Study of Improved Tissue Response on Titanium Alloys

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1. Abstract

This summer it was my objective to study bone cell tissue response on titanium alloys as used for biomaterials by assisting two graduate students with their research. Two different titanium alloys were investigated. First, titanium-tantalum alloys are a relatively new metal combination within the biomaterial community. A literature review was conducted on the alloy to verify biocompatibility, corrosion resistance, and other mechanical properties important for implant materials. Much is left to be desired where the study of tissue response is concerned on this new alloy. It is the effort of this material's study at SDSM&T to ascertain whether bone growth cells, or osteoblasts, will proliferate and adhere to a porous grid of this alloy. Secondly, titanium-15 molybdenum was studied to obtain qualitative data on osseointegration occurring on a porous grid of this alloy. Stain testing showed after a two week cell culture process that the sample grids strongly supported osteoblast proliferation and adhesion.

2. Introduction

The medical use of metals can be traced as far back as several thousand years ago in India; however, most of the knowledge collected on metals as biomaterials has been obtained in the time period during and since World Wars I and II. While polymers, ceramics, and electronic devices are also large areas of study within the medical community, here the focus will be placed on metals as used for implants, especially focusing on the bone-metal interaction.

Metals were chosen for study based on their biocompatibility-- that is the "acceptance of an artificial implant by the surrounding tissues and by the body as a whole."[8] A biomaterial should not degrade within the human body or cause any adverse reactions. The body's internal environment is extremely hostile to most metals, and the introduction of foreign objects comes with many risks and concerns. One concern is the corrosion of the metal implants. If the material begins to breakdown it may release harmful particles into the body, which can lead to infection and other harmful side effects.[8]

In addition to biocompatibility, scientists and engineers are concerned with the stress shielding phenomenon. This phenomenon is a result of "insufficient loading of bone due to the large difference in modulus between an implant device and its surrounding bone." By Wolff's law, bones continuously adjust and develop their internal structure and external configuration according to the external loading conditions to which they are exposed. For example, if the bone is exposed to a smaller load, the bone will undergo bone resorption due to the lack of mechanical stimuli to encourage bone growth. Below is a chart of the Young's modulus of pure Ti and various Ti alloys used in implants. The table demonstrates the large difference in Young's modulus between the materials and cortical bone, or compact bone that provides the primary support for the body.[7] The implant is able to carry a much larger load than natural bone due to its greater strength and modulus, which can lead to bone resorption and eventual failure of the implant. This concern has contributed itself as motivation to search for materials with improved mechanical properties, especially a significantly lower modulus of elasticity.[8]

