ANODIZED TITANIA: PROCESSING AND CHARACTERIZATION TO IMPROVE CELL-MATERIALS INTERACTIONS FOR LOAD BEARING IMPLANTS

By

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Materials Science Program

May 2007
To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of KAKOLI DAS find it satisfactory and recommend that it be accepted.

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Co-chair

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Co-chair
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ANODIZED TITANIA: PROCESSING AND CHARACTERIZATION TO IMPROVE CELL-MATERIALS INTERACTIONS FOR LOAD BEARING IMPLANTS

Abstracts
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The objective of this study is to investigate in vitro cell-materials interactions using human osteoblast cells on anodized titanium. Titanium is a bioinert material and, therefore, gets encapsulated after implantation into the living body by a fibrous tissue that isolates them from the surrounding tissues. In this work, bioactive nonporous and nanoporous TiO$_2$ layers were grown on commercially pure titanium substrate by anodization process using different electrolyte solutions namely (1) H$_3$PO$_4$, (2) HF and (3) H$_2$SO$_4$, (4) aqueous solution of citric acid, sodium fluoride and sulfuric acid. The first three electrolytes produced bioactive TiO$_2$ films with a nonporous structure showing three distinctive surface morphologies. Nanoporous morphology was obtained on Ti-surfaces from the fourth electrolyte at 20V for 4h. Cross-sectional view of the nanoporous surface reveals titania nanotubes of length 600 nm. It was found that increasing anodization time initially increased the height of the nanotubes while maintaining the tubular array structure, but beyond 4h, growth of nanotubes decreased with a collapsed array structure. Human osteoblast (HOB) cell attachment and growth behavior were studied using an osteoprecursor cell line (OPC 1) for 3, 7 and 11 days. Colonization of the cells was noticed with
distinctive cell-to-cell attachment on HF anodized surfaces. TiO$_2$ layer grown in H$_2$SO$_4$ electrolyte did not show significant cell growth on the surface, and some cell death was also noticed. Good cellular adherence with extracellular matrix extensions in between the cells was noticed for samples anodized with H$_3$PO$_4$ electrolyte and nanotube surface. Cell proliferation was excellent on anodized nanotube surfaces. An abundant amount of extracellular matrix (ECM) between the neighboring cells was also noticed on nanotube surfaces with filopodia extensions coming out from cells to grasp the nanoporous surface for anchorage. To better understand and compare cell-materials interactions, anodized nanoporous sample surfaces were etched with different patterns. Preferential cell attachment was noticed on nanotube surfaces compared to no cells on etched patterned surface. Cell adhesions and differentiation were more pronounced with vinculin protein and alkaline phosphatase, respectively, on anodized surfaces. MTT assays showed increase in living cell density and higher proliferation on H$_3$PO$_4$, HF and nanotube surfaces. When anodized surfaces were compared for cell materials interaction, it was noticed that each of the surfaces has different surface properties that led to variations in cell-materials interactions. It was clear that rough surface morphology, high surface energy, and low value of the contact angles were important factors for better cell materials interaction. Mineralization study was done in simulated body fluid (SBF) with ion concentration nearly equal to human blood plasma to further understand biomimetic apatite deposition behavior. Similar to cell-materials interaction, variation in mineral deposition behavior was also noticed for films grown with different electrolytes. These results clearly show that nonporous titania in H$_3$PO$_4$, HF electrolytes and nanotubes can significantly increase biocompatibility of Ti implants, which has the potential to reduce the healing time and increase in vivo lifetime for these implants.
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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Motivation

Titanium and its alloys are widely used for orthopedic and dental implants for their superior mechanical properties, low modulus, excellent corrosion resistance and good biocompatibility. Titanium, being bio-inert, cannot directly bond to bone. Although an oxide layer 10 nm is always present naturally on the titanium surface, still, fibrous tissues grow on its surface. Oxide layer thickness has been increased to improve the surface for better tissue-materials interaction. A crucial concept to understand about the tissue-biomaterials interface is that a lot of things happen there. The environment inside the body is chemically, electrically, and mechanically active, and the interface between an implanted biomaterial and the body is the location of a variety of dynamic biochemical processes and reactions. Figure 1.1 shows some of the atomic and molecular level events that happen when a metallic implant is placed in the body. Oxygen diffuses from the surface oxide into the bulk metal, and metal ions can diffuse from the bulk into the surface oxide as well. Biological ions can also be incorporated into the surface oxide. Interactions of biological molecules (protein, enzymes etc.) with the implant surface can cause transient or permanent changes in the conformation and thus function of these molecules.

Although the interface between a man-made, synthetic biomaterial and the body is complex enough, tissue-engineered products that incorporate living cells within a biomaterial matrix make things even more complicated. These tissue-engineered products have three distinct interfaces to consider: between the body and biomaterials, between the body and living cells, and between the cells and biomaterial. Each interface presents unique opportunities and potential problems related to the long-term viability of the tissue-engineered products. Designing biomaterial surface to
control subsequent cell and tissue function that truly integrate with the body’s natural tissues is challenging aspect.

![Diagram](image)

**Figure 1.1.** Molecular level events at the surface of metal implants. At the microscopic level, each surface probably varies in chemical composition and topology and is the location of a number of dynamic molecular surface interactions.

Implant materials in general, and metallic implant materials in particular, have a significant economic and clinical impact on the biomaterials field. The global market for orthopedic implants generated total revenues of $12.5 billion in 2005, representing a compound annual growth rate (CAGR) of 10.4% for the five-year period spanning 2001-2005. Looking forward, the global orthopedics market is forecast to continue its acceleration, with an anticipated CAGR of 8.4% for the period 2005-2010 expected to drive total revenues to $3.1 billion by the end of 2010. Hip and knee replacements are mature markets in the US and Europe with knee procedures and revenues growing at a higher rate than hip replacement procedures and revenues. The 2005 revenues for hip implants in the US were $2 billion and $1.4 billion in
Europe. Knee implant revenues for 2005 were $2.4 billion in the US and $774m in Europe. Valued at $2.48 billion in 2005, the European market for orthopedic implants is expected to be worth $3.78 billion in 2012. In view of this wide utilization of implants, many of which are metallic, the current study with metallic implants was to understand the role that biomaterials play in the larger problem of design, proper utilization of medical devices and tissue interactions.

**The Biomaterials and Healthcare Market**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total U.S. healthcare expenditure (2000)</td>
<td>$1,400,000,000,000</td>
</tr>
<tr>
<td>Total U.S. health research and development (2001)</td>
<td>$82,000,000,000</td>
</tr>
<tr>
<td>Number of employees in medical device industry (2003)</td>
<td>$300,000</td>
</tr>
<tr>
<td>Registered U.S. medical device manufacturers (2003)</td>
<td>$13,000</td>
</tr>
<tr>
<td>Total U.S. medical device market (2002)</td>
<td>$77,000,000,000</td>
</tr>
<tr>
<td>U.S. market for disposable medical supplies (2003)</td>
<td>$48,600,000,000</td>
</tr>
<tr>
<td>U.S. market for biomaterials (2000)</td>
<td>$9,000,000,000</td>
</tr>
</tbody>
</table>

Researchers are working to understand the improvements in controlling the tissue interaction at the interface. The research is motivated by the growing market demands for biomaterials and challenges in improving the tissue-materials interactions for long-term stability and shorter healing time. As of yet, there has been little success in mimicking bone in terms of its mechanical and biological properties. To replace a specific part of bone with implant materials and to study the interface interactions it is very important to have some basic understanding about human bone.
1.2. Bone

Bone is a living material composed of cells and a blood supply encased in a strong, interwoven composite structure. Bone tissue can be classified into two categories: (1) cortical or dense and (2) cancellous bone or spongy. Cancellous bone differs from cortical bone in being open-spaced and trabecular. The trabecular features represent “unrolled” osteons on both surfaces, which are in apposition to a central framework of interstitial bone. The solid framework and pore network are continuous and interconnected domains.
Table 1.1- Microstructural comparison of human cancellous bone studied by two different groups

<table>
<thead>
<tr>
<th></th>
<th>Iliac bone$^3$</th>
<th>Iliac bone$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume fraction (%)</td>
<td>20.5 ± 0.4</td>
<td>20.3 ± 0.4</td>
</tr>
<tr>
<td>Surface Area</td>
<td>3.0 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Ratio of surface area to volume fraction</td>
<td>14.6 ± 0.6</td>
<td>17.3 ± 0.2</td>
</tr>
<tr>
<td>Mean trabecular width</td>
<td>136.6 ± 4.5</td>
<td>120.3 ± 1.6</td>
</tr>
<tr>
<td>Mean pore width</td>
<td>529.6 ± 22.9</td>
<td>468.2 ± 27.2</td>
</tr>
</tbody>
</table>

Bone has several functions in the human body. The main functions of human bones are 1) providing a rigid support for the human body structure; 2) protecting internal organs of the human body; 3) assisting in movement of human body parts with the help of muscles and tendons; and 4) serving as a storage area for minerals such as calcium and phosphorus. When excess minerals are present in the blood, buildup will occur within the bones. When the supply of these minerals within the blood is low, it will be withdrawn from the bones to replenish the supply; 5) thereby producing blood cells by bone marrow located in the bones. An average of 2.6 million red blood cells is produced each second by the bone marrow to replace those worn out and destroyed by the liver.

There are three types of cells that contribute to bone formation. Osteoblasts are bone-forming cells, osteoclasts resorb or break down bone, and osteocytes are mature bone cells. Equilibrium between osteoblasts and osteoclasts maintains bone tissue. Compact bone consists of closely packed osteons or haversian systems. The osteon consists of a central canal called the osteonic (haversian) canal, which is surrounded by concentric rings (lamellae) of matrix. Between the rings of matrix, the bone cells (osteocytes) are located in spaces called lacunae. Small channels (canaliculi) radiate from the lacunae to the osteonic (haversian) canal to provide passageways through the hard matrix. In compact bone, the haversian systems are packed tightly together to form what appears to be a solid mass. The osteonic canals contain blood vessels that are parallel
to the long axis of the bone. These blood vessels are interconnected, by way of perforating canals, with vessels on the surface of the bone.

The main constituents of human bone are 20 wt% collagen (a natural polymer) and 69 wt% calcium phosphate (main inorganic component) and 9 wt% water. Additionally, other organic materials such as proteins, polysaccharide and lipids are present in small quantities. Collagen, in the form of small microfibers, forms the matrix of bone structure by 3-dimensional network formation. The diameter of collagen microfibers varies from 100 to 2000 nm. Presence of calcium phosphate in the form of crystalline hydroxyapatite (HA) and/or amorphous calcium phosphate (ACP) provides stiffness to the bone. HA crystals are present in the collagen matrix in the form of platelets or needles, which are 40–60 nm long, 20 nm wide and 1.5-5 nm thick. These crystals are deposited parallel to the collagen fibers such that the larger dimensions of the crystals are along the long axis of the polymer fiber. The deposited HA crystals are connected to each other and form a 3-D network structure.
Table 1.2 – Chemical composition of human bone

<table>
<thead>
<tr>
<th>Composition</th>
<th>Enamel</th>
<th>Dentine</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>36.5</td>
<td>35.1</td>
<td>34.8</td>
</tr>
<tr>
<td>P</td>
<td>17.7</td>
<td>16.9</td>
<td>15.2</td>
</tr>
<tr>
<td>Ca/P molar ratio</td>
<td>1.63</td>
<td>1.61</td>
<td>1.71</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.5</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.44</td>
<td>1.23</td>
<td>0.72</td>
</tr>
<tr>
<td>K</td>
<td>0.08</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>3.5</td>
<td>5.6</td>
<td>7.4</td>
</tr>
<tr>
<td>F⁻</td>
<td>0.01</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.30</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>P₂O₇⁴⁻</td>
<td>0.02</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Total inorganic</td>
<td>97</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Total organic</td>
<td>1.5</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Adsorbed H₂O</td>
<td>1.5</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Mechanical properties of human bone are given in Table 1.3. Because of its lower density, cancellous bone has a lower modulus of elasticity, and lower compressive and tensile strength than cortical bone.

Table 1.3 – Mechanical properties of human bone

<table>
<thead>
<tr>
<th>Properties</th>
<th>Cortical bone</th>
<th>Cancellous bone</th>
<th>Dentine</th>
<th>Enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressive strength (MPa)</td>
<td>130-180</td>
<td>2-12</td>
<td>250-350</td>
<td>95-370</td>
</tr>
<tr>
<td>Tensile strength (MPa)</td>
<td>60-160</td>
<td>10-20</td>
<td>21-53</td>
<td>10</td>
</tr>
<tr>
<td>Young’s (tensile) modulus (GPa)</td>
<td>3-30</td>
<td>0.05-0.5</td>
<td>11-19</td>
<td>-</td>
</tr>
<tr>
<td>Fracture toughness (Kfc)</td>
<td>2-12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hardness (Knoop)</td>
<td>132-166</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1.3. Biomaterials

A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems (Williams, 1987). In general it addresses the properties and applications of materials (synthetic and natural) that are used in contact with biological systems. Although biomaterials are primarily used for medical application they are also used to grow cells in...
culture, to assays for blood proteins in the clinical laboratory, and in equipment for processing biomolecules for biotechnological applications. However, artificial materials that simply come into contact with the skin, such as hearing aids and wearable artificial limbs, are not included in biomaterials, since they are not in contact with body fluid.

Biomaterials science is the physical and biological study of materials and their interaction with the biological environment. Traditionally, the most intense development and investigation have been directed toward biomaterials synthesis, optimization, characterization, testing and biology of host-material interactions. Most biomaterials focus on a nonspecific, stereotyped biological reaction. Considerable current effort is directed toward the development of engineered surfaces that could elicit rapid and highly precise reactions with cells and proteins, tailored to a specific application. Indeed a complementary definition essential for understanding the goal of biomaterials science is that of “biocompatibility”. Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application (William, 1987). Examples of appropriate host responses include resistance to blood clotting, resistance to bacterial colonization and normal, uncomplicated healing. Biocompatibility is determined by the extent of chemical and biological interaction between host and implant, and stability (mechanical integrity) of the implant. A compatible implant would have no effect on the adjacent tissue, the nearby cells would show no abnormalities, no variant cell types would appear, there would be no inflammatory reactions, and there would be no cell necrosis. Biocompatibility of devices (and, by necessity, of their constituents materials) must be established (that is, tested and documented by manufacturers) and approved by regulatory agencies, for example Food and Drug Administration (FDA), International Organization for Standardization (ISO) before any
biomedical devices are marketed and used clinically. Table 1.4 lists a few applications for synthetic materials in the body.

**Table 1.4-** Some Applications of synthetic Materials and Modified Natural Materials in Medicine.

<table>
<thead>
<tr>
<th>Applications</th>
<th>Types of materials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skeletal systems</strong></td>
<td></td>
</tr>
<tr>
<td>Joint replacements (hip, Knee)</td>
<td>Titanium, Ti-Al-V alloy, Stainless steel.</td>
</tr>
<tr>
<td>Bone plate for fracture fixation</td>
<td>Stainless steel, cobalt-chromium alloy</td>
</tr>
<tr>
<td>Bone cement</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>Artificial tendon and ligament</td>
<td>Teflon, Dacron</td>
</tr>
<tr>
<td>Dental implant for tooth fixation</td>
<td>Titanium, Ti-Al-V alloy, Stainless steel. Titanium, alumina, calcium phosphate</td>
</tr>
<tr>
<td><strong>Cardiovascular system</strong></td>
<td></td>
</tr>
<tr>
<td>Heart valve</td>
<td>Stainless steel, carbon, reciprocated tissue</td>
</tr>
<tr>
<td>Catheter</td>
<td>Teflon, silicone rubber, polyurethane</td>
</tr>
<tr>
<td><strong>Organs</strong></td>
<td></td>
</tr>
<tr>
<td>Artificial kidney</td>
<td>Cellulose, polyacrylonitrile</td>
</tr>
<tr>
<td>Skin repair template</td>
<td>Silicone-collagen composite</td>
</tr>
<tr>
<td><strong>Senses</strong></td>
<td></td>
</tr>
<tr>
<td>Contact lens</td>
<td>Silicone-acrylate, hydrogel</td>
</tr>
<tr>
<td>Intraocular lens</td>
<td>Poly(methyl methacrylate), hydrogel, silicone rubber</td>
</tr>
</tbody>
</table>

Implantation of materials and devices in animals provides valuable information regarding interactions with blood compatibility, acute and chronic, as well as local and systemic inflammation sensitization. Table 1.5 illustrates a testing matrix based on FDA and ISO guidelines. To determine which tests should be conducted, the intended use (external, internal), type of tissue contacted, and duration (transient, short-term, or long-term) of contact must be specified.
Table 1.5- FDA/ISO test matrix^6

<table>
<thead>
<tr>
<th>Device Categories</th>
<th>Initial Evaluation</th>
<th>Supplemented Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact Duration</td>
<td>Cytotoxicity</td>
</tr>
<tr>
<td>Bone Contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue, Bone Dentine Communicating</td>
<td>A</td>
<td>●</td>
</tr>
<tr>
<td>Bone/Tissue</td>
<td>A</td>
<td>●</td>
</tr>
<tr>
<td>Blood</td>
<td>A</td>
<td>●</td>
</tr>
</tbody>
</table>

●: FDA and ISO evaluation tests; o: Additional tests required by the FDA.

A: Limited exposure (≤24 hours); B: Prolonged exposure (24 hours-30 days); C: Permanent Contact (>30 days).

