Biocompatibility and Hardness of Ti-Hf and Diamond-like Carbon Coatings for Orthopedics

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Abstract

Every year, about 300,000 total hip replacement (THR) surgeries are performed in the United States. The typical lifespan of an implant ranges between 10 and 20 years, with implant failures largely due to materials issues such as biocompatibility, wear, corrosion, and premature stress failures. The objective of this research is to examine the feasibility of using a new class of materials, namely Ti-Hf alloys and low friction diamond-like carbon (DLC) coatings, for improving the performance of orthopedic devices. Biocompatibility and hardness tests were performed, which showed Hf did not adversely affect the biocompatibility of Ti and that the DLC coating did not adversely affect the biocompatibility of the Ti-Hf alloy. Furthermore, the biocompatibility of the Ti-Hf alloy was comparable to that of Ti-6Al-4V. The research was done as a collaborative effort between the College of Engineering and the School of Veterinary Medicine at the University of Wisconsin – Madison and NASA Glenn, Cleveland, OH.

Keywords: biocompatibility, diamond-like carbon, hemocytometer, orthopedic, Ti-Hf

Introduction

Every year, about 300,000 total hip replacement (THR) surgeries are performed in the United States. The cost of a single procedure can exceed $20,000.[1] The most common recipients of THR's are between the ages of 65 and 70; however, it is becoming more prevalent in the younger demographic.[2] The durability of a THR, is in the range of 10 to 20 years depending on the patient's age, weight, and physical activity. One of the major issues associated with THR's is that retrieval surgeries are often needed to replace corroded, worn, or fractured parts from the original implant. These procedures tend to be more expensive and debilitating. The orthopedics industry aspires to extend the life of the implant and has concentrated on developing new materials and surface modifications that are more biocompatible and corrosion and wear resistant, as well as those that exhibit high strength.

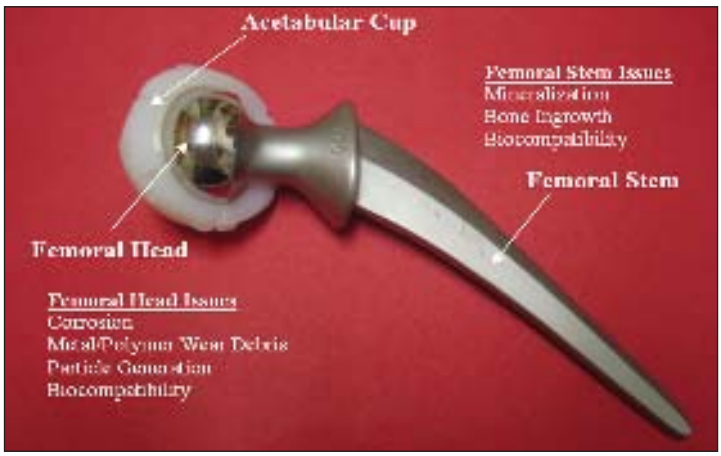

Figure 1. Components of a THR

A THR consists of three main components, the acetabular cup, femoral head, and femoral stem, which can be seen in Figure 1. The acetabular cup is polymeric and is usually made of ultra high molecular weight polyethylene (UHMWPE). The femoral head and stem are metallic and usually made of either Co-Cr or Ti-6Al-4V alloys.

Figure 1 summarizes some of the issues and their respective locations on the implant. There is significant wear at the poly-

mer/metal interface. The alloy used for the femoral head is significantly harder than the polymer used for the acetabular cup. This causes the generation of wear particles up to about 5μm in size, thus causing adverse tissue reactions. The rate of wear can range from 0.1 to 0.5 mm per year depending on the weight and activity of the patient.[3] Fibrosis, an immune response eliciting fibroblasts to combat foreign objects inserted in the body, is well-researched in the orthopedics industry.[4] Fibroblast cells are widely used in medical device research (ATCC), so 3T3 mouse fibroblasts were selected for biocompatibility testing.

The materials used for orthopedic implants must be biocompatible as well as exhibit good wear and corrosion resistance. Material selection is only part of the issue concerning biocompatibility and implant longevity. Surface modifications are also being studied as a means of reducing friction, minimizing wear, and promoting bone ingrowth. Research has shown that diamond-like carbon (DLC) coatings reduce polymer wear and increase corrosion resistance in synovial fluid, the body's natural lubricant.[5] DLC is a composite of carbon in the graphite and diamond states, which has a low friction coefficient and high hardness, and would be deposited on the femoral head.[6] The biocompatibility and hardness of Hf and DLC coatings were assessed via biological testing and hardness measurements. Table 1 lists the materials that were used for testing.