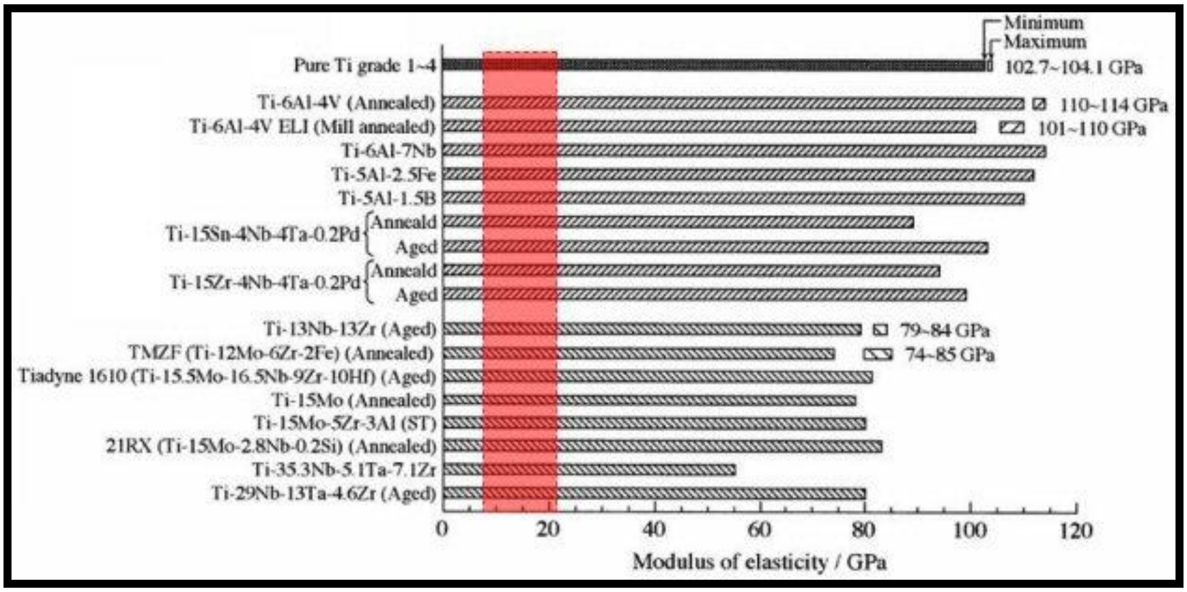

Figure 1. Modulus of elasticity of pure Ti and Ti alloys compared to cordical bone.[7]

All factors considered the materials that are most commonly used and studied given their biocompatibility and other qualifying characteristics are stainless steels, cobalt, titanium, and their alloys. No metal or its alloys, however, can be considered perfectly biocompatible and different alloys can produce different responses in an identical biological milieu. "Bone growth to metal implants is also dependent on the metal alloy itself".[13] Among these metals, commercially pure titanium (cp-Ti) is the ideal choice when osseointegration is considered, and it is considered inert in the body where biocompatibility is concerned.

Originally a metal of importance within the aeronautics' community, titanium made the transition to a biomaterial beginning in the 1960s due to its high strength, low weight, low modulus of elasticity, high melting temperature, and high corrosion resistance.[2]

Ti-6Al-4V is the most commonly used Ti alloy. A call for a new and improvement material, however, has been issued due to the toxic nature of the metal ions- aluminum and valadium- that can cause serious health problem should they be released into the body.

In additional to the search for metals with better mechanical properties, biocompatibility, and corrosion resistance, the biomedical community also seeks to improve the bone-metal interface. It is the goal to see a stronger bond and bone growth on metal implants. The optimal bone-implant interface should exhibit intimate apposition or even chemical bonding between bone and implant. Porous coated implants are being considered to encourage a stronger interface. Inert materials will allow bony tissues to grow into any space large enough to accommodate osteons, which requires a space of 75 μm in diameter.[8] The porous metal implants would allow for direct bonding of bone and metal known as osseointegration.[13] A porous surface, however, increases the surface area of the implant which in turn increases the risk of corrosion.

At the South Dakota School of Mines two different combinations of titanium alloys are being studied for use as porous coatings on medical implants. Kirsten William is pursuing research on titanium-15 molybdenum, or Ti-15Mo, in an effort to find improved tissue response. This material has a low modulus of elasticity, good corrosion properties, and is being considered for use as a dental implant. Graduate student, Mary Huber, is investigating titanium-tantalum alloys. Both pure titanium and pure tantalum have been metals used for medical applications due to their sought after properties of biocompatibility and corrosion resistance. It is a somewhat novel idea to combine the two materials for biomedical uses. Only limited attention has been paid to this combination, which is to some degree the motivation behind the study of this alloy.

3. Broader Impact

The ever expanding knowledge, technologies, and practices of the medical community are leading to a world population with greater quality of and extended length of life. As a result the world's population is living longer, thus increasing the quantity of elderly persons. The lifestyle of the population as whole, however, is leading to an increasing percentage of the population suffering from obesity. There is also an increasing presence of degenerative diseases like osteoarthritis. Musculoskeletal disorders continue to affect persons of all ages and lifestyles and severely affect quality of life. Artificial bone substitute materials can restore the presence of lost structures and function of diseased bone among such patients.[1] The biomedical community has made large advancements since the first generation of devices. There is the current ability to provide prosthetic devices and biomedical implants that increase comfort and function to individuals of all ages suffering from discomfort and wear and tear of their joints and bones.[10] The overall goal for new generations is to find devices and biomaterials with the ability to last longer, heal faster, and be more compatible for both older and younger members of the world's population.