**A. Historical Background**

Use of biomaterials did not become practical until the advent of the aseptic surgical technique developed by Dr. J. Lister in the 1860s. Earlier surgical methods involved in tissue replacement were generally unsuccessful as a result of infection. The earliest successful implants, as well as a large fraction of modern implants, were in the skeletal system. Bone plates were introduced in the early 1900s to aid in long bone fracture. Many of these implants did not work well due to poor material properties and poor design. Several improvements and research studies have been
done to get better performance of the implant structure. Table 1.6 shows the notable developments of implant research and applications.\textsuperscript{7}

**Table 1.6 - Notable development relating to implants**

<table>
<thead>
<tr>
<th>Year</th>
<th>Investigators</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late 18th-19th century</td>
<td>-</td>
<td>Various metal devices to fix bone fractures; wires and pins from Fe, Au, Ag and Pt</td>
</tr>
<tr>
<td>1860-1870</td>
<td>J. Lister</td>
<td>Aseptic surgical technique</td>
</tr>
<tr>
<td>1866</td>
<td>H. Hansmann</td>
<td>Ni-plated steel bone fracture plate</td>
</tr>
<tr>
<td>1912</td>
<td>W. D. Sherman</td>
<td>Vanadium steel plates first developed for medical use</td>
</tr>
<tr>
<td>1924</td>
<td>A. A. Zierold</td>
<td>Introduced Stellites (CoCrMo alloy)</td>
</tr>
<tr>
<td>1931</td>
<td>M. N. Smith-Peterson</td>
<td>First femoral neck fracture fixation device made of stainless steel</td>
</tr>
<tr>
<td>1936</td>
<td>C. S. Venable, W. G Stuck</td>
<td>Introduced Vitallium (19-9 stainless steel), later changed the material to CoCr alloy</td>
</tr>
<tr>
<td>1938</td>
<td>P. Wiles</td>
<td>First total hip replacement</td>
</tr>
<tr>
<td>1946</td>
<td>J. and R. Judet</td>
<td>First biomechanically designed femoral head replacement prosthesis. First plastic (PMMA) used in joint replacement</td>
</tr>
<tr>
<td>1947</td>
<td>J. Cotton</td>
<td>Introduced Ti and its alloy</td>
</tr>
</tbody>
</table>

The development and application of surgical and orthopedic implants made it necessary to give increasing attention to the materials used to manufacture such implants. These materials, which are also referred to as biomaterials, must meet certain chemical, physical and biological requirements, in order to ensure optimum and lasting function of implants and success of the implantation procedure. No material implanted in living tissues is inert; all materials elicit a response from the host tissue. The response that occurs at the tissue-implant interface depends on factors like composition of implant, phases in implant, surface morphologies, surface porosity, chemical reactions, closeness of fit and mechanical load factors. There are four general types of implant-tissue response as summarized in Table 1.7.
Table 1.7- Consequences of Implant-Tissue Interactions

<table>
<thead>
<tr>
<th>Implant-Tissue Reaction</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic</td>
<td>Tissue dies</td>
</tr>
<tr>
<td>Biologically nearly inert</td>
<td>Tissue forms a non-adherent fibrous capsule around the implant</td>
</tr>
<tr>
<td>Bioactive</td>
<td>Tissue forms an interfacial bond with the implant</td>
</tr>
<tr>
<td>Dissolution of implant</td>
<td>Tissue replaces implant</td>
</tr>
</tbody>
</table>

B. Different types of biomaterials

Synthetic materials currently used for biomedical applications include ceramics, polymers, and, metals and alloys. Because the structures of these materials differ, they have different properties and, therefore, different uses in the body. Sometimes a combination of these materials is used as a composite structure.

Ceramic and Glass Biomaterials

Ceramics and glasses are used as components of hip implants, dental implants and heart valves. They are also designed and fabricated for repair and reconstruction of diseased, damaged or “worn out” parts of the body. Ceramics used for this purpose are called bioceramics. Some of the ceramics that have been used for biomedical applications are listed in Table 1.8. Overall, however, these biomaterials have been used less extensively than metals or polymers. The major drawback of ceramics and glasses is that they fail with little, if any, plastic deformation, and they are sensitive to the presence of cracks or other defects. Although they can have outstanding strength loaded in compression, they fail at low stress when loaded in tension or bending. Ceramics and glasses do not undergo corrosion, but they are susceptible to other forms of degradation when exposed to a physiological environment. The mechanism and rate of degradation, however, depend on the particular type of ceramic. Even alumina, which is considered a bioinert ceramic, experienced a time-dependent decrease in strength during
immersion in saline in vitro and after implantation. This process may result from a preferential dissolution of impurities that result in crack propagation. Bioactive ceramics and glasses are also degraded in the body. Because of the similarity of calcium phosphates to the mineral component of bone, bioactive ceramics may also be resorbed by osteoclasts (the cells that break down bone).

Table 1.8- Ceramics Used in Biomedical Applications

<table>
<thead>
<tr>
<th>Ceramic</th>
<th>Chemical Formula</th>
<th>Types of Attachment</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumina</td>
<td>Al₂O₃</td>
<td>Mechanical interlock (morphological fixation)</td>
<td>Bioinert</td>
</tr>
<tr>
<td>Zirconia</td>
<td>ZrO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrolytic carbon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioglass</td>
<td>Na₂OCaOPO₄O₃-SiO</td>
<td>Interfacial bonding with tissues (Bioactive fixation)</td>
<td>Bioactive</td>
</tr>
<tr>
<td>Hydroxyapatite (sintered</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>Ingrowths of tissues in pores (Biological fixation)</td>
<td>Biodegradable</td>
</tr>
<tr>
<td>high temperature)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite (sintered</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low temperature)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium phosphate</td>
<td>Ca₃(PO₄)₂</td>
<td>Replacement with tissues</td>
<td></td>
</tr>
</tbody>
</table>

The mechanical properties of calcium phosphates and bioactive glasses make them unsuitable as load-bearing implants. Table 1.9 shows some of the mechanical properties of ceramic biomaterials. Among the biomedical ceramics, alumina has the best mechanical properties, but its tensile properties are still below those of metallic biomaterials. Additional advantageous properties of alumina are its low coefficients of friction and wear rate. Because of these properties, alumina has been used as a bearing surface in joint replacements.

Table 1.9- Mechanical Properties of Ceramic Biomaterials

<table>
<thead>
<tr>
<th>Bioceramics</th>
<th>Young’s Modulus E (GPa)</th>
<th>Compressive Strength, σUCS (MPa)</th>
<th>Tensile Strength, σUCS (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumina</td>
<td>380</td>
<td>4500</td>
<td>350</td>
</tr>
<tr>
<td>Bioglass-ceramic</td>
<td>22</td>
<td>500</td>
<td>56-83</td>
</tr>
<tr>
<td>Calcium Phosphate</td>
<td>40-117</td>
<td>510-896</td>
<td>69-193</td>
</tr>
<tr>
<td>Pyrolytic carbon</td>
<td>18-28</td>
<td>517</td>
<td>280-560</td>
</tr>
</tbody>
</table>
Clinically, hydroxyapatite has been used as filler for bone defects and as an implant in load-free anatomic sites. In addition, hydroxyapatite has been used as a coating on metallic orthopedic and dental implants to promote their fixation in bone.

**Polymeric Biomaterials**

Polymers are mostly widely used materials in biomedical applications. They are the materials of choice for cardiovascular devices as well as for replacements and augmentation of various soft tissues. Current applications include vascular grafts, heart valves, artificial hearts, contact lenses, intraocular lenses, sutures, adhesives. Table 1.10 shows some of the polymers and their uses.

**Table 1.10- Examples of Biomedical Applications of Polymers**

<table>
<thead>
<tr>
<th>Applications</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular implants</td>
<td>Polyethylene, Polyvinyl chloride (PVC), polyester, silicone rubber, polytetrafluoroethylene</td>
</tr>
<tr>
<td>Orthopedic implants</td>
<td>Ultra-high-molecular-weight polyethylene (UHWMPE), polymethylmethacrylate</td>
</tr>
<tr>
<td>Drug release</td>
<td>Polylactide-co-glycolide</td>
</tr>
<tr>
<td>Tissue engineering</td>
<td>Polylactic acids, polyglycolic acid, Polylactide-co-glycolide</td>
</tr>
</tbody>
</table>

Polymers may contain various additives, traces of catalysts, inhibitors, and other chemical compounds needed for their synthesis. Over time in the physiological environment, these compounds can leach from the polymer surface. Chemicals released from polymers may induce adverse local and systemic host reactions that cause clinical complications. This release is concern for materials, such as bone cements, that are polymerized in the body and for flexible polymer such as PVC that contain low-molecular-weight (plasticizers) species to make them pliable.
The mechanical properties of polymers depend on several factors, including the composition and structure of the macromolecular chains and their molecular weight. Compared to ceramic and metals, polymers have much lower strength but they can be deformed to a greater extent before failure. Table 1.11 shows some of the mechanical properties of selected polymeric biomaterials. Ultra high molecular weight polyethylene is used for bearing surface in hip and knee replacements.

**Table 1.11 - Mechanical Properties of Polymers**

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Tensile strength $\sigma_{UTS}$ (MPa)</th>
<th>Young’s Modulus $E$ (GPa)</th>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymethylmethacrylate</td>
<td>30</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Nylon 6/6</td>
<td>76</td>
<td>2.8</td>
<td>90</td>
</tr>
<tr>
<td>Polylactic acid</td>
<td>28-50</td>
<td>1.2-3</td>
<td>2-6</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>28-36</td>
<td>1.1-1.55</td>
<td>400-900</td>
</tr>
<tr>
<td>Polytetrafluoroethylene</td>
<td>17-28</td>
<td>0.5</td>
<td>120-350</td>
</tr>
<tr>
<td>Silicone rubber</td>
<td>2.8</td>
<td>Up to 10</td>
<td>160</td>
</tr>
</tbody>
</table>

**Metallic Biomaterials**

Metals have been used almost exclusively for load-bearing implants, such as hip and knee prostheses and fracture fixation wires, pins, screws and plates. Metals have also been used as parts of artificial heart valves, as vascular stents, and as pacemaker leads. Although pure metals are sometimes used, alloys (metals containing two or more elements) frequently provide improvement in materials properties, such as strength and corrosion resistance. Three material groups dominate biomedical metals: 316L stainless steel, cobalt-chromium-molybdenum alloy, and pure titanium and titanium alloys (Table 1.12). The main considerations in selecting metals and alloys for biomedical applications are biocompatibility, appropriate mechanical properties, corrosion resistance, and reasonable cost.
Table 1.12 - Surgical Implant alloy Compositions (wt %)\(^6\)

<table>
<thead>
<tr>
<th>Element</th>
<th>316L Stainless Steel (ASTM F138, 139)</th>
<th>Co-Cr-Mo (ASTM F799)</th>
<th>Grade 4 Ti (ASTM F67)</th>
<th>Ti-6Al-4V (ASTM F136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.5-6.5</td>
</tr>
<tr>
<td>C</td>
<td>0.03 max</td>
<td>0.35 max</td>
<td>0.010 max</td>
<td>0.08 max</td>
</tr>
<tr>
<td>Co</td>
<td>-</td>
<td>Balance</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cr</td>
<td>17.0</td>
<td>26.0-30.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>Balance</td>
<td>0.75 max</td>
<td>0.30-0.50</td>
<td>0.25 max</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>-</td>
<td>0.0125-0.015</td>
<td>0.0125 max</td>
</tr>
<tr>
<td>Mo</td>
<td>2.00</td>
<td>5.0-7.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>2.00 max</td>
<td>1.0 max</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>-</td>
<td>0.25 max</td>
<td>0.03-0.05</td>
<td>0.05 max</td>
</tr>
<tr>
<td>Ni</td>
<td>10.00</td>
<td>1.0 max</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>-</td>
<td>-</td>
<td>0.18-0.40</td>
<td>0.13 max</td>
</tr>
<tr>
<td>P</td>
<td>0.03 max</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>0.03 max</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Si</td>
<td>0.75 max</td>
<td>1.0 max</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ti</td>
<td>-</td>
<td>-</td>
<td>Balance</td>
<td>Balance</td>
</tr>
<tr>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.5-4.5</td>
</tr>
<tr>
<td>W</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The mechanical properties make metals the appropriate choice for many biomedical applications, susceptibility to chemical degradation is an aspect that must be considered. The physiological environment is typically modeled as a 37 °C aqueous solution, at pH 7.3, with dissolved gases (such as oxygen), electrolytes, cells, and proteins. Immersion of metals in this environment can lead to corrosion, which is deterioration, and removal of the metal by chemical reactions. During the electrochemical process of corrosion, metallic biomaterials can release ions which may reduce the biocompatibility of materials and jeopardize the fate of implants. For example, the type and concentration of released corrosion products can alter the functions of cells in the vicinity of implants as well as of cells at remote locations after transport of the corrosion by-products to distant sites inside the body. Even before implantation, via chemical reaction of metals with oxygen in ambient air or by oxidation in an acidic solution, an oxide surface film forms on their surface. Because oxides are ceramics which are electrical and thermal insulators,
then electrochemical reactions that lead to corrosion are reduced or prevented. In other words, oxidized metallic surfaces are “passivated". In fact, the stability of the oxides present on different metals determines their overall corrosion resistance. For example, even though 316L stainless steel implants perform satisfactorily in short-term applications, such as fracture fixation, they are susceptible to crevice corrosion and pitting when implanted for longer periods. Titanium and its alloys, as well as cobalt-chromium alloys, have favorable corrosion resistance for long-term implant application such as joint and dental prostheses.

The mechanical properties of materials are of great importance when designing load-bearing orthopedic and dental implants. Mechanical properties of metallic biomaterials are listed in Table 1.13. With a few exceptions, the high tensile and fatigue strength of metals, compared to ceramics and polymers, make them materials of choice for implants that carry mechanical loads.

**Table 1.13- Select Properties of Metallic Biomaterials**

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s Modulus E (GPa)</th>
<th>Yield Strength σy (MPa)</th>
<th>Tensile Strength σUTS (MPa)</th>
<th>Fatigue Limits σend (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless steel</td>
<td>190</td>
<td>221-1,213</td>
<td>586-1,351</td>
<td>241-820</td>
</tr>
<tr>
<td>Co-Cr alloys</td>
<td>210-253</td>
<td>448-1,606</td>
<td>655-1,896</td>
<td>207-950</td>
</tr>
<tr>
<td>Ti</td>
<td>110</td>
<td>485</td>
<td>760</td>
<td>300</td>
</tr>
<tr>
<td>Ti-6Al-4V</td>
<td>116</td>
<td>896-1,034</td>
<td>965-1,103</td>
<td>620</td>
</tr>
<tr>
<td>Cortical bone</td>
<td>15-30</td>
<td>30-70</td>
<td>70-150</td>
<td></td>
</tr>
</tbody>
</table>

The elastic moduli of the metals listed in Table 1.13 are at least seven times greater than that of natural bone. This mismatch of mechanical properties can cause stress-shielding of either condition characterized by bone resorption (loss of bone) in the vicinity of implants. Compared to the elastic moduli of either stainless or cobalt-chromium molybdenum alloys, Ti and Ti-6Al-4V have much lower moduli that are still almost an order of magnitude higher that of bone. Another advantage of Ti-based metals as bone implants is their favorable strength-to-density ratio. Stainless steel and Co-Cr alloys have densities of approximately 8.8 g/cm³ and 7.8 g/cm³,
respectively. Because Ti has a density of 4.5 g/cm³, its strength-to-density ratio is larger. Disadvantages of titanium for medical use include relatively low shear strength, poor wear resistance, and difficulties in fabrication. The stable, coherent titanium oxide (TiO₂) film that forms on titanium and its alloys gives them superior corrosion resistance compared with stainless steel and Co-Cr alloys. The oxidized surface is also believed to be responsible for Ti implants becoming osseointegrated \textit{in vivo}, a process whereby bone is aposed to the implant without chronic inflammation and without an intervening fibrous capsule.

1.4. Metal-Protein-surface Interactions

The behavior of proteins at surfaces plays a vital role in determining the nature of the tissue-implant interface. Adsorbed protein can affect bacterial and cell adhesion. Table 1.14 below shows some important interactions of protein with surfaces.

\textbf{Table 1.14-} Properties of surfaces that affect their interaction with protein

<table>
<thead>
<tr>
<th>Feature</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topography</td>
<td>Greater textures exposes more surface area for interaction with proteins</td>
</tr>
<tr>
<td>Composition</td>
<td>Chemical makeup of a surface will determine the types of intermolecular forces governing interaction with protein</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>Hydrophobic surfaces tend to bind more protein</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>Nonuniformity of surface characteristics results in domain that can interact differently with proteins</td>
</tr>
<tr>
<td>Potential</td>
<td>Surface potential will influence the distribution of ions in solution and interaction with proteins.</td>
</tr>
</tbody>
</table>

Surface properties, are grouped into three categories: geometric, chemical and electrical. Substrates with more topographical features will expose more surface area for possible interaction with proteins. Surfaces with grooves or pores have greater surface areas compared to smooth surfaces. Other surface features, such as machine marks introduced during processing, provide additional sites for protein interaction. The surface chemical composition will determine
which functional materials are available for interaction with biomolecules. The oxidized surface of a metallic biomaterial exposes metal and oxygen ions. Similarly, ceramic, and some glass, surfaces include metal and nonmetal ions. A variety of functional materials, such as amino, carbonyl, and carboxyl groups, can be present on polymeric materials. Depending on which species are exposed, biomolecules may have different affinities for various surfaces. The surface potential influences the structure and composition of the electrolyte solution adjacent to the biomaterial.
CHAPTER TWO

2.1. Ti alloys

Titanium use for implant fabrication dated to the late 1930s. There are four grades of unalloyed commercially pure titanium for surgical implant application in Table 2.1. The impurity contents separate them; oxygen, iron and nitrogen must be controlled carefully. Oxygen has great influence on ductility and strength.

Table 2.1- Chemical composition of Titanium and its alloys [American Society for testing and Materials, F67-89, p39; F136-84, p55, 1992]

<table>
<thead>
<tr>
<th>Element</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Ti6Al4V*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.0125</td>
</tr>
<tr>
<td>Iron</td>
<td>0.20</td>
<td>0.30</td>
<td>0.30</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.18</td>
<td>0.25</td>
<td>0.35</td>
<td>0.40</td>
<td>0.13</td>
</tr>
<tr>
<td>Titanium</td>
<td>Balance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Aluminum 5.5-6.5%, Vanadium 3.5-4.5% and other elements 0.1% maximum or 0.4% total. All are maximum allowable weight percent.

Titanium is an allotropic material, which exists as a hexagonal closely packed structure 882 °C, and body-centered cubic structure above that temperature. Titanium alloys can be strengthened and mechanical properties varied by controlled composition and thermomechanical processing techniques. The mechanical properties of the commercially pure titanium and its alloys are given in Table 2.2.