1. Materials Selection

The Ti-6Al-4V alloy was selected for evaluation because it is commercially used for THR's and exhibits good biocompatibility and strength-to-weight ratio. Aluminum and V are added primarily to increase the strength of Ti, but the toxicity of V is much greater than that of Ti.[7] Therefore, V was chosen as a negative control, while Ti was chosen as a positive control for biocompatibility testing. Hafnium was selected for evaluation because, similar to Ti, it forms a passive oxide layer and is in the same group as Ti in the periodic table, thus likely to exhibit comparable properties. Furthermore, Hf is expected to increase the strength of Ti because of the large difference in atomic radii. Large solute atoms create localized stress fields, which impede dislocation motion, thus increasing the strength of the material.[8] The Ti-Hf phase diagram shows very narrow two-phase regions, which implies that there is less segregation upon solidification, and a lower tendency for galvanic corrosion. Limited studies have shown evidence that Hf is biocompatible when used as a coating for biomedical applications, but no studies were found for which Hf was used in the bulk material.[9]

DLC coatings were selected for evaluation for the low friction coefficient in attempt to improve wear resistance.

2. Procedure

2.1 Sample Preparation

The metallic samples varied in surface roughness R_a, weight, and surface area; therefore, they had to be polished to a comparable R_a value in order to eliminate that variable during testing. The samples were polished according to the procedure outlined in Table 2. Weight was a non-issue since the biocompatibility test protocol, created in part of the research project, requires the cells be seeded on top of the surface of interest. The total surface area was taken into consideration when analyzing the cell viability results.

Materials for Biological Testing		
Initial	*Material Research*	*Surface Modification Research*
Ti	20wt%Hf-80wt%Ti	20wt%Hf-80wt%Ti
V	Ti-6Al-4V	
Hf		

Table 1. Materials for biological testing

Sample Preparation Procedure									
Material	Pretreatment	Mount	Remove from Mounting Press	Polish					
				SiC Paper					Colloidal Silica
				320	400	600	800	1200	
Ti	Roughen sample surface (convex side up) with 180 SiC	Phenolic Mounting Powder T = 138-149° C P = 29 MPa Premold = 5 min Mold = 10 min	Immediately	N/A	N/A	N/A	x	x	x
V			Immediately	N/A	N/A	N/A	x	x	x
Hf			After 30 min.	N/A	N/A	N/A	x	x	x
80%Ti-20%Hf	N/A		Immediately	x	x	x	x	x	x
60%Ti-40%Hf	N/A		Immediately	x	x	x	x	x	x
40%Ti-60%Hf	N/A		Immediately	x	x	x	x	x	x
20%Ti-80%Hf	N/A		Immediately	x	x	x	x	x	x

Table 2. Sample preparation procedure

The samples were then removed from the mounts and ultrasonically cleaned in acetone and ethanol for 3 mins each. Profilometry measurements were performed to ensure that the surface roughness was comparable (0.05 – 0.1 μm) for all samples.

2.2 Knoop Microhardness

Knoop microhardness measurements were performed for the Ti-Hf alloy and Ti-6AL-4V at a 50g load for 12 seconds.

2.3 Diamond-like Carbon Thin Film Deposition

Plasma Immersion Ion Implantation and Deposition (PIIID) technology, pioneered at the University of Wisconsin – Madison in the late 1980's, was used to coat Ti-Hf alloy samples. An Ar plasma cleaned the surface of the sample, and was followed by an acetylene (C_2H_2) plasma, which created a DLC film, with a 0.5 μm thickness, on the substrate (Figure 2).[10]

2.4 Cell Cultures

The samples were individually packaged and placed in an autoclave chamber at 125°C for one hr, to be sterilized prior to *in vitro* testing.

The cells were seeded on the surface of a flask and about 20 mL of growth medium was added to cover the bottom of the flask, and changed every two days. Growth medium is a solution containing essential nutrients for cell growth, and consisted of Dulbecco's Modified Eagles' Media with 2 mM glutamine, 25 mM HEPES buffer, 1% non-essential amino acids, 10% fetal calf serum, 50 mgl^{-1} ascorbic acid and supplemented with 1% penicillin/streptomycin antibiotic.[11,12] The flask was placed in an incubator at 37°C and 5% CO_2 for one week or until cells became confluent. Confluent cells grow as a monolayer to cover the surface of the flask. The 3T3 mouse fibroblast cell line is categorized as adherent, indicating that the cells grow on a substrate, and not in suspension. In order to transfer the cells from one substrate to another, they had to be trypsinized. Trypsin is an enzyme that breaks down the proteins that bind cells to a substrate, thus suspending them in the growth medium allowing them to be transferred. The Biocompatibility Test Protocol, outlined in Appendix I, was developed and carried out for each sample.