4. Literature Review

As previously stated, a search for new Ti alloys is being conducted. The element, tantalum, is beginning to receive attention as a potential alloying agent in combination with titanium. Pure Ta and pure Ti have been used as biomaterials for decades. Pure Ta has been used for surgical implants such as sutures, bone screws and plates. Separately these two materials have relatively poor mechanical properties preventing any extensive use. However, when the two are alloyed together their mechanical properties improve. Additionally, porous Ta by itself has been studied and found to have desirable mechanical properties and good tissue adhesion. Studies show that Ta enhances the strength and reduces the modulus of elasticity in combination with Ti. One such study by Zhou et. al. investigated the effects of Ta content on the microstructure, Young's modulus and tensile properties of quenched Ti-Ta alloys with adjusted Ta content in increments of 10 mass%. The study found that Ti-30% Ta and 70% Ta have a modulus value almost one half of the modulus value for Ti-6Al-4V and, therefore, have the greatest potential as a new biomedical application candidate. Additionally, the corrosion resistance of the alloys increases as the Ta content increases and does not produce any toxicity.[14] Since this initial study, Zhou et. al. have conducted more studies on the mechanical properties of Ti-Ta alloys, each time focusing on a singular content of Ta including 25, 30, and 50 mass%.[15,16]

Other studies have also been conducted on porous Ta. Levine et. al. found this new metal to have 70-80% volumetric porosity, a modulus of elasticity of 3 MPa, and high frictional characteristics. With good biocompatibility porous tantalum has potential applications as primary femoral stems, salvage prostheses, total shoulder components, and use in arthrodeses of the wrist.[6] Welldon et. al. studied the interaction of human osteoblasts (HOBs) on porous Ta, and their results conclude that in addition to having desirable mechanical properties, HOBs were found to have good attachment, growth and differentiated function on porous Ta metal. [11]

5. Procedures

While at two very different stages in their research, each graduate student is testing bone cell adhesion on sample grids of their particular porous Ti alloy. The grids are made by graduate student, Jake Fuerst, using the micro Laser Additive Manufacturing (m-LAM) system. The system is a recent addition to the Additive Manufacturing Lab (AML) at the South Dakota School of Mines and Technology. It was developed in the summer of 2009 by Dr. Kalanovic.

The m-LAM serves the purpose of performing the process of depositing a material onto a substrate using a laser in an effort to create a three-dimensional object by building up layers of melted powdered material. The machine has six-degrees of robotic freedom for laser-powder deposition with the ability to make surface modifications on prosthetic devices at the 100 micron scale. Modifications at such a small scale are made with the hopes of improving osseointegration. The deposited layers form a porous coating onto a small area of a chosen substrate using a high, localized energy laser beam to melt a powder spray into a grid of chosen size and shape. The laser moves across the substrate surface depositing the powder into a weld bead at a high enough temperature to melt the powder without affecting the overall microstructure of the part.

For Kirsten, six Ti-15 Mo samples were made for the purpose of testing the quality of cell adhesion to the grids. The procedure conducted used Ti-15 Mo powder at -140 +325 mesh and deposited onto a Ti-6Al-4V substrate in an argon atmosphere, which is important in order to prevent the creation of oxidation layers during deposition. The system used a Nd:YAG laser piped through a 60μm fibre, collimated, and focused so that the focal plane was at the surface of the substrate. In total the samples were deposited with 5 layers of Ti-15 Mo melted powder.