Table 2.2- Mechanical properties of Ti and its alloys (ASTM F136) [American Society for Testing and Materials, F67-89, F136-84, p.55, 1992 and (Davidson et al., 1994]

<table>
<thead>
<tr>
<th>Properties</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Ti6Al4V</th>
<th>Ti13Nb13Zr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength (MPa)</td>
<td>240</td>
<td>345</td>
<td>450</td>
<td>550</td>
<td>860</td>
<td>1030</td>
</tr>
<tr>
<td>Yield strength (0.2% offset)(MPa)</td>
<td>170</td>
<td>275</td>
<td>380</td>
<td>485</td>
<td>795</td>
<td>900</td>
</tr>
<tr>
<td>Elongation (%)</td>
<td>24</td>
<td>20</td>
<td>18</td>
<td>15</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Reduction of area (%)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>25</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>
The above table shows that higher impurity content of the cp-Ti leads to higher strength and reduced ductility.

Titanium implant surfaces consist of a thin oxide layer and the biological fluid of water molecules, dissolved ions, and biomolecules (proteins with surrounding water shell) as shown in Figure 2.1.

Figure 2.1. (a) Interface between a titanium implant and bioliquid and (b) the cell surface interaction

The microarchitecture (microgeometry, roughness, etc.) of the surface and its chemical composition are important for the following reasons:
1. Physical nature of the surface either at the atomic, molecular, or higher level relative to the dimensions of the biological units may cause different contact areas with biomolecules, cells, etc. The different contact areas, in turn, may produce different perturbations and types of bonding of the biological units, which may influence their conformation and function.

2. Chemical compositions of the surface may produce different types of bonding to the biomolecules, which may then also affect their properties and function. Metals undergo chemical reactions at the surface depending on the environment, which can cause the difficulties for understanding the nature of the interactions.

The surface-tissue interaction is dynamic rather than static, i.e., it will develop into new stages as time passes, especially during the initial period after implantation. During the initial few seconds after implantation, there will be only water, dissolved ions, and free biomolecules in the closest proximity of the surfaces but no cells. The composition of the biofluid changes which in turn probably causes changes in the composition of the adsorbed layer of biomolecules on the implant surface until quasi-equilibrium sets in. Cells and tissues will approach the surface and, depending on the nature of the adsorbed layer, they will respond in specific ways that may further modify the adsorbed biomolecules. The type of cells closest to the surface and their activities will change with time. Initial interaction may result in fibrous tissue surrounding the implant surface.
2.2 Literature review

One of the major concerns for biomedical devices made of Ti is that it gets encapsulated after implantation into the living body by a fibrous tissue that isolates them from the surrounding bone, because Ti is a bioinert material.

Figure 2.2. As-machined Ti implant, sections after 84 days (v. Kossa/Paragon stain): (a) overview, position of the implant; (b) thin frame of bone; (c) different density of soft tissue, only some macrophages at the interface; no attachment between bone and implant surface; border of a cyst-like structure in the upper part of this figure.8

Bone tissue can form on a titanium surface with a very thin cementum layer in between.9-10 This phenomenon has been named osseointegration first introduced by Branemark.11-12 It takes several months for titanium implants and bone tissue to reach integration. Hence, there is a growing interest in shortening the process toward osseointegration, thereby reducing surgical restrictions. Researchers all over the world have been trying to develop surface modification of the implant for faster osseointegration. Pre-oxidation of titanium surface, either thermal or anodic, can accelerate the bone bonding process considerably. It was believed that it is the titanium oxide on
the titanium surface that induces formation of Ca-P layer and eventually achieves osseointegration. Osseointegration provides a firm fixation between titanium implant and bone. Various surface modifications, including chemical treatment\textsuperscript{13-15}, thermal treatment\textsuperscript{16}, electrochemical methods\textsuperscript{17} and anodization\textsuperscript{18, 19} have been applied to form a bioactive titanium oxide layer on the metal surface. In chemical treatment processes the Ti-substrate is immersed in NaOH or H\textsubscript{2}O\textsubscript{2} solution for a few hours. The chemical treatment results in oxide coating on Ti-substrate. One of the major problem is that the coating thickness is not uniform and morphology of the surface cannot be controlled over a wide range. Thermal Treatment Ti-alloys are heat-treated at different temperatures. These kinds of heat treatment resulted in different phases of the oxide. It is reported that anatase is more pronounced toward mineralization than the rutile phases. It is difficult to control the phases. Moreover the oxide morphology is not uniform over a wide area of the surface. In some cases Ti-6Al-4V is ion implanted with Mg or Zn ions to a nominal dose of 1x 10\textsuperscript{7} ionscm\textsuperscript{-2} using a metal vapor vacuum arc ion source by ion-beam implantation.\textsuperscript{20}

To modify the Ti-surface the surface is coated with Ca and P sol via sol-gel technique. Ca and P precursor solutions are prepared from ethanol solution of Ca(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O and P\textsubscript{2}O\textsubscript{5}. Ti6AL4V substrates are dipped into the sols and withdrawn at a rate of 8 cm/min. The films are dried at 150 °C for 15 min and then heated at 600 °C for 15 min. The dipping coat procedures are repeated 5 times. The sol-gel process requires lot of parameters to be optimized like different precursors, dipping techniques and post heat treatment to get a particular morphology on the Ti-substrate.\textsuperscript{21} It also shows weak interfacial strength and poor control over thickness. Evaluation of the above processed films, in simulated body fluid (SBF), demonstrated that apatite deposition depends on chemical composition, surface properties, concentration of the electrolytes and crystalline phases present in the film.\textsuperscript{22} Anodization approach is able to build a porous as well as
nonporous titanium oxide film of controllable pore size, good uniformity and conformability over large areas at low cost. These oxides are in situ and they will show higher interfacial strength on the metal substrate than on the coated surface. The morphology of the oxide surface can be controlled by electrolyte solutions or anodization voltage, and will eventually help to control the cellular adherence on the surface. It has been shown that micro-arc oxidized and hydrothermal synthesized surfaces have higher cell adhesion and proliferation rates than do untreated Ti-surface. Several in vitro and in vivo studies have shown that modified surfaces have a higher early level of cell attachment than a untreated Ti-surface. Hydrothermal treatment of anodized Ti-substrate with calcium glycerophosphate and calcium acetate as electrolytes also led to higher protein production than untreated Ti-surface. Also shown that titanium oxide surface had greater number of osteoblasts with higher cell activity than bare Ti surface. Another approach to modify the Ti surface was to coat with bioceramics, e.g. hydroxyapatite and/or TiO₂, using plasma spray or alternative processes. For these coatings typically a bioactive ceramic such as hydroxyapatite (HA) powders is heated at extremely high temperatures and deposited with high velocity on the metal surface. The coating quality, composition and crystallinity are difficult to control. Typical plasma sprayed HA ceramic coatings are porous with low mechanical strengths, fracture toughness and weak interfacial shear strength. Therefore, the tendency for cracking or peeling/flaking off under the influence of bending or shearing forces is higher and detachments at the metal-coating interface are often present. Lack of optimal performance in the long term of such coatings has been related to adhesion problems of the deposits to substrates. As a result, researchers are still trying to investigate alternative ways to ensure faster cell-materials adhesion with various surface modifications.
Anodic oxidation is a relatively simple and well-established process that is known for its industrial viability. Implant design (macro scale) and aspects related to the surface properties in micro- and nano-metric scale can affect osseointegration. Anodic oxidation is especially useful for an implant where fast healing is required. Anodic oxidized Ti-substrate in electrolytes of $\text{H}_3\text{PO}_4$, $\text{H}_2\text{SO}_4$ and acetic acid shows bone healing by a gradual mineralization process. Further thermal treatment of these oxides retreats a more pronounced effect in the apatite deposition process.\textsuperscript{17} Crystalline phases of the anodized \textit{in situ} oxides enhanced the mineralization process.\textsuperscript{31-33} Microstructural topography, chemical composition and surface properties of the oxide plays an important role in the interactions of bone cells with implant surfaces. In some cases it was noticed that anodization in a mixture of sodium β glycerophosphate and calcium acetate at high voltage resulted microporous topography.\textsuperscript{31} Heat treatment of these anodized surfaces at 300 °C showed higher bone to implant contact than the untreated Ti-surface.\textsuperscript{31} It was also noticed that phenotypic expression of osteoblasts was enhanced by anodization in sodium glycerolphosphate and calcium acetate electrolytic solutions and higher bone contact in anodized hydrothermal treated samples.\textsuperscript{33-34}

\textbf{2.3 Objectives and Research Plan}

The objective of this research was to modify commercially pure Ti (Cp-Ti) surfaces to improve cell-materials interactions on surfaces and faster osseointegration. Titanium oxide layers were grown on a Ti-surface via anodization using different acid electrolytes. Oxide layer growth varied from 200 to 400 nm in thickness. The \textit{in situ} oxide film had higher interfacial strengths than the as-coated oxide film on Ti-substrates. The research has been focused on three different areas:
1) **Nonporous oxide layer**: Titanium oxide layer was grown on a Ti-surface by the anodization process using H$_2$SO$_4$, HF and H$_3$PO$_4$ electrolytes at anodized voltages of 20 V. Oxide layers different surface morphology and significantly different cell interaction at the surface.

2) **Nanoporous oxide layer**: Titania nanotubes were grown on a Ti-surface via the anodization process, using citric acid, sulfuric acid and sodium fluoride as constituents of electrolytic solutions. Anodized voltages were kept constant at 20V and time was varied from 2h to 10h. Nanotube surfaces revealed enhanced cell interactions.

3) **Study of biological and surface properties**: Biological properties of these anodized surfaces have been evaluated with an *in vitro* osteoblast precursor cell line (OPC1) and in simulated body fluid (SBF) for 3, 7, 14 and 21 days. Although non-uniform apatite precipitation was observed on substrates for all anodized surfaces after incubation in SBF, each surface gave rise to different cellular responses due to variations in surface properties. Protein expression with vinculin molecules and enzymatic behavior with alkaline phosphatase both were evaluated on different morphology at nonporous and nanoporous surfaces. Surface properties like roughness, surface energy and contact angles were also evaluated on the nonporous and nanoporous surfaces. Characterization of surface properties was needed to relate important surface characteristics to biological responses.
3.1 Sample preparation

Thin titanium (from Supra alloys, CA) sheets of 0.5 mm thickness and 99.8% purity were used for fabrication of titania nanotubes. Circular samples of 12 mm diameter were machined using a waterjet. Each of these samples was abraded with SiC paper in successive grades from 400, 600 to 1200 grit (Leco Corporation, MI) and then ultrasonically cleaned in distilled water and dried. The final polishing of the sample was performed with a cotton polishing cloth with 1 μm alumina suspension. Polished titanium specimens were ultrasonically cleaned in distilled water followed by isopropanol prior to anodization. The polished commercial material was used as control (Ti).

![Schematic of the experimental set-up used for anodization process.](image)

**Figure 3.1** Schematic of the experimental set-up used for anodization process.

**Electrolytes**

H$_2$SO$_4$, (Fischer Scientific), 1 (N) phosphoric acid (H$_3$PO$_4$, Fischer Scientific) and 0.25 (N) hydrofluoric acid (HF, J. T. Baker). Hydrofluoric acid from 0.25 (N) to 1 (N) was initially tried.
A high concentration from 0.5 to 0.75 (N) lead to high corrosion and the anode became thinner with increase in concentration. At 1(N) HF the anode was completely dissolved in the electrolytic solution. In the case of hydrofluoric acid all the experiments was performed in 0.25 (N). Anodization time was also varied from 2 min to 60 minutes. Samples anodized for 60 minute show complete coverage with an oxide surface. Different experimental parameters are shown in Table 3.1.

Table 3.1- Experimental parameters

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Concentrations (N)</th>
<th>pH</th>
<th>Volt</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂SO₄</td>
<td>0.5, 1, 2</td>
<td>0.43-0.45</td>
<td>5,10,20</td>
<td>2, 15, 30, 45, 60</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>0.5, 1, 2</td>
<td>1.23</td>
<td>5,10,20</td>
<td>2, 15, 30, 45, 60</td>
</tr>
<tr>
<td>HF</td>
<td>0.15, 0.25,0.5</td>
<td>2.72</td>
<td>5,10, 20</td>
<td>2, 15, 30, 45, 60</td>
</tr>
</tbody>
</table>

A fourth electrolyte was made by dissolving sodium fluoride (NaF, Sigma-Aldrich), citric acid (J.T. Baker), 1(M) sulfuric acid (Fischer Scientific) in a ratio such that the final electrolyte components had F⁻: 0.1mol/L, SO₄²⁻: 1.0 mol/L and citric acid: 0.2 mol/L.

The electrolyte pH was adjusted to 4.5 using NaOH solution. A fourth electrolyte named electrolyte-C showed nanoporous morphology. To understand this particular nanoporous morphology different compositions were also tested and are shown later in the 5.1B result section of the thesis. Different calculations are shown below.
Figure 3.2 Samples after anodization in different electrolytic solution (a) H$_2$SO$_4$, (b) H$_3$PO$_4$, (c) HF and (d) 0.1 mole/L NaF, 1.0 mole/L H$_2$SO$_4$, 0.2 mole/L of citric acid.
Calculations

NaF: 0.1 mole/L=0.42gm
H₂SO₄: 1 mol/L=5.5 cc of stock directly in electrolyte solution (36 N H₂SO₄ stock)
  0.1 mol/L= 10 cc from solution A (A: 5.5 cc of stock + 94.5 cc of D.I water)
H₃PO₄: 1 mole/L=11.1 cc of stock directly in electrolyte solution (26 N H₃PO₄)
  0.1 mole/L= 10 cc of solution B (11.1 cc of stock +88.9 cc of D.I. water)
CH₃COOH: 1 mole/L=7.5 cc of stock directly in electrolyte solution (13.33 M stock)
  0.1 mole/L= 10 cc of solution C (7.5 cc of stock + 92.5 cc of D. I. water).

Calculation CH₃COOH (Liquid)
F.W: 60
Stock 80%; Strength of stock: 13.33 (M)
1 mole= 60 gm
60 gm is equal to 1 mole. Specific gravity=1.04
80 gm (as it says 80%) is equal to 80/60 mole present in 100cc of stock solution
1000 cc of stock contains 80/60 x10=800/60=13.3 mole/L=13.33M is the strength of stock.
Reqd.: 1 mole/L; present in stock: 13.33 mole /L of stock. Wants to prepare 100 cc of electrolyte
13.33 x p =1x100
p=7.5cc of stock needed to add in 100 cc of electrolyte solution.

Calculation for NaF (Solid)
F.W :42g
1000cc NaF reqd. in 0.1 (M)
100cc NaF reqd in 0.01(M)
1(M) of NaF corresponds to 42g
0.01(M) of NaF corresponds to 0.42g
to get 0.1mole/L NaF=0.42g NaF added in 100 cc of electrolytic solution.

Calculation for H₂SO₄ (Liquid)
Stock Strength: 18 (M)
1(M) in 1000/18=55 cc stock
1 mole/L=1(M) in 1000 cc we have to add 55 cc of stock in 945 cc of water.
To get 1 mole/L in 100cc
To get 1 mole/L in 100 cc we have to add 5.5 cc of stock in 94.5 cc of water.

Calculation for H₃PO₄ (Liquid)
F.W: 98
Stock 85%; Strength: 8.67 (M)
1 mole=98gm
98 gm is equal to 1 mole
85 gm is equal to 85/98= 0.867 mole present in 100 cc of stock solution
1000 cc of stock solution contains 85/98x10=8.67 mole/L=8.67 (M) is the strength of the stock
Reqd. : 1 mole/L; Present in stock: 8.67 mole/L.
8.67xq=1/100
q=11.5 cc of stock needed to add in 100 cc of electrolyte solution.
**Calculation for Citric acid (Solid)**

F.W: 210

1(M) of citric acid corresponds to 210g in 1000cc

0.2 (M) of citric acid corresponds to 210 x 0.2 = 42 g in 1000cc

100 cc of electrolyte will have 42/1000*100 = 4.2g

To get 0.2 mole/L citric acid need to add 4.2 g in 100 cc electrolyte batch.

**Composition of 0.1 mole/L of NaF, 1.0 mole/L H₂SO₄**

Electrolyte solution with above composition was prepared from the stock solution in the lab. In 250 ml polypropylene beaker 80 ml of water is added followed by 0.42 gm of NaF and 5.5 ml of H₂SO₄ stock solution. Finally water is adjusted to make the resulted electrolyte solution to 95 ml.

Electrolyte pH was adjusted to with concentrated H₂SO₄ to make resulted solution pH close to 1.06.

For the anodization process, the electrolytic cell consisted of a three-neck round bottomed flask. A cp-Ti anode was suspended from the centre of the cell and a platinum cathode was suspended from one of the necks by a platinum wire, and a thermometer was placed through the other neck.

A dc power supply (Hewlett Packard 0-60V/0-50A, 1000W) was used to vary the applied voltage.

### 3.2. Surface Characterization and Phase Analysis

TiO₂ anodized films were characterized using a scanning electron microscope (SEM) [Hitachi’s-570] and a field emission scanning electron microscope (FESEM, FEI, SIRION, OR) fitted with an EDX. Operating voltage for both SEM and FESEM was 20kV. Energy dispersive spectroscopy (EDS) was performed to qualitatively identify the composition of the film. Oxide film thicknesses were measured in SEM from cross sectional view of the Ti-foils. Three samples were used to give the average thickness value for each anodized condition. Glancing angle x-ray diffraction (GAXRD) was conducted using a Bruker/Seimens platform system at the University
of Wisconsin, Madison. GAXRD studies were carried out from a sealed Cu tube operating at 40 KeV and 20 mA. Glancing angles of 5° and 10° were used for all the samples. Each of these samples was scanned in the 2θ range of 20 to 80°.

3.3 Surface analysis

A. Surface roughness

Surface roughness of each Ti-control and anodized samples were measured using a surface profilometer (SPN Technology, Goleta, CA). The scan was performed on each sample three times with a scan distance of 4 mm at different place of the sample. For each anodized condition three samples were used. The stylus radius was 25 μm. Roughness data 4 mm scan was based 5000 points of the tip of the profilometer. From all of these 5000 points the root mean square data (r.m.s) were calculated. The average of 9 r.m.s values (three from each sample) for each anodized conditions along with standard deviation were determined.

