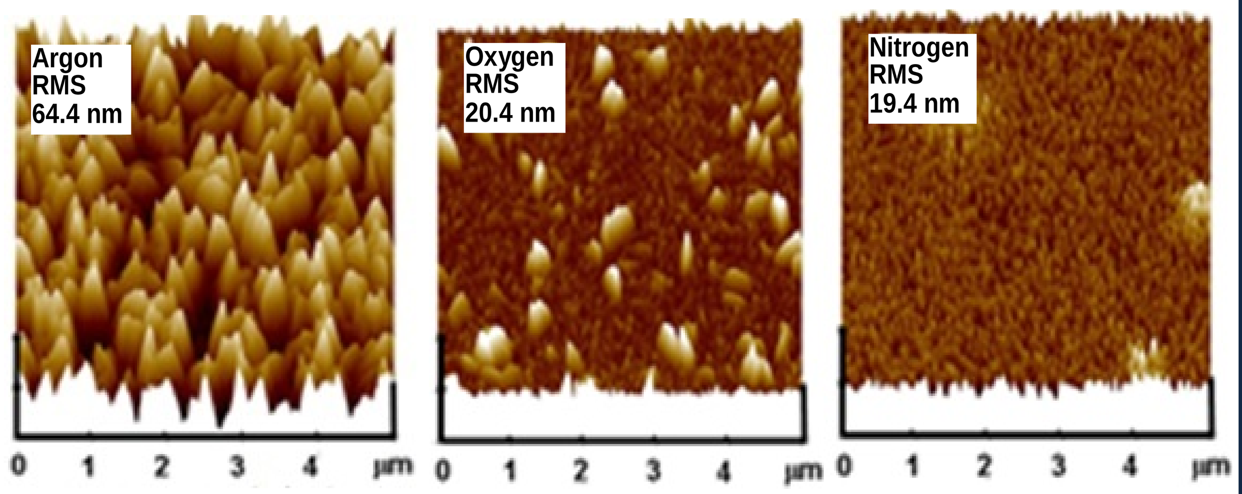



**Fig. 3: Atomic Force Microscopy Images of Nano-structure $TiO_2$ Films Deposited on Silicon Substrates Using IBAD with Different Beams**

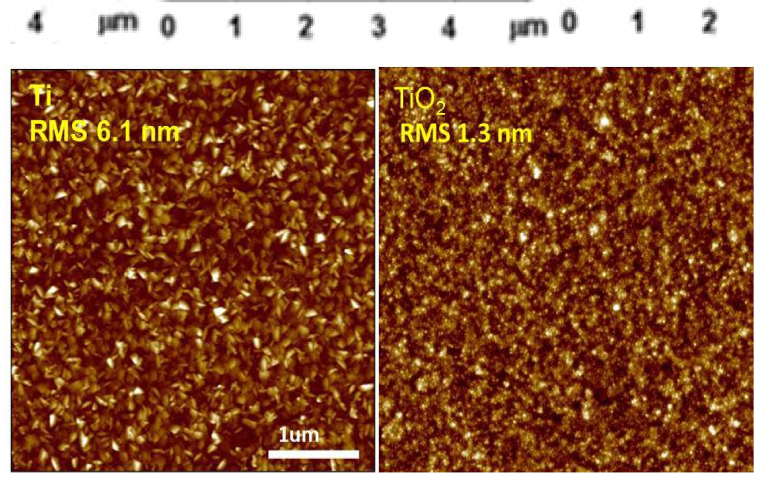



**Fig. 4: Atomic Force Microscopy Images(5μm Scan Size) of Ion Beam Deposited Nanocrystalline Ti and $TiO_2$ Deposited at Room Temperature**

## METHODS (con't)

➢Cell culture
The SAOS-2 cell line was used, which is a human osteosarcoma cell line, and maintains the cellular features of the osteoblasts with the ability to deposit extracellular matrix and mineralization.

➢Comparing cell adhesion and growth
Cell counting was done using DAPI (6-diamidino-2-phenylindole) fluorescent staining and ImageJ software.

➢Comparing cell morphology
Immunofluorescence staining was done to monitor actin stress fiber shapes.

➢Comparing calcium deposition
Alizarin red assay was used to detect calcium compounds on surfaces using a standard plate reader.