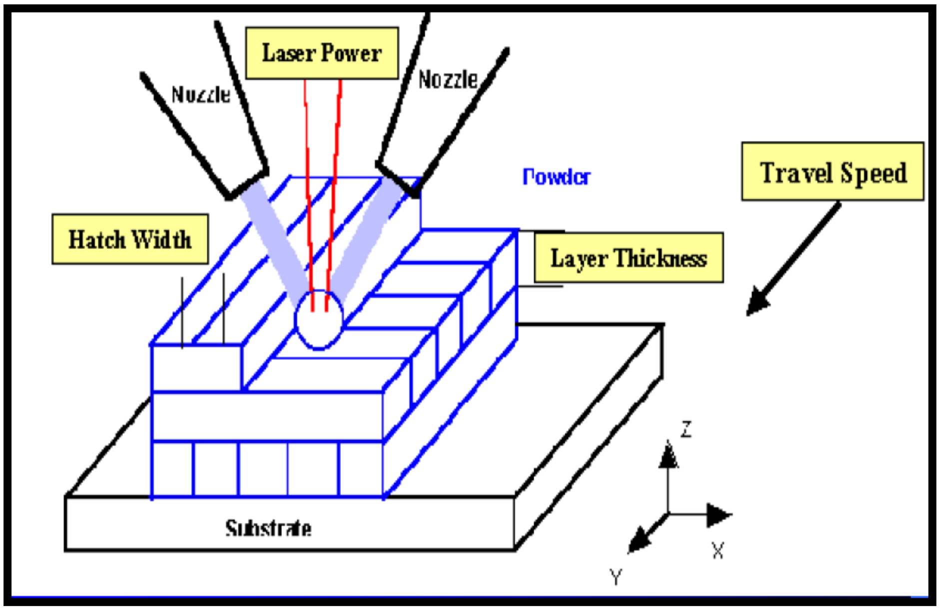

Figure 2. M-LAM deposition diagram

Figure 3. M-LAM system in Additive Manufacturing Laboratory

The samples were then wire brushed in order to remove excess powder. Then, they were placed in a polypropylene container containing sufficient 40% HNO_3 solution to submerge all samples, in an ultrasonic bath in a fume hood. A premeasured volume, sufficient for a 2% final concentration, of HF was then added to the HNO_3 and allowed to sonicate for 5 minutes. The samples were then rinsed in a saturated $NaHCO_3$ solution to neutralize the acid, rinsed with water, then rinsed with acetone. As a final step, the samples were autoclaved in an effort to sterilize and remove any unwanted debris.

A cell culture then takes place on the grids over the period of two weeks. The culture methods of samples are as follows:

CRL2593- mouse osteoblast differentiated cells were obtained from Fischer Scientific, and grown in Hyclone Mem Alpha Modification 1X medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin antibiotic solution. Cells are first removed from storage in liquid nitrogen and cultured for two days. Next, a process of cell splitting is completed twice. The first set of cells after splitting is used to seed the test substrate, while the second set is frozen in order to replace the set originally removed. [Note: A detailed procedure can be found in reference 12]. Each of Ti-15Mo grids is seeded with the cell line. The cell media is exchanged every other day. After the 14 day culture period, the sample grid/substrate is removed and tested for osteoblast proliferation. Three tests, using the technique of colorimetry, are completed to qualitatively prove bone cell proliferation: von Kossa, Alizarin Red, and Alkaline Phosphatase staining. The Von Kossa staining method has been used since the 19th century. Currently, it is used as a detector of osteoclast and osteoblast activity among in vitro experiments. For the purposes of our study, the von Kossa stain will be used to demonstrate that calcium deposits are present on the samples.[4] [Note: Full procedure for all staining can be found in reference 12].

The second procedure, Alizarin Red, is also a qualitative detector of calcium deposits on the samples. The nuclei of the calcium cell deposits will appear red after staining and the cytoplasm will appear pink. The final staining that will be performed is an alkaline phosphatase test.[3] Alkaline phosphatase is an enzyme that is released in large quantity during the bone growth process. It is needed in order for the proper deposition of minerals in bones and teeth. This stain should appear purple if the enzyme is present, therefore, confirming that bone growth is taking place on the substrate.