B. Contact angle measurement

Contact angles were measured using the sessile drop method on a face contact angle set-up equipped with a microscope and a camera. A 0.5-1.0 μl droplet of distilled water and McCoys 5A solution at pH 7.4 (cell culture medium) was suspended from the tip of the microliter syringe. The syringe tip was advanced toward the disk surface until the droplets made contact with the disk surface. Images were collected using the camera and the contact angle between the drop and the substrate was measured from the magnified image. Three samples for each anodized conditions were used to collect the contact angle data in water and cell media. For each samples again 4 data points were used.
Figure 3.3. Schematic of contact angle measurement apparatus.

Figure 3.4 Water droplets on the Ti-control surface. Contact Angle was 70°.
**C. Surface Energy**

Contact angles were determined with three different liquids to calculate surface energy. Apolar liquid diiodomethane and two polar liquids formamide and glycerol were used in the following equation to calculate the surface energy.

\[
\gamma_L(1 + \cos \theta) = 2 (\gamma_{S}^{LW} \gamma_{L}^{LW})^{1/2} + 2(\gamma_{S}^{+} \gamma_{L}^{-})^{1/2} + 2(\gamma_{S}^{-} \gamma_{L}^{+})^{1/2}
\]  

(1)

In equation (1) \( \theta \) the contact angle, of liquid L and solid S, \( \gamma_{L}^{LW} \) is the apolar component of the surface energy, \( \gamma^{+} \) is the Lewis acid component (electron acceptor) and \( \gamma^{-} \) Lewis base component (electron donor).\(^{36}\)

**Table 3.3-** Surface Tension Data (mJ/m\(^2\)) on the three contact angle liquids.\(^{36}\)

<table>
<thead>
<tr>
<th>Liquids Used</th>
<th>( \gamma_{L} ) (mJ/m(^2))</th>
<th>( \gamma_{L}^{LW} ) (mJ/m(^2))</th>
<th>( \gamma_{L}^{+} ) (mJ/m(^2))</th>
<th>( \gamma_{L}^{-} ) (mJ/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diiodomethane</td>
<td>50.8</td>
<td>50.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>64</td>
<td>34</td>
<td>3.92</td>
<td>57.4</td>
</tr>
<tr>
<td>Formamide</td>
<td>58</td>
<td>39</td>
<td>2.28</td>
<td>39.6</td>
</tr>
</tbody>
</table>

**Example for the calculation of surface energy for anodized H\(_3\)PO\(_4\) surface**

**Table 3.4-** Contact angle values of different liquids on anodized H\(_3\)PO\(_4\) surface

<table>
<thead>
<tr>
<th>Contact Angle (Degree)</th>
<th>Diiodomethane</th>
<th>Formamide</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

For diiodomethane

Inserting the contact angle values from the Table 2.4 and surface tension data from table 2.2 in equation (1)

\[
\gamma_{S}^{LW} = 50.8 (1+\cos 1^{\circ})^{2}/4
\]
\( \gamma_{LW} = 50.79 \) \hspace{1cm} (2)

Similarly inserting the contact angle values and data in equation (1) for formamide

\[ 6.3 \gamma_{S}^{+1/2} + 1.5 \gamma_{S}^{-1/2} = 13.37 \] \hspace{1cm} (3)

For glycerol

\[ 7.97 \gamma_{S}^{+1/2} + 1.97 \gamma_{S}^{-1/2} = 17.87 \] \hspace{1cm} (4)

Solving equation (3) and (4)

\[ \gamma_{S}^{+} = 0.188; \gamma_{S}^{-} = 50.30. \] \hspace{1cm} (5)

\[ \gamma_{S} = \gamma_{LW}^{S} + \gamma_{S}^{+} + \gamma_{S}^{-} \]

\[ \gamma_{H3PO4}^{S} = \gamma_{LW}^{S} + \gamma_{S}^{+} + \gamma_{S}^{-} = 50.79 + 0.188 + 50.30 \]

\[ \gamma_{H3PO4} = 101.27 \text{ mJ/m}^2 \]

Surface energy is a measure of the extent to which bonds are satisfied at the surface of a material. At the surface, however, there is an asymmetric force field which results in a net attraction of surface atoms into the bulk. This tends to deplete the surface of atoms, putting the surface in tension. When a liquid drop is placed onto a solid surface or another liquid surface two things may happen: The liquid may sit on the surface in the form of droplet, or it may spread out over the entire surface. Which event occurs depends upon the relative interfacial free energies of the two substances. The interfacial free energy is analogous to surface free energy but accounts for the interactions of the materials on either side of the interface.

3.4 Osteoblastic precursor cell line

For cell-materials interaction studies, human osteoblast cells were used. Cells were derived from an osteoblastic precursor cell line (OPC1) established from human fetal bone tissue. Cells were plated at a density of \( 10^5 /\text{cm}^2 \) in 100 mm tissue culture plates, and were cultured in McCoy’s 5A medium (with L-glutamine, without phenol red and sodium bicarbonate). Composition of the
medium is shown in Table 2.5. Cells were maintained at 37 °C under an atmosphere of 5% CO₂ and 95% air to attain the confluency. These cells were then splitted by the following steps and seeded onto each sample surface.

**Table 3.5-** Compositions of cell media for 1000 ml batch

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>900 ml</td>
</tr>
<tr>
<td>Mc Coys 5A</td>
<td>11.9 g</td>
</tr>
<tr>
<td>Sodium Carbonate</td>
<td>2.2 g</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Penicilin</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Bovine Calf Serum</td>
<td>50 ml</td>
</tr>
<tr>
<td>Fetal Calf Serum</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

**A. Seeding cells onto the surface to begin experiment**

Old medium onto the confluent culture plate was aspirates off. The plate was rinsed with phosphate based buffered saline and then 2 mL of trypsin enzyme was added onto the plate and incubated for 3-5 minutes. Trypsin helps in digesting the attachment proteins of the cell from the culture plate. Fresh 6 mL of the McCoy’s 5A medium was added to inactivate the trypsin. Different steps of cell splitting were shown in **Figure 3.5**. Part of the trypsinzed cells were used to seeded on the surface of the sample and another part was kept for future use in a cryogenic atmosphere.
Figure 3.5. (a) Nonconfluent state- cells were thawed and were less in number, (b) Confluent state- cell increase in number and are dense, (c) Trypsinized state- cells detach from the surface of the culture plate.

The whole 8 mL solution with cell suspension was transferred to a conical vial. Approximately 7.5 ml of cell suspensions were spread under the cover slip on each side of the haemocytometer. There were 8 grids in haemocytometer and cells were counted on each of the grids using. The following calculations were performed to find the volume of cell suspension needed on each of the samples.
Calculations to determine the volume of the cell suspension

Number of cells/8 grids = A

A \times (1.0 \times 10^4) = B, \text{cell count, cells/ml}

For 12-well plate: \((2 \times 10^4)/B\) ml of the volume of cell suspension to seed onto each substrate in each well.

For 100 mm plate: \((1 \times 10^5)/B\)

**Example of the calculation** is provided below:

No. of cells counted/grid = 459

No. of cells counter/grids = 459/8 = 57.375 = A

57.375 \times (1 \times 10^4) = B

For 100 mm plate: \((1 \times 10^4) / (57.375 \times 10^4) = 0.174\ \text{ml} = 174\ \mu\text{l}

Anodized samples were sterilized by autoclaving (Amerex Instruments Ltd, CA) for 20 minutes at 121 °C before cell culture. OPC1 cells were seeded onto the sterilized anodized titania surface. Culture medium was changed every 2-3 days for the duration of experiment. Samples were then removed from culture at 3, 7 and 11 days of incubation to study cell-materials interactions under SEM.

Tissue preparation for electron microscopy can be divided into six major steps: primary fixation, washing, secondary fixation, rinsing, dehydration, critical point drying. Ideally, one purpose of fixation is to preserve the structure of living tissue with no alteration from living state. Additionally fixation should protect tissues against disruption during embedding and sectioning and subsequent exposure to the electron beam. Fixation protocols developed subsequently as a two step basic procedure described above. The primary fixative a combined 2% glutaraldehyde and a low concentration of formaldehyde (2%), which allowed more rapid initial fixation of the
tissue because formaldehyde penetrates the tissue more readily than glutaraldehyde (1 mm per hour) does. Glutaraldehydes attribute as a fixative is in its ability to cross-link protein by virtue of the terminal aldehyde groups. Constituents of the cell would be unified into a single interlocking structure or mesh work held together by a multitude of glutaraldehyde molecules. Secondary fixative, osmium tetroxide, worked by reacting primarily with lipid moieties. It is widely believed that the unsaturated bonds of fatty acids are oxidized by osmium tetroxide, with osmium tetroxide being reduced to black metallic osmium.\(^{38}\) This reduced heavy metal adds density and contrast to the biological tissue. Penetration of osmium tetroxide is slower than glutaraldehyde (about 0.5 mm in one hour). Buffers like cacodylate and phosphates are used along with fixative. As fixative lowers the pH of the tissues during the fixative process, artifacts may be produced. The buffering system maintains physiologic pH (e.g. 7.2 to 7.4) resulting in fewer artifacts. After primary fixation with glutaraldehyde, the tissue is usually washed in the same buffer vesicle used in glutaraldehyde fixation step. Washing is extremely important because it eliminates any free unreacted glutaraldehyde that remains within tissues. Aldehydes remaining from the primary fixation will be oxidized by osmium tetroxide. Dehydration is the process of replacing the water in cells with a fluid that acts as a solvent between the aqueous environment of the cell and the hydrophobic embedding media. Water is a highly polar molecule that is, by far, the major component of virtually all cells. Common dehydrating agents are ethanol or acetone. Ethanol is a widely used dehydrating agent for acetone because anhydrous acetone absorbs water from the atmosphere and is a more powerful extractor of lipids within the cell. Usually 30% ethanol is the first solvent that tissue is exposed to after secondary fixation followed by 50%, 70%, 95% and 100%. As one reaches higher concentrations of the dehydrating
agents, the time that tissue is exposed to the dehydration agent are increased in order to eliminate the small amount of water remaining in the tissues.

For SEM observation, anodized cell-cultured samples were placed in 0.1M phosphate buffered saline (PBS) and rinsed quickly. Samples were subsequently fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4 °C. Following three rinses in 0.1 M cacodylate buffer, each sample was post-fixed in a secondary fixative (2% osmium tetroxide (OsO₄) for two hours at room temperature). The fixed sample was then again rinsed three times in 0.1 M cacodylate and dehydrated in an ethanol series (30%, 50%, 70%, 95% and 100% three times). Samples were then critical-point dried using acetone and hexamethyldisilazane (HMDS). Samples were mounted on aluminum stubs, gold coated (Technis Hummer, San Jose, CA), and observed in SEM.

**B. Cell proliferation using MTT assay**

A 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay was used to evaluate cell proliferation on anodized samples. Duplicate samples per group were evaluated. MTT (Sigma, St. Louis, Mo) solution of 5 mg/ml was prepared by dissolving MTT in PBS, and filter sterilized. MTT was diluted with 50 μl of the solution in 450 μl of serum free phenol red-free Dulbeco’s Minimum Essential medium (DME). 500 μl of the above-diluted solution were used in each sample in 12-well plates to form formazan by mitochondrial dehydrogenases. After 2-hour incubation at 37 °C, 500 μl of the solubilization solution (10% triton X-100, 0.1N HCl and isopropanol) were added in each well plate to dissolve the formazan crystals. The 100 μl of the solution was then transferred to new 96 wells plate and 5 data points were obtained from each sample. The purple color of the formazan provides an optical density number which is also a measure of cell density. Optical density of the solution in each well was measured at a
wavelength of 570 nm using a Microplate reader (Cambridge Tech., Inc., EIA). Data are presented as mean of 10 values with standard deviation.

C. Immunochemistry and confocal microscopy

Cells cultured on anodized samples for a pre-specified number of days were fixed in 4% paraformaldehyde in 0.1M phosphate buffer and were kept for 24h at 4 °C for future use. Those samples were rinsed in Triton for 10 minutes and blocked with TBST/BSA (tris-buffered saline with 1% bovine serum albumin, 250 mM NaCl, pH 8.3) for 1 hour. Primary antibody alkaline phosphatase (ALP) (Sigma, St. Louis, MO) or Vinculin (Sigma-Aldrich, MO) was added at a 1:100 dilution and incubated at room temperature overnight. ALP was used for cell differentiation, while vinculin was used for cell attachment. The following day, samples were rinsed with TBST/BSA three times for 10 minutes each. The secondary antibody, Oregon green goat anti-mouse (GAM) (Molecular Probes, Eugene, OR), was added at 1:100 dilution and incubated at room temperature for one hour. Samples were then mounted on coverslips with Vectashield Mounting Medium (Vector Labs, Burlingame, CA) with propidium iodide (PI) and observed in confocal scanning laser microscopy (BioRad 1024 RMC). Figure 3.6 shows the schematic of the protocols of alkaline phosphatase study on cell cultured samples.
Samples are fixed with primary fixative 4% PFA in 0.1 M phosphate buffer

Samples were rinsed in 0.1(M) triton in 0.1M PBS for 10 minutes

Samples were rinsed for 10 min with 1(M) PBS for three times

Blocked with 100 μm/ sample TBST.BSA (10mM TRIS+250 mM NaCl+ 0.3% TWEEN at pH 8.2) for 1h.

Samples were washed thrice with

Primary antibody vinculin added along with TBST*BSA in a ratio 1:100. For each sample 100 μm of the above antibody solution was

Following day samples were rinsed for 10 min with TBST*BSA thrice

Samples were rinsed 1(M) PBS for 10 minute for three times

Secondary antibody Oregon Green anti mouse was added along with TBST*BSA in a ratio 1:100.

Samples were mounted with vecta-shield propidium iodide with cell side down onto the coverslip

**Figure 3.6** Different steps involved for samples to be prepared in immunochemistry study
3.5. Mineralization study in simulated body fluids (SBF)

Bioactivity of anodized titanium samples was evaluated by immersion in SBF, which has a similar ionic composition to human blood plasma. The solution was prepared by dissolving NaCl, KCl, NaHCO₃, MgCl₂·6H₂O, CaCl₂·2H₂O, Na₂SO₄·10H₂O and K₂HPO₄ into distilled water and buffered at pH 7.35 with tris-hydroxymethyl aminomethane (TRIS) and 1 (N) HCl at 37 °C. The detailed compositions of different salts used are given in Table 2.7. Samples were immersed in the glass vial containing 10 ml of SBF solutions and were kept under thermostatic conditions inside a biological thermostat at 37 °C for 3, 7, 14 and 21 days. All experiments were performed in duplicate, by running two independent glass vials simultaneously. After exposure, samples were washed with distilled water and then dried at 150 °C for 24h.

| Table 3.6- Concentration of ions in simulated body fluid (SBF) and in human plasma.³⁹ |
|-----------------------------------|------------------|---------------|-----------------|---------------|---------------|------------|
| Ions                             | Conc. (m.mol/l)  | Na⁺           | K⁺             | Mg²⁺          | Ca²⁺          | HCO₃⁻      | HPO₄²⁻    | SO₄²⁻   |
| Human Plasma                     | 142              | 5             | 1.5            | 2.5           | 103           | 27         | 27        | 0.5     |

| Table 3.7- Different amount of reagents to make SBF |
|-----------------------------------|------------------|------------------|
| Order | Reagent            | Amount (g/litre) |
| 1     | NaCl               | 7.996            |
| 2     | NaHCO₃             | 0.35             |
| 3     | KCl                | 0.224            |
| 4     | K₂HPO₄             | 0.171            |
| 5     | MgCl₂·6H₂O         | 0.305            |
| 6     | 1 (N) HCl          | 40 ml            |
| 7     | CaCl₂·2H₂O         | 0.368            |
| 8     | Na₂SO₄·10H₂O       | 0.161            |
| 9     | Tris base          | 6.057            |
Polished Ti Specimen

Anodized in different electrolyte solution

Characterization
- FESEM, EDS, SEM

Mineralization
- Surface Properties: Roughness, surface energy, Contact Angles

Osteoblast Cell Study
- Cell Culture
- Cell Adhesion with Vinculin; Cell Proliferation with MTT assays; Cell Differentiation with ALP

Figure 3.7. Schematic outline of research
CHAPTER FOUR

RESULTS

4.1. TiO$_2$ nonporous surface on Ti-surface

Commercially pure Titanium (Cp-Ti) surface was anodized in different electrolytes at 20V for 60 minutes. Table 2.1 in the experimental section shows the different conditions, which have been used to study the anodization process. Three different electrolytes (a) 1 (N) sulfuric acid H$_2$SO$_4$, (b) 1 (N) phosphoric acid H$_3$PO$_4$ and (c) 0.25 (N) hydrofluoric acid HF were used for anodization of the Ti-substrates that led to three different surface morphologies.

A. Surface morphology

![Figure 4.1. FESEM micrographs showing morphology of (a) Ti surface, anodized oxides surfaces prepared by anodization in (b) H$_2$SO$_4$ (c) H$_3$PO$_4$ and (d) HF electrolytes.](image)

Figure 4.1. FESEM micrographs showing morphology of (a) Ti surface, anodized oxides surfaces prepared by anodization in (b) H$_2$SO$_4$ (c) H$_3$PO$_4$ and (d) HF electrolytes.
All the anodic surfaces were nonporous with patterned micrographs. Figure 4.1 shows representative scanning electron micrographs of anodized and Ti-control surfaces. Ti-control, being metal, shows a smooth surface as shown in Figure 4.1a. Although the H₂SO₄ anodized surface was nonporous, a few bigger pores were noticed in some regions of the undulated topography as shown in Figure 4.1b. H₃PO₄ anodized surface was rough with a flowery pattern, as shown in Figure 4.1c, while HF anodized surface shows a rough granulated debris pattern, as shown in Figure 4.1d. Average coating thickness of the film was between 200 and 400 nm. Samples anodized for 60 min showed complete coverage of TiO₂ on Ti for all electrolytes whereas samples anodized for <60 min were partially covered with the oxide film as shown in Figure 4.2.

![Figure 4.2](image)

**Figure 4.2.** FESEM micrograph for anodized samples in HF for (a) 2 min, (c) 45 min and anodized in H₃PO₄ electrolyte for (b) 2 min and (d) 45 min respectively. Microstructure is not uniform as unanodized Ti substrate is visible.
4.2. Phase Analysis

Glancing angle diffraction has been performed on all the anodized surfaces in different electrolyte.