## RESULTS

**Fig. 5: Number of Nuclei by DAPI**

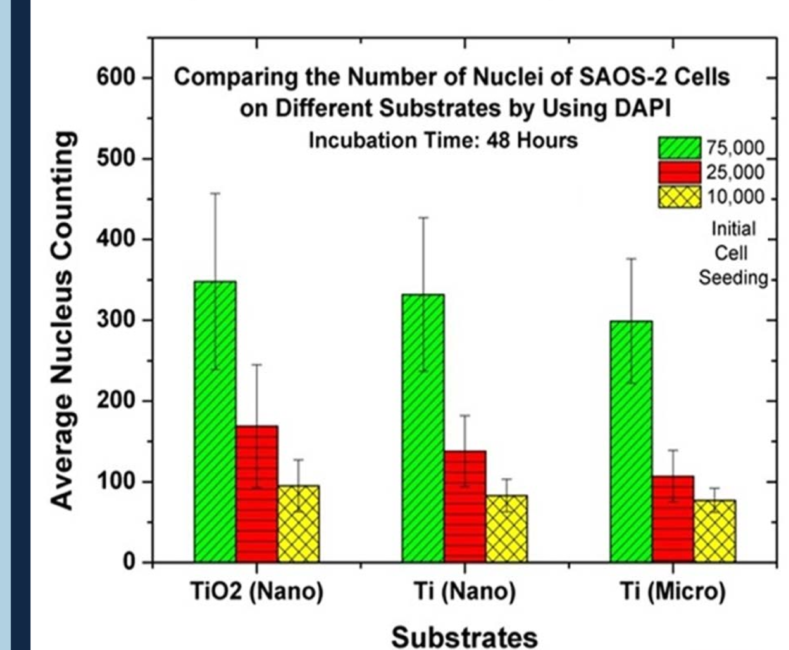



**Fig. 6: Comparing Calcium Deposition on Different Substrates Using Alizarin Red Assay for 7 and 14 Days**

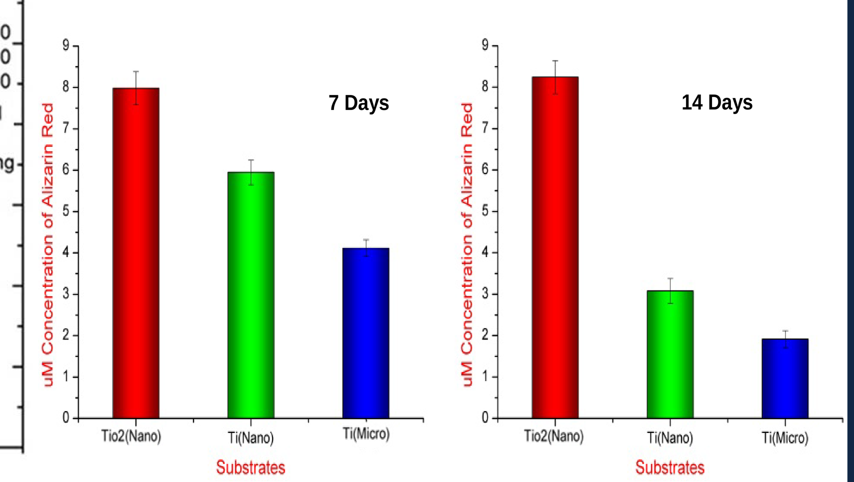



**Fig. 7: Merged Actin and DAPI Stained Cells on Nanocrystalline $TiO_2$ and Microcrystalline Ti, Respectively**

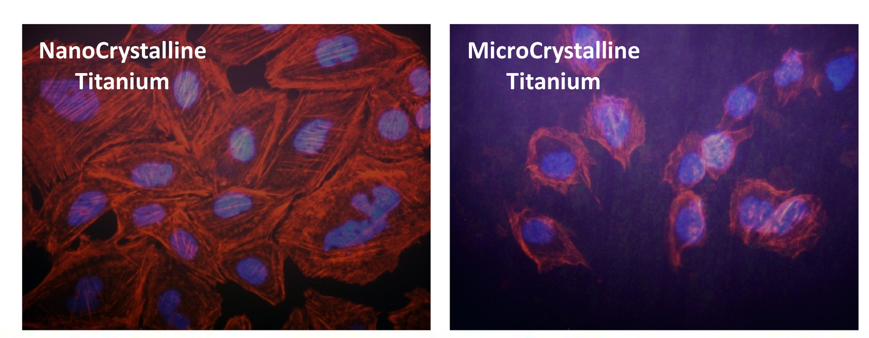
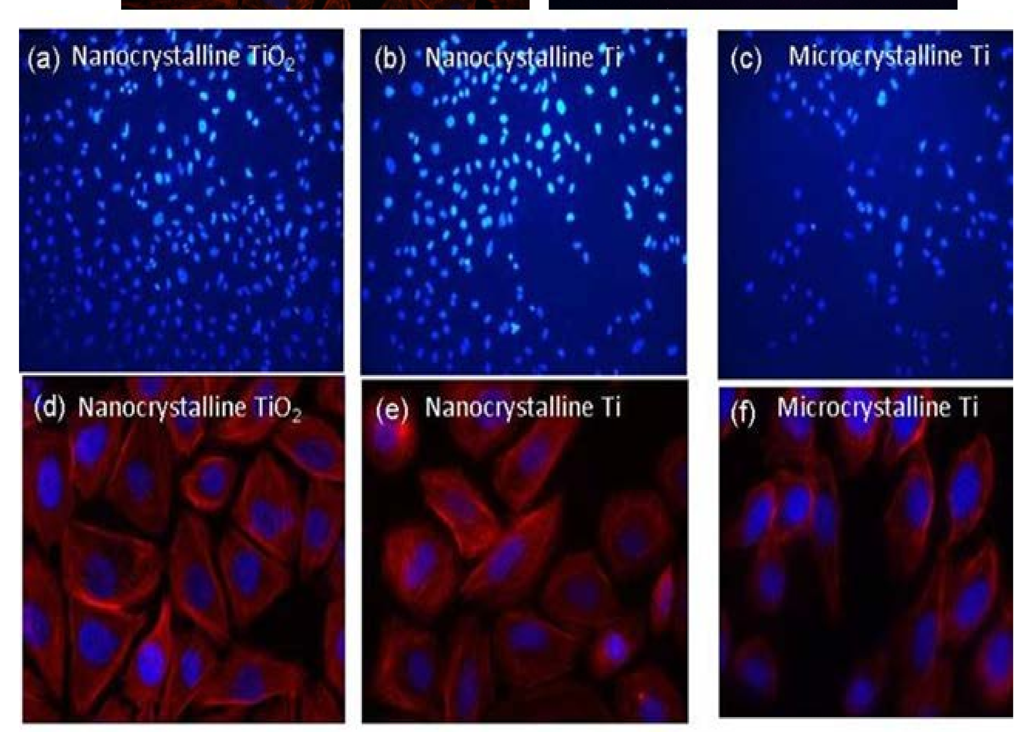



**Fig. 8: Comparing Cell Adhesion on Nanocrystalline $TiO_2$, Ti, and Biomedical Grade of Ti by Fluorescence Images Microscopy. 50000 Cells Were Incubated for 48 Hours. Images (a), (b) and (c) are DAPI and (d), (e) and (f) are Actin Stain Experiments**




## DISCUSSION

➢Figure 5 shows a greater number of DAPI-stained cells on nanocrystalline TiO2 and Ti compared to microcrystalline Ti, which indicates more adhesion and growth on nano surfaces.

➢Figure 6 indicates the difference in calcium deposition on nano-engineered TiO2 compared to nanocrystalline Ti and orthopaedic grade Ti.

➢ Besides DAPI staining, actin stress fiber shapes were also monitored (Figure 7) because by only observing nuclei, it is impossible to know if cells are healthy and prolific on the surface or not.

➢Figure 8 shows cell morphology on different substrates. Cells look more extended and healthier on nano-surfaces ($TiO_2$ and Ti) compared to microcrystalline Ti.

## CONCLUSION

➢ Nanocrystalline surfaces ($TiO_2$ and Ti) are superior in supporting growth, adhesion, and proliferation as compared to microcrystalline Ti due to the nano-crystal film characteristics that affect the wettability and mechanical properties of the coatings.

➢ Nano-engineered $TiO_2$ is superior to both nano and biomedical grade Ti because of the improved quality of the oxide layer by employing an IBAD technique.

➢ Osteoblastic cells deposit calcium in order to support bone construction. The cells cultured on the nano surfaces showed more calcium deposition (brighter red color in alizarin red staining), which implies a higher degree of the differentiation of the cells. Therefore, enhanced bone formation ability can be expected from the engineered nano structured surfaces.

## ACKNOWLEDGMENT

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