6. Results

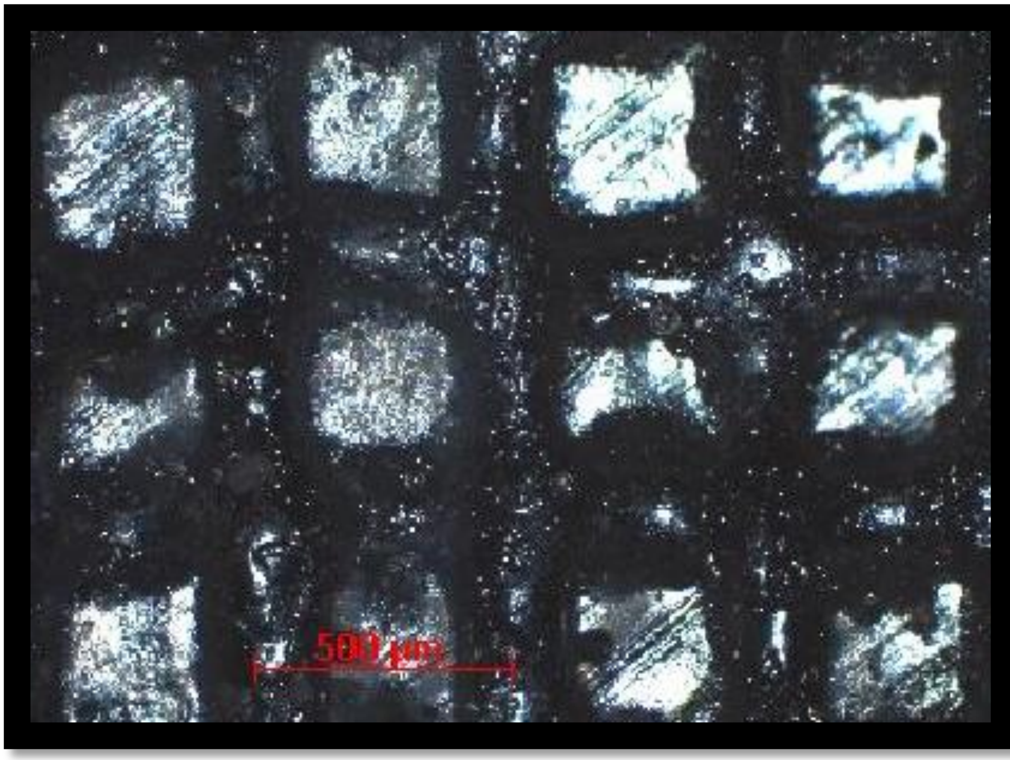

Figure 4. Ti-15Mo grid used for cell culture

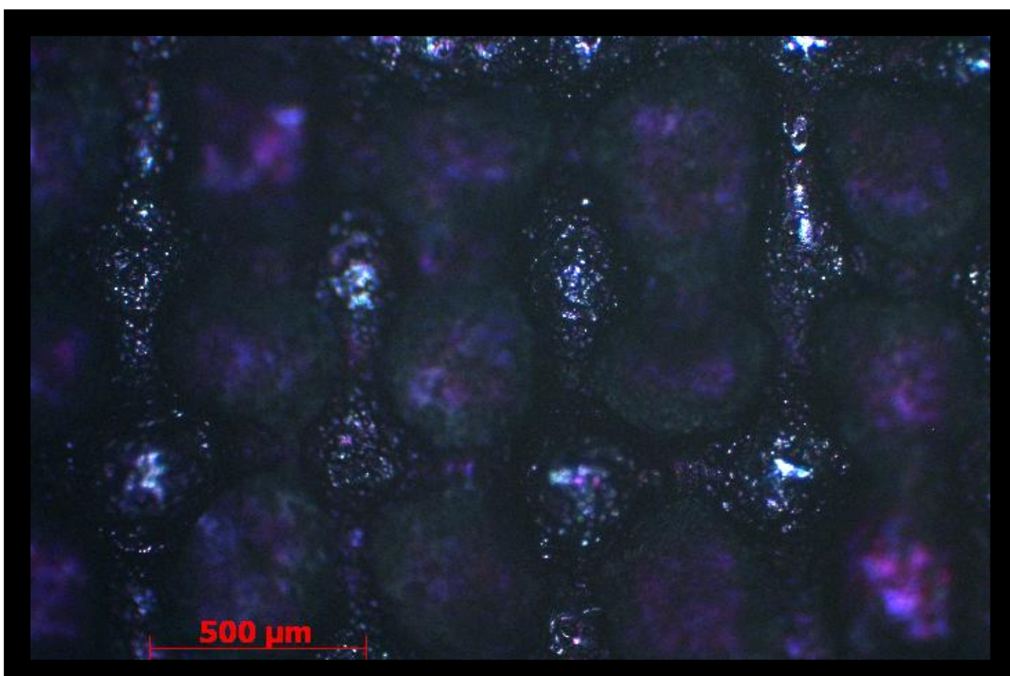

Figure 5. Ti-15Mo grid after Alkaline Phosphaase staining

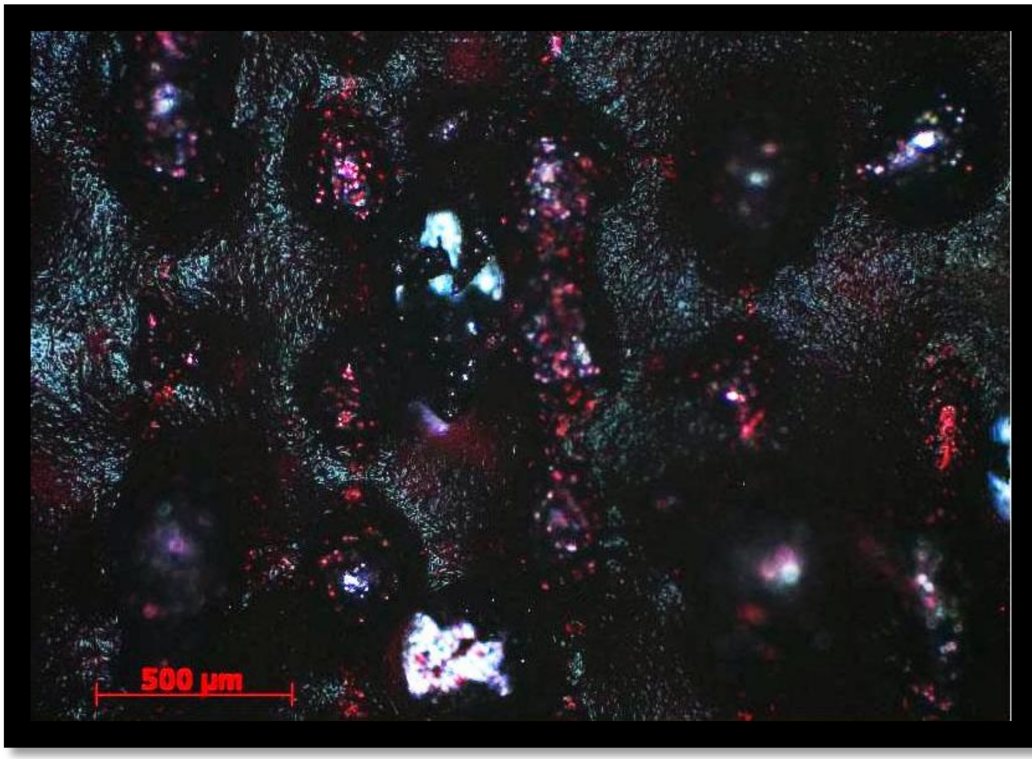

Figure 6. Ti-15Mo grid after Alizarin Red staining

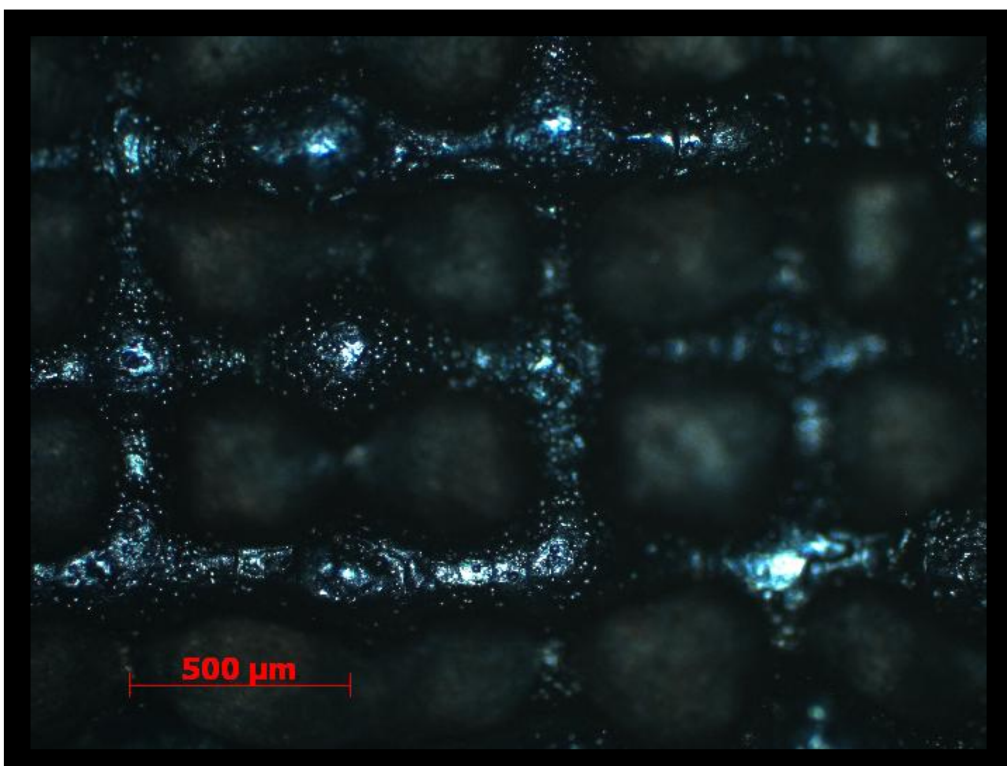

Figure 7. Ti-15Mo grid after von Kossa staining

7. Discussion

Each of the three staining test presented results that verified osteoblast proliferation. Osteoblasts are the cell responsible for forming the protein matrix necessary for bone formation. On the surface of the osteoblasts is a bone-specific tetrameric glycoprotein called alkaline phosphatase that is a biochemical indicator of bone growth..