Figure 4.3. GAXRD results on oxide film anodized in (a) H₃PO₄ (b) heat treated at 400 °C and

Figure 4.3 shows the glancing angle x-ray diffraction results on the anodized films. Most of the oxide films show some anatase phase in an amorphous background, which is also given in Table 4.1. The oxide surface shows anatase phase at 400 °C and 600 °C.

Table 4.1 - GAXRD results analysis for different phases in the anodized and heat treated sample

<table>
<thead>
<tr>
<th>Sample Condition</th>
<th>Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂SO₄</td>
<td>Amorphous/Anatase</td>
</tr>
<tr>
<td>H₂SO₄-400 °C</td>
<td>Anatase</td>
</tr>
<tr>
<td>H₂SO₄-600 °C</td>
<td>Anatase</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>Amorphous/Anatase</td>
</tr>
<tr>
<td>H₃PO₄-400 °C</td>
<td>Anatase</td>
</tr>
<tr>
<td>H₃PO₄-600 °C</td>
<td>Anatase</td>
</tr>
<tr>
<td>HF</td>
<td>Anatase</td>
</tr>
</tbody>
</table>
4.3. Surface properties

Biomaterials interact with the body through their surfaces. Consequently, the anodized outermost layers of a material are critically important in determining both biological responses to implants and material responses to the physiological environment. Our goal is to design a surface that elicits the desired interfacial behaviors. Below are describe the different techniques for characterizing surfaces, approaches for modifying surfaces to control biological responses, and responses of surface to cell-material interactions.

A. Surface Roughness: Surface profilometer was used to scan a stylus of 25 μm on a scan 4 mm at different places of the anodized surfaces. Details of the number of scans and data points are described in experimental section 3.3A. As Table 4.2 indicates there was almost an order of difference in roughness of the anodized surfaces and Ti-control from the profilometer data.

Table 4.2: Surface Roughness data for different anodized conditions samples

<table>
<thead>
<tr>
<th>Surfaces</th>
<th>Average Roughness</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-Control</td>
<td>0.007414</td>
<td>0.000101</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>0.0250</td>
<td>0.00145</td>
</tr>
<tr>
<td>HF</td>
<td>0.0830</td>
<td>0.00415</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>0.0157</td>
<td>0.00075</td>
</tr>
</tbody>
</table>

B. Contact Angle and Surface Energy: Contact angles analysis involves measuring the angle (θ) of contact between a liquid and a surface. When a drop of liquid is placed on a surface, it will spread to reach a force of equilibrium, in which the sum of the interfacial tensions in the plane of the surface is zero.
Figure 4.4. Contact Angle of liquid droplets measured on the anodized and Ti-control samples

Contact angles were measured as shown in Figure 2.2 on the different anodized surfaces along with control-Ti surface using cell media and water. It was noticed that contact angle changes significantly for each nonporous surfaces. The Ti-control shows average values of 70° and 50° respectively in water and cell media, whereas anodized the H₃PO₄ surface shows a value of 25° and 10° as shown in Figure 4.4. With the help of contact angle surface energy for all the anodized surfaces was calculated using equation (1) in experimental section 3.3 C. To calculate the surface energy, three different liquids were used; one is apolar liquid diiodomethane and two are polar liquid formamide and glycerol.

Determination of materials surface energy better indicates surface properties. Surface energy, defined as the increased free energy per unit area for creating a new surface, is directly proportional to the tendency of molecules to adsorb. Calculations for surface energy from contact angles of three liquids were shown in Section 3.3 C in chapter 3 of the experimental portion. The
different values of contact angles along with surface energy data are presented in Table 4.3. A high value of surface energy of 101.27 mJ/m² for H₃PO₄ anodized surfaces and low value of 55.6 mJ/m² for H₂SO₄ surfaces were obtained.

Table 4.3- Surface energy of the nonporous samples anodized in three different acid electrolytes

<table>
<thead>
<tr>
<th>Samples</th>
<th>Contact Angles (degrees)</th>
<th>Surface Energy (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formamide</td>
<td>Glycerol</td>
</tr>
<tr>
<td>Ti-Control</td>
<td>18±1.15</td>
<td>40±2.04</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>21±1</td>
<td>41±1.15</td>
</tr>
<tr>
<td>HF</td>
<td>11±3.05</td>
<td>39±4.61</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>5±0.57</td>
<td>31±1.73</td>
</tr>
</tbody>
</table>
4.4. Cell-materials interactions on nonporous surfaces

A. Cell morphology: OPC1 cell attachments on sample surfaces were analyzed for cell-materials interactions using a scanning electron microscope.

Figure 4.5. SEM micrograph illustrating OPC1 cell adhesion after 3 days of culture on (a) control-Ti and oxide surfaces anodized in (b) H₂SO₄ (c) H₃PO₄ and (d) HF electrolytes.

Figure 4.5 shows SEM of cell morphologies on Ti-control and anodized surfaces after 3 days of cell culture. Cells on Ti-control in Figure 4.5 show an elongated, flattened morphology. Cells on anodized H₂SO₄ surface shows rounded up morphology and some cell death was also noticed, as shown in Figure 4.5b. The H₃PO₄ surface shows good cell attachment in Figure 4.5 but cells are
confined to one region and not extended like the TiO$_2$ surface from HF electrolyte. HF anodized surface is entirely covered with OPC1 cell layers and filopodia extensions were observed coming out from the cell to grasp the granulated pattern rough surface for anchorage as shown in Figure 4.5.

![Image](image1)

**Figure 4.6.** Anodized surface covered with osteoblast human bone cells cultured for 11 days (a) Ti-control, (b) H$_2$SO$_4$, (c) H$_3$PO$_4$ and (d) HF surfaces.

To further understand the osteoconductivity of the anodized surfaces, cells were cultured for 11 days as shown in Figure 4.6 and Figure 4.7 at low magnification and at high magnification respectively. Figure 4.6 shows a fewer number of cells as noticed in Ti-control. The cell number increased on anodized HF and H$_3$PO$_4$ surfaces. Figure 4.7a shows cells on the Ti-control in
which flattened, elongated cell morphology with many filopodia extensions from the cell to the substrate and small calcified nodule in 11 days cell culture can be seen.

Figure 4.7. SEM micrographs showing the cellular adhesion and proliferation on (a) control Ti, oxide surfaces anodized in (b) H₂SO₄ (c) H₃PO₄ and (d) HF electrolytes after 11 days of incubation.

It can be noticed in Figure 4.7b that cells minimized the surface area of attachment in H₂SO₄ anodized surface. However, cell spreading and proliferation are excellent for H₃PO₄ anodized surface shown in Figure 4.7c. An abundant amount of extracellular matrix (ECM) can be seen between the neighboring cells on the anodized H₃PO₄ surface, forming a three-dimensional fibril network. Cells adhere to implant *in vitro* by means of specialized cell adhesions or focal

54
contacts. They do not adhere directly to the metal surface, but rather to extracellular matrix (ECM) components that become adsorbed to the metal. Although often considered as artifacts of cell culture, many characteristics of focal contacts indicate that they are structurally and functionally equivalent to adhesions made by cells in vivo. Colonization of the cells was noticed with cells developing a double net-like layer structure in HF anodized surface after 11 days of cell culture (Figure 4.7d). Distinctive cell–to-cell attachment was noticed with small calcified nodules at an early sign of cell differentiation after 11 days in both HF and H₃PO₄ anodized surfaces.

**B. Cell Adhesions:** Vinculin aids in the assemblage of focal contacts by cross-linking and recruiting other proteins to form adhesive plagues. Vinculin also acts as an adhesion molecule between the cells and substratum.

![Figure 4.8](image-url)

**Figure 4.8.** Vinculin protein expressions after culture for 3 days at low magnification for (a) Ti-control, (b) H₂SO₄, (c) H₃PO₄ and (d) HF.
Cells adhere to implants *in vitro* by means of specialized cell adhesion or focal contacts. Many characteristics of focal contacts indicate that they are structurally and functionally equivalent to adhesions made to cells *in vivo*.\(^1\) As cells attach to one another and to the substratum, adhesive proteins interact with and form bonds to adhesion receptors within the cellular membrane. They can then spread out, migrate, and release specific proteins.\(^2\) Cells growing on different surfaces showed vinculin localization at focal contacts, but the intensity and distribution of the strain varied considerably between the different surface morphologies. Antibody bound to vinculin expressed green fluorescence and nuclei stained with propidium iodide (PI) in the mounting medium expressed red fluorescence. Adhesion of cells in this confocal image showed higher intensity and increase in cell number for H\(_3\)PO\(_4\) and HF anodized surfaces.

![Image](image-url)

**Figure 4.9.** Vinculin intensity increases as the culture time increases to 5 days, as green fluorescence increases for (a) Ti-control, (b) H\(_2\)SO\(_4\), (c) H\(_3\)PO\(_4\) and (d) HF anodized surfaces.
Figure 4.8. Figure 4.9 shows the vinculin study for 3 day and 5 days. To better understand this process, vinculin expressions were analyzed by using confocal imaging at high magnification as in Figure 4.10. Vinculin expression shows fewer of cells that attach to one another and to the substratum in Ti-control and H$_2$SO$_4$ anodized samples, as shown in Figure 4.10 a and b, than in the H$_3$PO$_4$ and HF surfaces, shown in Figure 4.10 c and d, respectively.

Figure 4.10. Immunolocalization of adhesive molecule vinculin in OPC1 cells for 5 days culture on (a) Ti-control, (b) H$_2$SO$_4$, (c) H$_3$PO$_4$, (d) HF surfaces; Green fluorescence (indicating antibody bound to vinculin) and red fluorescence (indicating antibody bound to DNA (nucleus)).
C. Cell proliferation using MTT assay: The MTT assay was used to determine the OPC1 cell proliferation on control-Ti and different anodized surfaces. Figure 4.11 shows a comparison of cell densities on the different anodized surfaces for 5, 11 and 16 days.

Figure 4.11. Optical density measured after culture for 5, 11 and 16 days at a wavelength of 570 nm by reader. There were significant differences in optical density after 11 days in the different anodized conditions samples. Probability $p^* <0.001$ for $\text{H}_3\text{PO}_4$, HF and $p^{**}<0.01$ for $\text{H}_2\text{SO}_4$ compared with Ti-control at 11 days of culture.

For 5 days of culture a slightly higher value in optical density was noticed in $\text{H}_3\text{PO}_4$ and HF anodized surfaces in comparison to Ti-control and $\text{H}_2\text{SO}_4$ anodized surfaces. After 11 days of culture, a significant difference in optical density was noticed. Cell density for the anodic oxide increased in the order $\text{H}_3\text{PO}_4< \text{HF} < \text{H}_2\text{SO}_4< \text{Ti-control}$. A slight increase in density was noticed with increase in culture time to 16 days for each anodic condition when compared with 11 days.
However, the numbers of cells on H₃PO₄ and HF anodic surfaces were always more than those of H₂SO₄ and Ti-control surfaces.

**Table 4.4-** Student t-test table for 11 days data

<table>
<thead>
<tr>
<th>Statistical data</th>
<th>Ti-control</th>
<th>H₂SO₄</th>
<th>H₃PO₄</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Values</td>
<td>0.108</td>
<td>0.1365</td>
<td>0.1912</td>
<td>0.17625</td>
</tr>
<tr>
<td>Sum = ( \sum x )</td>
<td>0.432</td>
<td>0.546</td>
<td>0.765</td>
<td>0.705</td>
</tr>
<tr>
<td>( (\sum x)^2 )</td>
<td>0.186624</td>
<td>0.298116</td>
<td>0.585225</td>
<td>0.497025</td>
</tr>
<tr>
<td>( \sum x^2 ) = ( x_1^2 + x_2^2 + x_3^2 + \ldots )</td>
<td>0.04681</td>
<td>0.074708</td>
<td>0.146493</td>
<td>0.124263</td>
</tr>
<tr>
<td>( (\sum x)^2/n )</td>
<td>0.046656</td>
<td>0.074529</td>
<td>0.14631</td>
<td>0.12425625</td>
</tr>
<tr>
<td>( \sum d^2 = [\sum x^2 - (\sum x)^2/n] )</td>
<td>0.000154</td>
<td>0.000179</td>
<td>0.000183</td>
<td>6.75x10⁻⁶</td>
</tr>
<tr>
<td>( \sigma^2 = \sum d^2/(n-1) )</td>
<td>5.1333x10⁻³</td>
<td>5.96667x10⁻³</td>
<td>6.1x10⁻⁵</td>
<td>2.25x10⁻⁶</td>
</tr>
<tr>
<td>( \sigma_d^2 = (\sigma_1^2/n_1) + (\sigma_2^2/n_2) )</td>
<td>-</td>
<td>2.77x10⁻⁵</td>
<td>2.81x10⁻⁵</td>
<td>1.34x10⁻⁵</td>
</tr>
<tr>
<td>( \sigma_d )</td>
<td>-</td>
<td>0.005267</td>
<td>0.005299</td>
<td>0.00366</td>
</tr>
<tr>
<td>( t = [x_1 - x_2]/\sigma_d )</td>
<td>-</td>
<td>5.41</td>
<td>15.709</td>
<td>18.64</td>
</tr>
</tbody>
</table>

*Note:* \( \sigma_d \) for all anodized surfaces calculated considering \( \sigma_1 \) for Ti-control and \( \sigma_2 \) for the respective anodized surface.

From the Student t-test data and degrees of freedom \((n_1 + n_2 - 2)\) the probability is matched from the table. For H₃PO₄ and HF anodized surfaces, there was no difference (\( p^* < 0.001 \)) in cell spreading but there was a significant difference between cells on H₂SO₄ (\( p^{**} < 0.01 \)) and Ti-control at 11 days of culture.

**D. Cell Differentiation:** Alkaline phosphatase is an early marker for osteoblast differentiation and is thought to play a major role in bone formation.⁴³ While this enzyme activity is present in all
cell membranes, it is found mostly in higher levels in cells which mineralize their matrix such as osteoblasts.\textsuperscript{44}

**Figure 4.12.** Confocal micrographs of ALP expression for 5 days in OPC1 cells cultured on (a) Ti-control, (b) H\textsubscript{2}SO\textsubscript{4}, (c) H\textsubscript{3}PO\textsubscript{4} (d) HF for 5 days. Green fluorescence indicating antibody bound to ALP, red fluorescence indicating antibody bound to DNA (nucleus).

Enzyme activity varied with surface roughness and morphology on the three different conditions of anodized surfaces. Cell layers from cells cultured on all different surfaces contained significantly more alkaline phosphatase specific activity than on Ti-controls. **Figure 4.12a-d** shows ALP activity for samples after 5 days in culture. Little difference was found in Ti-control
and anodized surface in H$_2$SO$_4$ as shown in Figure 4.12a and b, respectively. Figures 4.12c and d show that the ALP enzymatic activity was higher in H$_3$PO$_4$ and HF anodized surfaces.

Figure 4.13. Confocal micrographs of ALP expression for 11 days in OPC1 cells cultured on (a) Ti-control (b) H$_2$SO$_4$, (c) H$_3$PO$_4$ (d) HF for 11 days.

With increase in cell culture time from day 5 to day 11, the ALP activity increased progressively at 11 days culture in all the anodized oxide surfaces, as shown in Figure 4.13a-d. At day 11, all
anodized surfaces showed better cell differentiation activity compared to control-Ti in Figure 4.13a. The qualitative analysis of ALP was repeated in duplicate, with similar results.

4.5. Bone mineralization effect on nonporous anodized film

For comparison of biocompatibility, anodized samples and control Ti were immersed in SBF for 3, 7, 14 and 21 days. Figure 4.14a-d show surface micrographs of the samples after immersion in SBF for 21 days. It can be noticed that anodized surfaces have low ability for Ca-apatite deposition in SBF. No apparent changes appeared on the polished surface of the Ti-control, even after 21 days. It has been reported that anodized samples, heat treated at higher temperature, results in more apatite deposition.19

![Figure 4.14](image)

**Figure 4.14.** FESEM micrographs demonstrating the deposition of precipitates in SBF for 21 days on (a) Ti-Control, oxidized titanium surface anodized in (b) H$_2$SO$_4$, (c) H$_3$PO$_4$ and (d) HF electrolytes.
Therefore, in the present study influence of temperature on the surface TiO$_2$ layer for apatite deposition was investigated by heat-treating the anodized samples at 400 and 600 °C for 2h. It was found that heat treatment in anatase titanium oxide phases with different surface morphologies compared to the anodized surface as shown in Figure 4.15a-f.

**Figure 4.15.** FESEM micrograph showing the surface morphology of TiO$_2$ layer prepared by heat treatment at (a) 400 °C, (b) 600 °C anodized H$_2$SO$_4$ surface, (c) 400 °C, (d) 600 °C anodized in H$_3$PO$_4$ and (e) 400 °C, (f) 600 °C anodized in HF electrolytes.

**Figure 4.16.** Mineralization study for 7 days on the anodized heat treatment at 400 °C and 600 °C surface condition above samples.
Heat-treated samples showed significant Ca-apatite deposition in SBF after 7 days, as shown in Figure 4.16a-f. Precipitates were more pronounced in heat-treated anodized samples than in the anodized surfaces. EDS were performed on the anodized and heat treated SBF immersed samples.

**Figure 4.16a-f.** Precipitates were more pronounced in heat-treated anodized samples than in the anodized surfaces. EDS were performed on the anodized and heat treated SBF immersed samples.

**Figure 4.17.** (a) EDS analysis for the apatite deposition on anodized surface in SBF. Calcium to Phosphorous ratio: Ca/P=1.36. (b) IR data for H$_3$PO$_4$ oxide surface immersed in SBF for 21 days.
From EDS analysis in Figure 4.17a, it was observed that the precipitated particles on the surface of anodic film were Ca, P and Mg compounds, as reported in Table 4.5. IR results in Figure 4.17b show the presence of OH\(^-\), PO\(_4\)^{2-} and CO\(_3\)^{2-} groups and FTIR peaks for the respective groups are shown in Table 4.6.