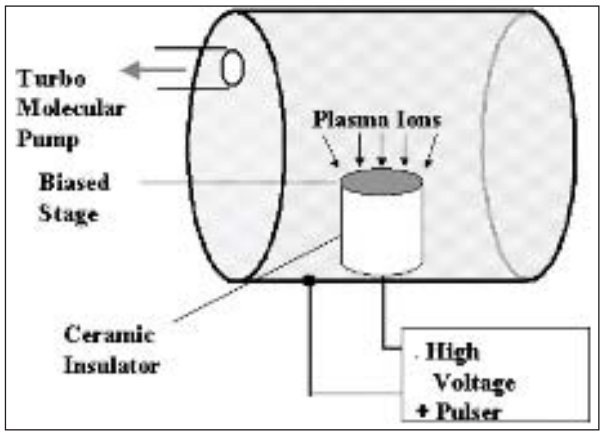

Figure 2. Plasma immersion ion implantation and deposition (PIIID)[10]

3. Results and Discussion

3.1 Knoop Microhardness

Figure 3 shows that the hardness of 80Ti-20Hf is comparable to that of Ti-6Al-4V. Five measurements were taken for each sample and an error of ±1 standard deviation was assessed for each sample. There is a large error bar associated with Ti-6Al-4V, and it is most likely attributed to the presence of multiple phases that vary in hardness. Conversely, Ti-Hf has a much smaller error bar, which can be attributed to the single phase microstructure.

3.2 Cell Cultures

Figure 4 shows that the biocompatibility of Ti is much greater than that of V, which confirms that the test methods were rigorous enough to adequately differentiate between Ti and V, as well as the Ti-Hf alloy

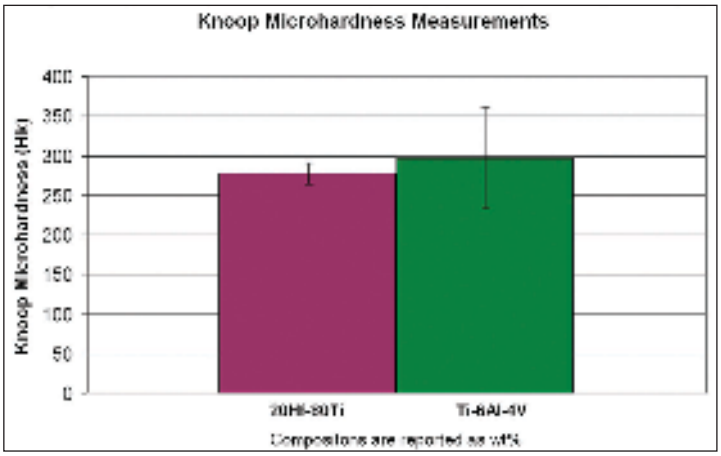

Figure 3. Knoop microhardness measurements

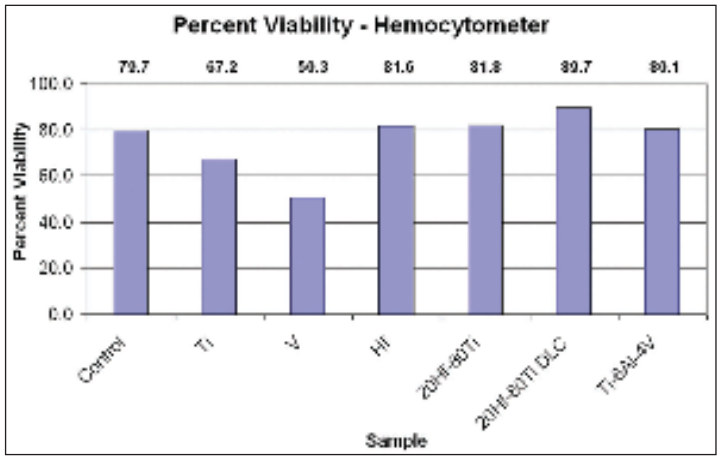

Figure 4. Percent viability - hemocytometer

and the other samples. The biocompatibility of the Ti-Hf alloy is comparable to that of Ti, which means that Hf does not adversely affect the biocompatibility of Ti. The biocompatibility of the DLC coated Ti-Hf alloy is comparable to that of the non-coated alloy, which indicates that DLC coatings do not adversely affect the biocompatibility of the bulk material. Lastly, the biocompatibility of both the DLC-coated and non-coated Ti-Hf was comparable to Ti-6Al-4V.