[9] The first stain for alkaline phosphatase produced a purple stain, which indicates that the alkaline phosphatase enzyme is present on the grid (Figure 5). The Alizarin Red stain test produced a red stain on the grids (Figure 6). This reaction identifies calcium deposits formed in the protein matrix by calcium salts of osteoblasts in the early stage of development before the formation of true calcified bone. Finally, the von Kossa test produced a black/brown stain (Figure 7). This is an indicator that deposits of calcium or calcium salt specific to osteoblasts are once again present. All the tests combined verify that bone cell growth is taking place on the grids.

8. Conclusions

While previous research and studies report that Ti-15Mo possesses biocompatibility and good mechanical properties, it was still left to be determined how osteoblasts would grow on the porous grid. The stain tests show that the grids will fully support bone cell growth. Only qualitative results, however, were acquired in this study and quantitative data is still needed. In the future, nine more Ti-15Mo grids will be created as samples for testing. Cell count tests will be performed to check for osteoblast proliferation and adhesion are taking place at 1, 4, and 7 days.

For the Ti-Ta alloys, it has been concluded at this time to melt non-porous pure Ta powder on a Ti-6Al-4V substrate. Bone cells prefer to grow on an ordered surface, and the pure

Ta grid will provide that. Additionally, the Ta content will be adjusted and tested in the future with the same techniques reported by this study in order to gain qualitative and quantitative data.

Acknowledgements

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