### Table 4.5- EDS results for SBF immersed samples at different conditions

<table>
<thead>
<tr>
<th>Sample Condition</th>
<th>Wt. %</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
<td>O</td>
<td>Ca</td>
<td>P</td>
<td>Mg</td>
<td>Na</td>
<td>Cl</td>
</tr>
<tr>
<td>H(_2)SO(_4)</td>
<td>62.29</td>
<td>12.19</td>
<td>1.05</td>
<td>0.78</td>
<td>0.58</td>
<td>3.64</td>
<td>1.67</td>
</tr>
<tr>
<td>H(_2)SO(_4)-400 °C</td>
<td>67.68</td>
<td>12.91</td>
<td>1.52</td>
<td>1.30</td>
<td>1.31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H(_2)SO(_4)-600 °C</td>
<td>74.08</td>
<td>13.53</td>
<td>0.87</td>
<td>1.25</td>
<td>0.85</td>
<td>4.28</td>
<td>9.42</td>
</tr>
<tr>
<td>H(_3)PO(_4)</td>
<td>91.7</td>
<td>5.09</td>
<td>0.36</td>
<td>0.56</td>
<td>2.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H(_3)PO(_4)-400 °C</td>
<td>67.16</td>
<td>15.65</td>
<td>0.70</td>
<td>0.38</td>
<td>1.68</td>
<td>14.43</td>
<td></td>
</tr>
<tr>
<td>H(_3)PO(_4)-600 °C</td>
<td>81.13</td>
<td>11.97</td>
<td>0.47</td>
<td>0.65</td>
<td>5.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>45.73</td>
<td>21.47</td>
<td>1.17</td>
<td>0.86</td>
<td>0.82</td>
<td>29.95</td>
<td></td>
</tr>
<tr>
<td>HF-400 °C</td>
<td>65.58</td>
<td>20.16</td>
<td>2.10</td>
<td>1.60</td>
<td>0.52</td>
<td>10.04</td>
<td></td>
</tr>
<tr>
<td>HF-600°C</td>
<td>72.26</td>
<td>16.81</td>
<td>0.52</td>
<td>0.62</td>
<td>0.76</td>
<td>9.03</td>
<td></td>
</tr>
</tbody>
</table>

| Sample Condition | At. % |  |  |  |  |  |  |
|------------------|-------|---|---|---|---|---|
|                  | Ti    | O  | Ca | P  | Mg | Na | Cl | C  |
| H\(_2\)SO\(_4\)   | 33.95 | 19.90 | 0.68 | 0.65 | 0.73 | 4.14 | 1.23 | 38.72 |
| H\(_2\)SO\(_4\)-400 °C | 38.89 | 22.21 | 1.35 | 1.06 | 1.48 | - | 35.01 |
| H\(_2\)SO\(_4\)-600 °C | 47.19 | 25.8 | 0.96 | 1.03 | 1.06 | 23.96 |
| H\(_3\)PO\(_4\)   | 77.93 | 12.95 | 0.64 | 0.73 | 7.75 |
| H\(_3\)PO\(_4\)-400 °C | 38.18 | 26.64 | 0.57 | 0.47 | 1.88 | 32.26 |
| H\(_3\)PO\(_4\)-600 °C | 57.25 | 25.30 | 0.55 | 0.64 | 16.26 |
| HF               | 73.51 | 11.53 | 1.22 | 0.70 | 0.97 | 12.07 |
| HF-400 °C       | 37.89 | 34.88 | 1.94 | 1.43 | 0.65 | 23.21 |
| HF-600°C        | 44.70 | 31.13 | 0.38 | 0.59 | 0.93 | 22.27 |

### Table 4.6- FTIR peaks for apatite layer

<table>
<thead>
<tr>
<th>System</th>
<th>Wave number (cm(^{-1}))</th>
<th>Wave number (cm(^{-1})) Literature(^{45-46})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bending mode of O-P-O bonds of (PO(_4))(^{3-})</td>
<td>601</td>
<td>601-597</td>
</tr>
<tr>
<td>Vibrational mode of OH(^-)</td>
<td>717</td>
<td>635-628</td>
</tr>
<tr>
<td>Stretching mode of P-O bond in (PO(_4))(^{3-})</td>
<td>1020</td>
<td>1040-1014</td>
</tr>
<tr>
<td>Vibration mode of C-O of CO(_3)^{2-}</td>
<td>1400, 1470</td>
<td>1373, 1453</td>
</tr>
<tr>
<td>Stretching mode of OH(^-)</td>
<td>3670</td>
<td>3574</td>
</tr>
<tr>
<td>Stretching mode HPO(_4)^{2-}</td>
<td>1227</td>
<td>1250</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.1. Nanoporous surface morphology

Anodization of Ti-substrate in an electrolyte composition of citric acid, sulfuric acid and sodium fluoride salts resulted in nanoporous morphology, as described in detail in experimental section 3.1. The samples were anodized at 20 V for two different times for 2h and 4h.

A. Porous nanotube array structure

Microstructure shows spongy appearance with pores of nanometer in range. Table 5.1 shows the results for the average pore diameter, wall thickness and length of the nanotube at various anodized time periods. Measurements were made from randomly selected areas in the FESEM micrograph. The diameter of the nanotube increases from 2h to 4h.

![Figure 5.1](image)

**Figure 5.1.** (a) FESEM top-view images of porous titanium oxide films anodized at 20V at for 4h and (b) Cross-sectional view shows the length of nanotube anodized in electrolyte composition of 0.1 mole/L NaF, 1.0 mole/L H₂SO₄, 0.2 mole/L citric acid, for 4h.

**Figure 5.1** (a) shows the high magnification top view microstructure of the titanium oxide nanotube. Microscopic analysis showed that nanotubes were 50 nm in diameter with 15 nm wall thickness. Typical length of nanotubes varied between 300 nm to 600 nm based on anodization.
times. The length of the nanotube arrays was approximately 600 nm for 4h anodized with the electrolytes at pH 4.5 as shown in Figure 5.1 (b). Table 5.1 shows the different anodized nanotube dimensions.

Table 5.1- Dimensions of the nanotube at different anodized times

<table>
<thead>
<tr>
<th>Sample Anodized time</th>
<th>Internal Diameter Average</th>
<th>Standard deviation</th>
<th>Wall Thickness Average</th>
<th>Standard deviations</th>
<th>Length</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>54.2 nm</td>
<td>5.5 nm</td>
<td>39.6 nm</td>
<td>4.7 nm</td>
<td>298 nm</td>
<td>35 nm</td>
</tr>
<tr>
<td>4 h</td>
<td>51.1 nm</td>
<td>11.0 nm</td>
<td>50.6 nm</td>
<td>5.6 nm</td>
<td>592.5 nm</td>
<td>48 nm</td>
</tr>
</tbody>
</table>

To better understand the different steps of nanotube formation and the condition, which causes nanotube, different kinetics parameters were studied carefully. The effect of different electrolyte chemistries on the formation of nanotubes was evaluated on the next section. Nanotubes were already formed in 2 hours so it was important to understand how the morphology changes below 2 hours from smooth Ti-control surface to nanoporous morphology. One of the variable parameters varied was the anodized time from 5 minutes to 2 hours by using the above electrolyte conditions of citric acid, sodium fluoride and sulfuric acid at 20V. Below are the results of two important parameters.

B. Effect of electrolyte chemistry on the fabrication of nanotube arrays

To understand the conditions at which nanoporous surfaces morphology can be obtained electrolyte chemistry, concentration, time, and pH of electrolyte solutions were varied. Table 5.2 below shows the different parameters of the electrolyte compositions for nanotube formation.
Table 5.2- Different electrolyte chemistry varied for nanoporous morphology

<table>
<thead>
<tr>
<th>Sam</th>
<th>NaF (mole/L)</th>
<th>H₂SO₄ (mole/L)</th>
<th>H₃PO₄ (mole/L)</th>
<th>HF</th>
<th>Citric acid (mole/L)</th>
<th>CH₃COOH (mole/L)</th>
<th>Volt (min)</th>
<th>Time (min)</th>
<th>pH</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60,30</td>
<td>2.63</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60,30,15,5</td>
<td>1.06*</td>
<td>NT</td>
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<tr>
<td>3</td>
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<td></td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60</td>
<td>2.99</td>
<td>No</td>
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<tr>
<td>4</td>
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<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60,30,15</td>
<td>2.26</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td></td>
<td>0.5wt%</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60,30</td>
<td>2.09</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
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<td>1.0</td>
<td>0.5wt%</td>
<td></td>
<td></td>
<td></td>
<td>60,30</td>
<td>2.73</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60</td>
<td>1.22*</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td></td>
<td>1.0</td>
<td></td>
<td>0.5wt%</td>
<td></td>
<td>20</td>
<td>60</td>
<td>3.40</td>
<td>NT</td>
</tr>
<tr>
<td>9</td>
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<td>0.5wt%</td>
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<td>20</td>
<td>60</td>
<td>1.50</td>
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<tr>
<td>10</td>
<td>0.1</td>
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<td>0.5wt%</td>
<td></td>
<td></td>
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<td>20</td>
<td>60</td>
<td>0.85*</td>
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<tr>
<td>11</td>
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<td>0.1</td>
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<td></td>
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<td></td>
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<td>60</td>
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</tr>
<tr>
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<td>60</td>
<td>0.60</td>
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<tr>
<td>17</td>
<td>0.15 (M)</td>
<td>0.5 (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>30</td>
<td>3.23</td>
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<td>18</td>
<td>0.5 (M)</td>
<td>0.15 (M)</td>
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<td>30</td>
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<td>19</td>
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<td>0.15 (M)</td>
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<td></td>
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<td></td>
<td>20</td>
<td>60</td>
<td>5.62</td>
<td>No</td>
</tr>
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<td>0.1</td>
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<td></td>
<td>0.1</td>
<td></td>
<td>20</td>
<td>60</td>
<td>2.62</td>
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<td>NT</td>
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<td>0.2</td>
<td></td>
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<td></td>
<td>20</td>
<td>120</td>
<td>4.5*</td>
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<tr>
<td>24</td>
<td>-</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>60</td>
<td>1.91</td>
<td>No NT</td>
</tr>
</tbody>
</table>

*pH adjusted. NT nanotube

Table 5.3 Change in pH before and after nanotube formation

<table>
<thead>
<tr>
<th>Sam</th>
<th>Composition (mole/L)</th>
<th>Initial pH</th>
<th>pH adjusted</th>
<th>pH after anodization</th>
<th>C_H⁺ (from column 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.1 NaF, 1.0 H₂SO₄</td>
<td>1.71</td>
<td>1.06</td>
<td>0.56-0.61</td>
<td>0.34645</td>
</tr>
<tr>
<td>4</td>
<td>0.1 NaF, 1.0 H₃PO₄</td>
<td>2.26</td>
<td>2.26 (Not adjusted)</td>
<td>1.39</td>
<td>0.10435</td>
</tr>
<tr>
<td>8</td>
<td>0.1 NaF, 1.0 CH₃COOH</td>
<td>3.40</td>
<td>3.40 (Not adjusted)</td>
<td>3.16</td>
<td>0.03337</td>
</tr>
<tr>
<td>23</td>
<td>0.1 NaF, 0.1 H₂SO₄, 0.2 Citric acid</td>
<td>2.63</td>
<td>4.5</td>
<td>4.21</td>
<td>0.01111</td>
</tr>
<tr>
<td>24</td>
<td>1.0 CH₃COOH</td>
<td>1.91</td>
<td>1.91 (Not adjusted)</td>
<td>1.51</td>
<td>0.14808</td>
</tr>
</tbody>
</table>

**Note-** C_H⁺: Concentration H⁺ ions
Figure 5.2. Effect of electrolyte chemistries in formation of nanoporous morphology. (a) 0.1 mole/L NaF, 0.1 mole/L H$_2$SO$_4$; (b) 0.1 mole/L NaF, 1.0 mole/L H$_2$SO$_4$; (c) 0.1 mole/L NaF, 0.1 mole/L H$_3$PO$_4$; (d) 0.1 mole/L NaF, 1.0 mole/L CH$_3$COOH; (e) 0.1 mole/L NaF, 1.0 mole/L H$_2$SO$_4$, 1.0 mole/L CH$_3$COOH; (f) 0.1 mole/L NaF, 0.1 mole/L H$_2$SO$_4$, 1.0 mole/L CH$_3$COOH; (g) 0.1 mole/L NaF, 1.0 mole/L H$_2$SO$_4$, 0.1 mole/L citric acid; (h) 0.1 mole/L NaF, 0.1 mole/L citric acid.
Nanoporous morphology was obtained for 8 different electrolyte chemistries as shown in Figure 5.2. Nanopore size varied from 100 nm to 50 nm in internal diameter. Compositions 16 (1.0 mole/L H₂SO₄, 1.0 mole/L CH₃COOH) and 20 (0.1 mole/L NaF) do not give rise to nanoporous morphology. Based on the Table and microstructure it was noticed that fluorine ion is important for the nanoporous morphology. To verify the presence of fluorine ions 0.15 (M) hydrofluoric acid were used instead of sodium fluoride along with 0.5 (M) phosphoric acid, as shown in sample 18 in Figure 5.3a. Composition 20 (0.1 mole/L NaF) has only NaF as electrolyte constituents, composition 16 (1.0 mole/L H₂SO₄, 1.0 mole/L CH₃COOH) and composition 24 (1.0 mole/L CH₃COOH) has only an acid electrolyte. All of the above composition did not show any nanoporous surface morphology and are shown in Figure 5.3 b, c and d. Based on the above observations it can be concluded that fluorine ion is not the only factor which is necessary, but presence of an acid electrolyte is needed to fabricate the nanoporous morphology. Highly acidic electrolyte chemistry showed nanoporous morphology even within 30 and 15 minutes of anodized time in compositions 1 (0.1 mole/L NaF, 0.1 mole/L H₂SO₄), 2 (0.1 mole/L NaF, 0.1 mole/L H₂SO₄) and 4 (0.1 mole/L NaF, 1.0 mole/L H₃PO₄). Based on this result, specific criteria were established for electrolyte chemistry to create nanoporous surface morphologies.

(a) Required presence of fluoride ions e.g. NaF, HF and KF

(b) An acidic solution e.g. H₂SO₄, H₃PO₄, CH₃COOH.

Both of the above (a) and (b) conditions must be satisfied together to yield nanotubes.
Figure 5.3. (a) Any source of F⁻ ions, like HF solution and acid solution, gave rise to nanopores in composition 18 (0.15 (M) HF, 0.5 (M) H₃PO₄). Electrolyte chemistry does not give rise to nanoporous morphology. (b) 0.1 mole/L NaF, (c) 1.0 mole/L H₂SO₄, 1.0 mole/L CH₃COOH, (d) 1.0 mole/L CH₃COOH.

The effect of electrolyte pH, composition, and concentration on the formation of self-organized TiO₂ nanotube arrays by constant-potential anodization has been investigated. Nanotube arrays were formed in fluoride containing acidic electrolytes; in a 0.1 mol/L F⁻ solution. In strongly acidic solutions (pH <1) both the TiO₂ nanotube growth rate and Ti dissolution rate were increased. Increasing pH decreases the chemical dissolution rate, and apparently prolongs the time needed to reach equilibrium between the rate of nanotube growth and the dissolution rate; in weak acid electrolytes the nanotube length is time dependent as shown by samples 23 (0.1 mole/L NaF, 1.0 mole/L H₂SO₄, 0.2 mole/L citric acid). Length of the nanotube is 600 nm with composition 23 (0.1 mole/L NaF, 1.0 mole/L H₂SO₄, 0.2 mole/L citric acid) for 4h anodized time.
as pH adjusted with sodium hydroxide solution and is shown in Figure 5.1. Electrolyte pH affects both the behavior of the electrochemical etch and chemical dissolution, owing to the extent hydrolysis of titanium ions.\textsuperscript{35} With increasing the initial pH, the hydrolysis contents increases, which slows the rate of chemical dissolution resulting in a significant amount of hydrous titanic oxide precipitated on the nanotube surface.\textsuperscript{35} Table 5.3 shows the change in pH before and after nanotube formation with some of the selected composition. It was noticed pH decreases significantly with the formation of nanotube. Our studies showed that the best pH range for formation of relatively longer nanotubes is between pH 1 and pH 3; lower pH develops shorter but cleaner nanotubes, whereas higher pH values result in longer tubes that suffer from unwanted precipitates.

\textbf{C. Effect of anodized time at different stages of nanotube formation}

To help understand the process of nanotube formation, composition 23 (0.1 mole/L NaF, 1.0 mole/L H\textsubscript{2}SO\textsubscript{4}, 0.2 mole/L citric acid) in Table 5.2 were chosen to study the effect of anodized time. Longer time for the nanoporous morphology were evolved with electrolyte chemistry of 0.1 mole/L NaF, 0.2 mole/L citric acid, 1.0 mole/L H\textsubscript{2}SO\textsubscript{4} was selected. FESEM images of the surface of the samples were anodized at 20V for different durations from 5 minute to 120 minutes as shown in Figure 5.4. Figure 5.4a shows the image after 5 min anodization, which shows the initial oxide layer which were formed due to the interaction of the surface Ti\textsuperscript{4+} ions with oxygen ions in the electrolyte. The oxide layer spread uniformly across the surface. With increase in anodization time from 15 min to 45 min, these oxide layers grew and formed a more porous morphology, with small pits as shown in Figure 5.4b-d. In 60 minutes, anodization small pits had converted to bigger pores and pore density had increased, as shown in Figure 5.4e. With further increase in time at 120 minute the sample shows nanoporous morphology Figure 5.4f.
Figure 5.4. Microstructural topographies at different stages of nanotube formation with change in anodized time (a) 5, (b) 15, (c) 30, (d) 45, (e) 60 and (f) 120 min in electrolytes 0.1 mole/L NaF, 1.0 mole/L H$_2$SO$_4$ and 0.2 mole/L citric acid.
Mechanism for nanotube formation with a model has been described in detail in the discussion section of chapter six. Phases for the as grown nanotubes were evaluated using x-ray diffraction and energy dispersive spectroscopy.

5.2. Phase Analysis

EDS of the nanoporous samples shows Ti and O peaks. Gold peaks were seen in Figure 5.5 which came from the gold sputtered samples.