4. Conclusions

A protocol was established for testing the biocompatibility of metallic materials, and can be used for future work with considerable efficiency. Cell viability results from the hemocytometer showed that Hf does not adversely affect the biocompatibility of Ti. Similarly, DLC coatings do not adversely affect the biocompatibility of Ti-Hf alloys, suggesting that DLC coatings have the potential to be used along with Ti-Hf to improve wear resistance, thus minimizing the likelihood of adverse tissue reactions. The biocompatibility of the DLC-coated and non-coated Ti-Hf alloys were comparable to that of Ti-6Al-4V, proving these materials could potentially be used in orthopedic applications. Hardness measurements showed that Hf strengthens Ti via solid-solution strengthening, which could minimize stress-related failures in an orthopedic implant.

5. Future Work

This research covers the initial steps in exploring the potential use of Hf and DLC in orthopedic applications. Further testing should be done on a large sample set to establish statistical viability at a 24 hr incubation time. It would be beneficial to examine the long term effects of these materials on biological systems and to establish biocompatibility with respect to other relevant cell lines. Finally, *in vivo* biocompatibility testing would be another option for future research.

Appendix I: Biocompatibility Test Protocol

1. Growth medium was disposed and confluent cells from four flasks were trypsinized (5 mL/flask) for 10 mins.
2. Trypsinized cells were collected into a 50 mL tube and 15 mL growth medium was added.
3. The tube was placed in a centrifuge set to 23°C and spun at 1600 rev/min for 10 mins to produce a cell pellet.
4. Excess medium/trypsin was decanted and the cell pellet was separated.
5. 2 mL growth medium was added to the 50 mL tube and mixed.
6. A 1:5 trypan blue dilution (dilution factor = 5) was used to count the number of living cells/mL to determine the volume required so that each test well contained $5x10^5$ cells.
    a. 10 µL cell/growth medium solution and 40 µL trypan blue was pipetted into a 1.5 mL tube and mixed thoroughly.
    b. 20 µL was pipetted onto a hemocytometer slide and the viable cells were counted (cell count = 81 cells).
    c. Equation 1 was used to determine the number of cells/µL ($4.05x10^3$ cells/µL).

$$No.\ of\ cells\ /\ \mu L = cell\ count \cdot dilution\ factor \cdot 10 = 4.05x10^3\ cells\ /\ \mu L \quad (1)$$

    d. Equation 2 was used to determine the volume to be pipetted into each well to total $5x10^5$ cells (123 µL/well)

$$Volume\ of\ cells\ /\ well = \frac{number\ of\ cells\ desired}{number\ of\ cells\ /\ \mu L} \quad (2)$$

7. An autoclaved metal sample was placed in a labeled test well and growth medium was added to cover the surface of the metal sample (Note: For those wells that did not contain a metal sample, growth medium was added to cover the surface of the plate).
8. 120 µL cell/growth medium solution was pipetted into each well.
9. The plates were incubated for 24 hrs, followed by cell viability testing.
10. Growth medium (which may have contained dead cells that had detached from the substrate) was collected into a labeled 15 mL tube.
11. Cells were trypsinized for 10 mins (trypsin covered the surface of the substrate) and pipetted into the labeled 15 mL tube.
12. The tube was placed in a centrifuge set to 23°C and spun at 1600 rev/min for 10 mins to produce a cell pellet.
13. Excess growth medium/trypsin was decanted and the cell pellet was separated.
14. 250 µL of growth medium was added to the tube and mixed thoroughly.
15. A 1:1 trypan dilution was used to count the number of live and dead cells/mL to determine the percent viability.
    a. 10 µL trypan blue and 10 µL of cells/growth medium was pipetted into a 1.5 mL tube

and mixed thoroughly (the 15 mL tube was replaced on ice).

b. 20 µL was pipetted onto a hemocytometer slide and covered with a cover slip.
c. The live and dead cells were counted and recorded.
d. The hemocytometer slide and cover slip were rinsed and cleaned with ethanol.

Steps 7-15 were repeated for each metal sample.

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About the Author

Stephanie graduated with a Bachelor of Science in Materials Science and Engineering from the University of Wisconsin – Madison in the spring of 2007. During the summer of 2007, she plans to travel throughout Europe before returning to the U.S. to begin a full time career as an engineer at Kimberly-Clark Corp. in Roswell, GA.