![Figure 5.5](credan22.genesio/genesis/genesis/ebismap/15-May-08/16/025/12.png)

**Figure 5.5.** EDS on nanoporous surface shows only Ti and O peaks.

Glancing Angle x-ray diffraction did not show any crystalline phases from the samples. Presence of Ti, O on the samples in EDS and no crystalline phases indicates formation of an amorphous titania phase. Anodized amorphous samples were then heat treated at different temperatures from 300 to 600 °C. Crystalline phases started emerging in anodized and heat-treated conditions of 300, 480 and 580 °C samples. The nanotubes crystallized to the anatase phase at 480 °C, in and at 580 °C rutile phase emerge in XRD results as shown in **Figure 5.6**.
Figure 5.6. Phase analysis using GAXRD for nanoporous surfaces. The anodized sample shows only Ti-peaks whereas when heat treated (b) 480 °C and (c) at 580 °C it shows crystalline phases. T denotes the peak from Ti, A and R from anatase and rutile phases.

5.3. Surface properties

Surface roughness was measured on the nanoporous surfaces, which showed an r.m.s value of 0.0518 μm, much higher than the Ti-control (0.0074 μm). Contact angle on the anodized and Ti-control surfaces were measured using cell media and distilled water as shown in Figure 5.7. It was noticed that the contact angle changed significantly in the different steps of nanotube formation as anodization time varied from 5 to 240 minutes.
Surface morphology has an affect on the contact angle and wettability. Anodized surfaces started at 5 minutes show a contact angle of 25° in water media. Contact angle decreases with the onset of bigger pores in 60 minutes on anodized samples, as seen in the Figure 5.7 above samples. The contact angle in water decreases significantly to 7.5° and 4° for nanoporous morphology at 120 minute and 240 minutes. Contact angles were also measured on the nanopores and control Ti surfaces with diiodomethane, formamide and glycerol to calculate the surface energy (see chapter three-section 3.3C equation (1)). It was noticed that surface energy increases with the formation of the nanotube as seen in Figure 5.8. With an open porous structure, the surface energy for 240-minutes samples shows the highest value of 332.46 mJ/m².

Figure 5.7. Variation of contact angle at different stages of nanotube formation.
5.4. Mechanical properties of nanotubes

The mechanical properties of the TiO$_2$ nanotubes were probed by nanoindentation. A small-harmonic/high-frequency amplitude is superimposed over indentation loading and the contact stiffness of the sample is measured from the displacement response at the excitation frequency. From this contact stiffness, the Young’s modulus of the material has been derived. Thus, the modulus or hardness has been determined instantaneously as a function of depth. For all experiments, indentation was carried out using a Berkovich (three-sided pyramid) indenter. Nanoindentation on the TiO$_2$ nanotube structures was used to extract the apparent Young’s modulus and hardness from load and displacement curves as shown in Figure 5.9. Approximate hardness and Young’s modulus of TiO$_2$ nanotubes were respectively 1.2-1.7 GPa and 36–43 GPa. The term apparent is used because the measured modulus and hardness are not only a function of the nanotubes, but are also influenced by the substrate. None of the indentations for 2 or 4 h, however, showed signs of delamination as shown Figure 5.10. Thus, it is important to
note that coating delamination did not occur in thicker coatings (600–650 nm). This suggests that the adhesion strength may be enhanced with increases in coating thickness. In nanoindentation of thin films, it is common to determine the properties of the film at indentation depths less than 10% of the film thickness.\textsuperscript{48}

![Figure 5.9](image_url)

**Figure 5.9.** Characteristic (a) load, (b) apparent Young’s Modulus and (c) apparent hardness vs indentation displacements (depth) for nanotube samples.\textsuperscript{47}

This is to minimize contributions from the substrate. In our case, 10% of the film thickness is of the order of 60 nm or less. One of the simplest models is the “rule of mixtures,” commonly used for continuous fiber reinforced composites. It assumes an isostrain condition (longitudinal loading, parallel to the fibers) or isostress condition (transverse loading, perpendicular to the fibers). In the longitudinal orientation, the Young’s modulus of the composite, $E_c$, is given by:
\[ E_c = E_f V_f + E_m V_m; \]

where \( E \) and \( V \) correspond to Young’s modulus and volume fraction, respectively. The subscripts \( f \) and \( m \) denote fiber and matrix. For analysis we assume the volume fraction of pores in the coating is approximately 72% (from image analysis) and a modulus for dense amorphous TiO\(_2\) of 130–150 GPa (for the tubes themselves).\(^{48}\)

**Figure 5.10.** FESEM micrograph of nanoindentation on nanoporous TiO\(_2\) surface.\(^{48}\)

For analysis we assume the volume fraction of pores in the coating is approximately 72% (from image analysis) and a modulus for dense amorphous TiO\(_2\) of 130–150 GPa (for the tubes themselves).\(^{49}\)
5.5. *Cell-materials interactions on nanoporous surfaces*

A. *Cell morphology:* Figure 5.11 shows the osteoblast precursor cell attachment study on control Ti and on anodized titania nanotube surface for 3, 7, 11 days. The nanotube surface is osteoconductive as it allows attachment and proliferation of the osteoblast cells at its surface, in comparison to the control Ti.

![Figure 5.11](image)

**Figure 5.11.** Osteoblast cellular morphology on (a), (b), (c) Ti-control and (d), (e), (f) nanoporous surface for 3, 7 and 11 days.
**Figure 5.12.** (a) Nanoporous surface is entirely covered with osteoblast cells (a) low magnification and (b) high magnification shows nodules with extracellular matrix when cultured for 16 days.

**Figure 5.11a-c** shows the cells are elongated and flattened morphologically on the Ti-control surface. **Figure 5.11d-f** cells show a filamentous network structure with cell to cell attachment and cell spreading all along the nanoporous surface. Nodules are obtained as an early sign of differentiation after 11 days of cell culture in both Ti-control and nanoporous surface as shown in **Figure 5.11c** and **5.11f** respectively. Within 16 days of cell culture the nanoporous surface was completely covered with cells as shown in **Figure 5.12.** Extracellular matrix (ECM) was also visible on the nanoporous surface. High magnification microstructure imaging shows that filamentous microextensions spread by grasping the nanopores on the nanotube surface in **Figure 5.13.** Nonpores act as adhesion site for the filopodia extensions.
Figure 5.13. Human bone cell study after 11 days in nanoporous titania matrix at high magnification. Filopodia from cells use the nanoporous areas as anchorage sites for better cellular attachment.

Nanoporous surface gives cells adhesion sites for better cell attachment and proliferation. To further understand cell adhesion on the nanotube surface, we made microscopic-designed on the anodized surface. On the anodized titania surface some of the circular pockets of nanotubes was designed and the rest of the surface was etched out with HNA (a mixture of hydrofluoric acid, nitric acid and acetic acid) solution. This leaves us with a microstructure of nanopores on the circular region and the rest of the area exposed to the Ti-surface. Cells cultured for 5 and 11 days on these designed patterned samples showed cell attachment on the nanotube area with very few cells on the etched area as shown in Figure 5.14.
Figure 5.14. (a) Patterned surfaces shows good cell attachment in nanoporous structure compared to the etched surface where the Ti surface was exposed. (b), (c), are the nanoporous regions at low mag and high mag.; (d) etched Ti-areas at high mag.

A.1 Cellular behavior at different stages on nanotube formation: Cell culture for 5 days on different timed anodized surfaces shows different morphology. Figure 5.15a shows a rounded up morphology, with minimized surface area for attachment on the oxide surface anodized for 5
minutes, 15 minutes exposure to anodized surface shows more spreading but still cells show a circular morphology with a lower degree of bone-to-metal contact.

Figure 5.15. Osteoblast cells cultured for 5 days at different stages of nanotube formation. The cellular behavior on surfaces at different anodized times are (a) 5, (b) 15, (c) 30, (d) 45, (e) 60, (f) 90 and (g) 120 min with electrolytes 0.1 mole/L NaF, 1.0 mole/L H₂SO₄ and 0.2 mole/L citric acid.
Cell adhesion and proliferation improved with increase in anodization time from 30 min to 60 minute as shown in Figure 5.15c to 5.15e. Bigger pores in samples anodized for 60 minutes help in cell proliferation by using the filopodia extensions to grasp the surface. Further increase in anodized time to 90 minute cells shows spreading and covers the entire surface with small calcified nodules as an early sign of differentiation (Figure 5.15f). Different stages of nanotube formation influence cell attachment behavior as the surface morphology changes with the anodization time.

A.2 Cellular morphology on nanotubes formed with different chemistries: Cells cultured for 5 days on nanoporous surfaces with different electrolyte chemistries showed no significant difference in cellular behavior as shown in Figure 5.16.

Figure 5.16. Osteoblast cell interaction after 5 days of culture on the entire nanoporous surface fabricated with different electrolyte chemistries (Table 5.2). No significant difference in cellular behavior was noticed. (a) 0.1 mole/L NaF, 0.1 mole/L H₂SO₄, (b) 0.1 mole/L NaF, 1.0 mole/L H₂SO₄, (c) 0.1 mole/L NaF, 1.0 mole/L H₃PO₄, (d) 0.1 mole/L NaF, 1.0 mole/L CH₃COOH, (e) 0.1 mole/L NaF, 1.0 mole/L CH₃COOH, 1.0 mole/L H₂SO₄, (f) 0.1 mole/L NaF, 1.0 mole/L CH₃COOH, 1.0 mole/L H₂SO₄.
All of the nanoporous surface shows good spreading with extracellular matrix all over the surface. Nanoporous surfaces enhance the bone cell-materials interactions, much as pores give the cells additional focal contact points.

**B. Cell Adhesion:** Vinculin is a ubiquitous, highly conserved cytoskeletal protein that is localized in both cell-cell and cell-extracellular matrix (ECM) junctions.\(^4^9\) To understand the adhesion of OPC1 on the nanoporous surfaces, vinculin were used to evaluate the immunocytochemical activity with the help of confocal imaging. **Figure 5.17** shows vinculin expression on both the Ti-control and nanoporous surface after 5 days of culture.

![Figure 5.17](image)

**Figure 5.17.** Immunolocalization of adhesive molecule vinculin in OPC1 cells for 5 days culture on (a) Ti-control, (b) nanoporous surface. Arrows indicate the focal contact points.

It was noticed that dense vinculin-positive contacts on the nanoporous surface in **Figure 5.18b** compare to thin focal contacts on Ti-control surface as in **Figure 5.18a.** Confocal imaging with a higher number of focal contacts on nanoporous surface indicates that the cells attach more on the anodized surface. These results also show that osteoblast adhesion, spreading and formation of focal adhesion contacts, suggest initiation of adhesion-related cellular signaling, as two...
significantly different surfaces allow the nanoporous surface to be more conductive to osteoblastic cells.

C. Cell Differentiation: To further understand osteoblast differentiation, ALP enzymatic activity was evaluated on the nanoporous surface and compared with Ti-control. Figure 5.18 shows the ALP activity on the Ti-control and nanoporous surfaces for 5 and 11 days.

![Figure 5.18. Confocal micrographs of ALP expressions for 5 days in OPC1 cells cultured on (a) Ti-control (b) nanoporous and 11 days for (c) Ti-control (d) nanoporous surface.](image)
At day 5, the ALP activity of cells on a nanoporous surface in Figure 5.18b was higher than on a Ti-control surface in Figure 5.18a. With increase in culture time to day 11, ALP activity increased progressively with higher intensity of green fluorescence in nanoporous surfaces in Figure 5.18c.

D. Cell Proliferation: The MTT assay was used to determine the OPC1 cell proliferation on control-Ti and nanoporous surfaces. Figure 5.19 shows a comparison of cell densities on the different anodized surfaces for 5, 11 and 16 days. For 5 days of culture, a higher optical density was noticed in nanoporous than Ti-control surfaces.

![Figure 5.19](image)

Figure 5.19. Optical density measured after culture for 5, 11 and 16 days at a wavelength of 570 nm by reader. There was a significant difference in optical density after 11 days in the different anodized conditions samples. \( p^* < 0.001 \) compared with Ti-control at 11 days of cell culture.
After 11 days of culture a significant difference in optical density was noticed. A slight increase in density was noticed with increase in culture time to 16 days for anodic condition when compared with 11 days. However, the number of cells on the nanoporous surface was always high. Student t-tests were used to compare the mean spread area of the cells on nanoporous surface to cells growing on Ti-control for 11 days (p*<0.001) day. All these results suggest that cell adhesion, differentiation and cell spreading were increased by modifying the Ti surface with a porous titania morphology.

5.6. Apatite Deposition on a Nanoporous surface

The two different anodized time 4h samples were soaked in SBF for 7, 14 and 21 days.

![Apatite depositions on nanoporous surface for (a) 7, (b) 14, (c) 21 days. No precipitation is observed even after 21 days in (d) Ti-control.](image)

**Figure 5.20.** Apatite depositions on nanoporous surface for (a) 7, (b) 14, (c) 21 days. No precipitation is observed even after 21 days in (d) Ti-control.
Precipitation was observed on all the anodized surfaces from 7 to 21 days as shown in Figure 5.20a-c. No precipitation was observed in Figure 5.20 (d) on the Ti-control surface even after 21 days immersion. EDS results in Figure 5.21 show the deposition and presence of Ca, C and P other than Ti, O. It is mostly carbonate apatite.

Figure 5.21. EDS on nanoporous SBF immersed samples.
CHAPTER SIX

DISCUSSION

Formation of Nonporous oxide film: The different kinetic steps in growth of an anodic film reported by Vermileya\textsuperscript{50} are as follows: nucleation of the anodic film; growth of the crystals; possible formation of a continuous film; thickening of the film and limitation of the film thickness. Deplancke et al. described that there is always the presence of a natural continuous oxide film before anodization.\textsuperscript{51} Therefore the last two steps were changed as: thickening of the natural oxide film; oxygen evolution and dissolution of the film in acid. Anodic film formations are the result of the dissolution process. Electrodes acquire enough energy to surmount whatever energy barrier exists and dissolves into the electrolytic solution. The electrode surface becomes either rough and irregular or polished. In either case the surface contains many points of easy atom removal. The conditions leading to the formation of rough or polished surfaces are the following.\textsuperscript{50}

(a) If noncontinuous films are formed the interface becomes rough, because the reaction is confined to certain regions of the surface so that atom removal is not uniform.

(b) Continuous films, on the other hand, frequently grow by high-field ion conduction, the rate of growth being an exponential function of film thickness.

In general, oxide film begins inhibiting the active dissolution of titanium according to the following reaction:

\[ \text{Ti} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ + 4\text{e}^- \]

Maximum thickness to which an anodic film may be grown depends on the magnitude of the potential, which can be applied across the film. At low fields the current is proportional to the applied field, and at constant potential the thickness is proportional to the square root of time.\textsuperscript{50}
At high fields the current is an exponential function of the electric field strength and at constant potential the thickness is proportional to the logarithm of time. It is generally known that titanium oxide growth involves field-assisted migration of ions through the oxide films.\textsuperscript{52-53} Slower dissolution rate also creates a saturation effect on film thickness.

\textbf{Figure 6.1} Model to explain the nonporous oxide formation in acid electrolytes. (a) Formation of an oxide layer with active dissolution in titanium, (b) Titanium ions formed, (c) Ti-ions move from metal to the metal-oxide surface and oxygen ions move from electrolyte to electrolyte-oxide surface, (d) field assisted migration of ions take place, oxygen ions move towards the metal-oxide interface from the oxide-electrolyte interface and causes the growth of the oxide layer at metal-oxide interface.
Two theories have been proposed to explain the color of the anodized titanium sheets.51

(1) The color could be due to stoichiometric defects in the composition of the film.

(2) Interference phenomena may cause the coloration. Figure illustrates the second theory, which is called multiple interference theory.

If the incident beam 1 is a beam of white light, then the reflected beam, which is formed by the interfering beams 2, 5, 8, 11..., will be colored. This color will be the one reinforced by interference and will thus be the complementary color of the extinguished color. The value of the extinguished wavelength will be dependent on the thickness of the oxide film. Table 6.1 shows the conditions for extinction and reinforcement of wavelength in ideal conditions, i.e. a transparent oxide film (index of refraction, n2) with a constant thickness on an even and perfectly reflecting surface of titanium (index of refraction, n3).

**Table 6.1- Condition for the reinforcement and extinction of wavelength under ideal condition**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Reinforcement</th>
<th>Extinction</th>
</tr>
</thead>
<tbody>
<tr>
<td>n2 ≤ n3</td>
<td>(4π/λ) (n2d sina2)=2kπ</td>
<td>(4π/λ) (n2d sina2)=(2k+1)π</td>
</tr>
<tr>
<td>n2 &gt; n3</td>
<td>(4π/λ) (n2d sina2)=(2k+1)π</td>
<td>(4π/λ) (n2d sina2)=2kπ</td>
</tr>
</tbody>
</table>

When the value of the thickness d introduced in this formula is increased, a succession of colors is obtained. This theoretical succession is exactly the same as the succession that we observed experimentally, i.e. yellow, brown, dark blue, sky blue, green. The value of the most strongly absorbed wavelength increases with the thickness of the oxide film i.e. with the duration of anodization. The phenomena of interference are responsible for the colors of the films. For thick crystallized films these colors are a consequence of stoichiometric defects.
Formation of nanoporous oxide morphology: The mechanism for oxide nanoporous morphology in the presence of sodium fluoride and acid solution is completely different than the nonporous oxide morphology. It has been reported that the mechanism of formation of nanoporous alumina and titania are the same. In vacancy condensation mechanism, the growth and breakdown of anodic passive film were considered in terms of point defect models.\textsuperscript{54} Localized condensation of cations vacancies at the metal/film interface causes the breakdown of the anodic film to passivity. The condensation of cation takes place as there is enhanced ejection of cations from the film. The basic mechanism that leads to the formation of nanotubes involves two competing processes; one is electrochemical etch and the other is chemical dissolution.\textsuperscript{55-57}

In the first process, i.e., electrochemical etch, interaction of the metal with $O^{2-}/OH^-$ ions takes place which leads to an initial oxide layer formation in Figure 6.2a.\textsuperscript{56, 58} The oxide layer causes two interfaces-- metal-oxide interface and oxide-electrolyte interface. Under the application of an electric field, $Ti^{4+}$ cations migrate from the metal/oxide interface and move towards the oxide/electrolyte surface.\textsuperscript{59-61} The Ti-O bond undergoes polarization under the application of an electric field, thus promoting dissolution of metal cations. $Ti^{4+}$ cations dissolve into the electrolyte, and the free $O^{2-}$ anions migrate toward the metal-oxide interface to interact with the metal. Small pits are formed due to unequal distribution of ions in certain areas in the presence of $F^-$ ions by partially dissolving the oxide layer in Figure 6.2b with the following electrochemical reaction.\textsuperscript{35}

$$TiO_2 + 6F^- + 4H^+ \rightarrow TiF_6^{2-} + 2H_2O.$$  

At the bottom of the pits both electrochemical etching and chemical dissolution take place. These reduced the barrier layer and increase the electric field intensity in the process that results in further pore growth in Figure 6.2c. Chemical dissolution removes the top of the shallow pore
column on the surface of the oxide leaving an unanodized metallic region between the pores, which are available for electrochemical etching and chemical dissolution. Pores becomes deeper and enhances field assisted oxidation and dissolution Figure 6.2d. The channels are formed and separate pores from each other give rise to nanotubes in Figure 6.2e. The tube will grow until the electrochemical etch equals to the chemical dissolution at the bottom of the pore. Electrolytes were chosen to control the dissolution rate by adjusting the pH of the electrolyte using fluorine-containing salts such as sodium fluoride (NaF).

Overall electrochemical reactions for the formation of titania nanotube:

\[
4H_2O \rightarrow 4OH^- + 4H^+,
\]

\[
4OH^- \rightarrow 2H_2O + 2O^2^- + 4e^-
\]

\[
2H_2O \rightarrow 4H^+ + 4e^- + 2O^2-
\]

Anode: Ti + 2O^2- → TiO_2

Cathode: 4H^+ + 4e^- → 2H_2

Figure 6.2 A model depicting different stages of nanotube formation.
Generally the nanotubes were reported with hydrofluoric acid electrolyte. In this experimental procedure the electrolyte composition was chosen based on the three different functions of the electrolyte components in solution. Sulfuric acid has been the most widely used electrolyte, which forms nonporous or porous TiO$_2$ film respectively at low potentials and at high potentials. Nanotube length is restricted because of high chemical dissolution of the oxide in hydrofluoric acid containing the electrolyte. To control the dissolution rate sodium fluoride (NaF) was substituted for hydrofluoric acid.

Sodium fluoride totally dissociates in water with F$^-$ ions, which reacts with water to form HF and OH$^-$ ions. Dissociation constant ($K_{a1}$) of HF is $6.8 \times 10^{-4}$ is very low in comparison to strong acid like H$_2$SO$_4$ whose second dissociation constant ($K_{a2}$) is $1.2 \times 10^{-2}$ or H$_3$PO$_4$ with ($K_{a1}$) $7.5 \times 10^{-3}$. HF is weak acid in acidic solution. Most of the fluoride exists in the form of HF. Presence of these strong acids in electrolytes prevents HF from dissociation and thus prevents the chemical dissolution to be too high and maintains the nanoporous structure. It was noticed that electrolyte chemistry with the presence of F$^-$ ions from salts NaF, KF and HF were needed along with a low pH acidic solution like H$_2$SO$_4$ or H$_3$PO$_4$, CH$_3$COOH to form nanoporous morphology. Electrolyte chemistry with only fluoride ions or with only low pH grows only a non-porous TiO$_2$ film on Ti.

The electrolyte pH was adjusted using additives like citric acid and sodium hydroxide to 4.5.

\[
NaF \rightarrow Na^+ + F^- \quad \text{(1)}; \quad H_2O \rightarrow H^+ + OH^- \quad \text{(2)};
\]

Adding (1) and (2),

\[
NaF + H_2O \rightarrow Na^+ + OH^- + HF
\]

Rate constant for forward reaction $K_1 = [Na^+][OH^-][HF]/[NaF][H_2O]=$constant.
Adding NaOH causes the Na\(^+\) and OH\(^-\) ion concentration to increase and hence to make rate of forward reaction constant, the concentration of NaF should increase or concentration of HF should decreased. Hence the fluoride concentration decreases and so is the chemical dissolution and hence it takes long time (2h) to form nanotube. Increasing pH decreases the rate of chemical dissolution and long time for nanotube formation as well. For longer anodized time to 10h it was noticed a collapsed array of nanotubular structure causing nanotube pores to close at the top. This causes no more oxidation or dissolution at the bottom of the pore and nanotube length ceased to increases and so is the structure.

**Human-bone cell interactions:** Cell-materials interaction on the anodized nonporous and nanoporous morphology were evaluated and showed different cellular behavior. In some surfaces it was observed higher cell-materials interaction was observed, whereas surfaces anodized in H\(_2\)SO\(_4\) did show inferior interactions. This led me to investigate the co-relation between surface properties and cellular behavior as cells attached and proliferated on the surfaces. The surface of implant material has a crucial role for cell adhesion and cell proliferation. Different morphologies for the three different anodized surfaces lead to different cell-materials interactions. When a biomaterial is implanted in the body, its surface is immediately covered with blood and its serum proteins.\(^{62}\) Cell adhesive proteins such as fibronectin and vitronectin, found in high concentration in the blood, can provide attachment sites for cells. Materials that adsorb more attachment proteins can provide more sites for osteoblast precursor bonding to the implant, which then leads to faster bone in-growth and implant stabilization. Surface topography plays an important role as it gives focal adhesion points for the proteins to get attached and thus help in the cell adhesion process.\(^{63-64}\) The “Flowery effect” in H\(_3\)PO\(_4\), and a rough patterned HF anodized surface, gives more focal points than the smooth metallic Ti-control surface. Although
the three anodized surfaces are nonporous, only two of them gave better cell attachment. Nanoporous morphology with nanotubes also showed enhanced cell attachment and proliferation. FESEM images at high magnification in **Figure 5.13** show actin filaments of the cells anchoring at the numerous nanopores of the anodized matrix whose phase is titania. Ti-materials interaction with the osteoblast cells not only depends on the oxide surface, but also depends on the surface properties of the film. These observations lead us to investigate other factors that control cell-materials interactions.

Factors that also play a crucial role in cell attachment process are surface roughness, contact angles, wettability and surface energy. It is generally accepted that high roughness leads to better cell attachment. **Table 4.1** shows the average root mean square (r.m.s.) roughness values for different surfaces. There is almost an order of magnitude difference in roughness for the Ti-control and anodized surfaces. As-received and polished titanium samples show a smooth surface, with an r.m.s roughness value of 0.0074 μm. In the literature, it was also noticed that cellular adhesion, proliferation and detachment strength are sensitive to surface roughness. A roughness of 4.68 μm on HA disc-shaped pellets surface showed higher cellular adhesion than a roughness of 0.73 μm.65
Figure 6.3. Osteoblast cells exhibit different morphologies on different grades of rougher Ti-surface. Samples were graded at (a) 180, (b) 240, (c) 320, (d) 400, (e) 600 and (f) 1200 SiC paper.

Ti-surface was ground to different surface roughnesses using silicon carbide paper grade 180, 240, 320, 400, 600 and 1200. Human osteoblast precursor cells (OPC1) were seeded onto the different grounded sample surfaces, and cultured for 5 days. Cells showed elongated morphology and are extended parallel to the grooves in 180, 240, 320-grade roughness surfaces in Figure 6.3. Cells grown on grade 400 polished samples have long fine cytoplasmic extensions in multiple directions. Cells are oriented with respect to the polishing edges. Less rough sample surfaces at 600 and 1200-grade cells show round up morphology with minimum surface area in contact. Cell orientation is occasional in 600-grade polished samples and completely disappears in 1200-grade
polished surfaces. The above results reveal a clear influence of surface roughness of the Ti-surface in cell morphology. These results also validate the point that the rougher the surface the better the cells attach.

Contact Angle on the anodized and Ti-control surface was measured using cell media and distilled water as shown in Figure 4.4. It was noticed that the contact angle decreased significantly in the H₃PO₄ surface improving the wettability of the surface. Contact angles higher than 20° represent the hydrophobic nature of the surface. Since the cell media is water based, the cellular attachment is poor on any hydrophobic surface. An H₃PO₄ surface with improved wettability enhanced the cell attachment process. Webb et al. also noticed the high level of cell attachment on moderately hydrophilic surfaces. High contact angle with hydrophobic nature lead to poor cell attachment on H₂SO₄ surfaces. As the Ti-control has a smooth metallic material surface, the contact angle was as high as 70°. Spreading was mainly noticed on the nanoporous samples with a contact angle of 4° and 3° in distilled water and cell culture medium. Contact angle changes significantly during fabrication of nanoporous morphologies at different anodized times. Surface morphology changes from nonporous at 5 minutes anodized time, to nanoporous morphology for 60 minutes. Anodized oxide surfaces available for 5 minutes show a contact angle of 22°. With porous morphology in 60 minute and 90 minute samples, the contact angle in cell media decreases respectively to 5.5° and 6° as shown in Figure 5.7, improving wettability. Better cellular attachment was noticed for the porous 60 minute and 90 minute anodized porous samples. Increase in surface roughness improves wettability and thus helps in cell attachment by enhancing the formations of focal contacts or through adsorption of proteins. A porous matrix of titania nanotubes causes the liquid cell medium to penetrate and decrease the contact angle with
enhanced cell attachment. Low contact angle also leads to high surface energy, which is another factor that contributes to better cell attachment.

To better understand the cell-materials interaction effect of surface energies, the anodized surfaces were calculated using three different well-characterized liquids. The contact angles for each of the liquids on the samples are listed in Table 6.2. Contact angles in diiodomethane and formamide were $1^\circ$ and $5^\circ$ for H$_3$PO$_4$ anodized surfaces with a very high surface energy value, 101.27 mJ/m$^2$. The open microstructure of nanotubes also increases the surface energy, which also influences more osteoblast interactions. Significantly larger levels of cell attachment were noticed on rough nanoporous surfaces with high surface energy. The surface energy also changes significantly as shown in Figure 5.8. A low surface energy value of 62 mJ/m$^2$ in the first 5 minutes of oxidation to a high surface energy value of 332.42 mJ/m$^2$ in 240 minutes of anodized sample causes poor to improved cellular attachment behavior. A very low surface energy value of 55.60 mJ/m$^2$ leads to poor cellular attachment on the H$_2$SO$_4$ surface. Cell attachment and proliferation were inferior in Ti-control surface to anodized surfaces, indicating that surface energy plays an important role in cell-materials interaction. Other studies also showed that better cell attachment takes place on surfaces with higher energy. Redey et al. showed low-energy strata in carbonate apatite which lead to poorer human trabecular osteoblastic cell attachment and spreading than the high-energy strata of the hydroxyapatite surface. Lampin et al. also showed that influence of the degree of roughness affects the surface energy and hence cell adhesion process. These in vitro tests confirm that the anodized surface has higher osteoconductivity than that of Ti-control surface.

Osteoblasts are attachment-dependent cells i.e., they must attach first to the surface and then adhere spreads and mineralize their extracellular matrix. The attachment phase is an initial
stage that involves physicochemical linkage between cells and the attaching surface. Adhesion of
cells is affected by the attaching surface morphology, surface roughness, surface wettability and
surface energy.69-71 With an increase in roughness, the cell attachment is stronger as shown in 5
day cell culture results with vinculin protein expression, in Figure 4.9 and Figure 5.17, for
nonporous and nanoporous surfaces respectively. Both porous morphology and rougher surfaces
lead to increase in focal contacts on anodized surfaces, compared to Ti-control. One order of
higher roughness value in Table 6.1 on a nanoporous surface suggested synergistic effects of
vinculin expression. Ti6Al4V surfaces with degrees of roughness of grade 4000, 1200, 80 had
higher intensity of adhesion plaques than did the mirror-finish smooth surfaces reported by
Linez-Bataillon et al.72 Boyan et al. also observed a similar increase in focal adhesions formed
by MG63 osteoblast-like cells on a rougher Ti-surface.73 Focal contacts are distributed
throughout the cells, adhering to rough nanoporous surfaces, but are sparse and localized on the
smooth Ti-control. It is reported that osteoblast cells primarily attach to the implant surface with
the help of a receptor protein called integrin.74 These integrins not only help in anchoring cells to
their substrate, but also signals through these pathways as well, indicating that increased focal
adhesion on rougher surfaces contributes to physiologic response of the cells through this
mechanism. The ability of a substratum to promote the formation of more focal contacts is
therefore important for the implanted material, as focal cell adhesion formation can affect long-
term cell differentiation. Surface roughness affects the surface morphology, which in terms
affects the surface wettability. In the present case, cells were attached mainly to the hydrophilic
surface H3PO4 in Figure 4.6c than the hydrophobic surface Ti-control (Figure 4.6a). It is known
that the adhesion of the cells to the substrate involves (1) adsorption of serum proteins on the
substrate, (2) the contact of rounded cells with the substrate, (3) the attachment of the cells to the substrate and (4) the spreading of the cells on the substrate.\textsuperscript{75}

ALP is an early marker for osteoblast differentiation and it is generally accepted that differentiation increases with surface roughness.\textsuperscript{49-51} As osteoblasts mature, they produce extracellular matrix vesicles which are enriched in alkaline phosphatase specific activity; because of this specific enrichment, alkaline phosphatase is the marker enzyme for differentiation. It is found in higher levels of cells which mineralize their matrix, such as osteoblast.\textsuperscript{70} Boyan et al. found that with increase in surface roughness of the Ti substrate, the ALP activity of MG 63 cells was higher after 7 days.\textsuperscript{76} Similar results were observed in our 5 day results, which showed higher ALP expressions in “rougher” H\textsubscript{3}PO\textsubscript{4} and HF anodized surfaces than the smooth polished Ti-control and the H\textsubscript{2}SO\textsubscript{4} anodized surface seen in Figure 4.11a-d. ALP also plays a role in mineralization. With increase in culture time to 11 days there is an enhancement in ALP expression in all the anodized surfaces, indicating a beneficial effect of the surface by anodization on ECM mineralization. Cells exhibited more enhanced osteoblast differentiation when cultured on nanoporous surface than on the Ti-control. These observations indicate that the cells grown on the nanoporous surface start to initiate expression of the mature osteoblastic phenomenon at a higher rate than the Ti-control surface. One of the key reasons for such behavior is the modified surface and high roughness value of the nanoporous surface. Other researchers have also noticed enhanced cell differentiation on rough surfaces.\textsuperscript{62, 65} Porous morphology helps integrin receptor protein to anchor cells, thus making them an excellent model for signaling mechanisms used by osteoblasts in response to high surface roughness and hence enhancement of the differentiation process.
From the MTT assay study, we observed that osteoblast cell proliferation increased significantly when osteoblasts were cultured for 11 and 16 days. The proliferation of those cells usually follows a standard pattern: (1) initial lag-phase: period of adaptation during which cells replace elements of the cell surface; (2) log-phase: phase of exponential increase in cell number, and (3) a plateau phase: cell division is balanced by cell loss and bone cell phenotypic markers are expressed. Cell cultures were consistent and development a log phase after 5 days of culture high yield of proliferative activity was observed in osteoblast cells after 11 days. It was also noticed that cell proliferation was also affected by surface topography and roughness. Significant differences in cell density were observed on both anodized surfaces and flat polished Ti-control surfaces. The difference in cell density was statistically significant after 11 days in anodized H₃PO₄ and HF anodized surface, in comparison Ti-control.

**Bone-mineralization study:** Mineralization study of anodized samples showed the presence of Ca-compound on the oxide surface. It has been suggested that apatite, once nucleated, will grow spontaneously in the human body’s environment. Several reports of modeling for the apatite deposition on the TiO₂-surface have been identified. A TiO₂ layer forms Ti-OH groups in an aqueous solution and these groups serve as nucleation sites for apatite crystals, and favor deposition. Reaction of Ti-OH groups with aqueous solution changed the surface charge of the Ti-surface. The negatively charged OH⁻ interacts with positively charged calcium ions and thus reveals positive charge on the substrate surface. The surface then interacts with phosphate groups and forms amorphous calcium phosphate. These calcium phosphates eventually crystallize to bone-like apatite. A model at this process is provided to give the different steps involved in apatite deposition processes as in Figure 6.3 (i)-(vi). High ability for apatite deposition on heat-treated samples led us to study the influence of the surface properties on the kinetics of
nucleation of calcium-apatite. Surface properties were characterized by contact angle measurement on two different surfaces: one with an anatase phase heat-treated at 400 °C, and another anodized oxide surface with anatase/amorphous phase. The observed contact angles of various liquids are much larger in anatase/amorphous surfaces than just the anatase surface (Table 6.2).

![Diagram of apatite deposition](image)

**Figure 6.4.** Model depicting the different stages of apatite deposition on the anodic oxide surface.\(^8\)
In the process of nucleation, the tendency for a crystal to form depends on two thermodynamic factors- (a) Gibbs free energy and (b) an activation energy barrier to diffusion from bulk solution to substrate. The energy barrier, caused by interaction of Lewis acid-base interactions across the substrate interface, is reduced due to the high value of the Lewis base (γ⁺) parameter in anatase surfaces (Table 6.3).

**Table 6.2- Contact Angles (in Degrees) of various liquids on anatase and rutile surfaces**

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Anatase</th>
<th>Amorphous/Anatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diiodomethane</td>
<td>13 ± 0.82</td>
<td>30 ± 1.64</td>
</tr>
<tr>
<td>Glycerol</td>
<td>24 ± 1.70</td>
<td>28 ± 3.11</td>
</tr>
<tr>
<td>Formamide</td>
<td>10 ± 1.63</td>
<td>12 ± 1.71</td>
</tr>
<tr>
<td>Water</td>
<td>58 ± 2.08</td>
<td>60 ± 2</td>
</tr>
</tbody>
</table>

**Table 6.3- Surface energy components and parameters of Anatase and Anatase/Amorphous**

<table>
<thead>
<tr>
<th>Sample</th>
<th>γ₁,W (mJ/m²)</th>
<th>γ⁺ (mJ/m²)</th>
<th>γ⁻ (mJ/m²)</th>
<th>ΔG (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatase</td>
<td>49.5</td>
<td>57.57</td>
<td>51.97</td>
<td>-705.6</td>
</tr>
<tr>
<td>Anatase/Amorphous</td>
<td>44.22</td>
<td>4.57</td>
<td>2.53</td>
<td>-151.42</td>
</tr>
</tbody>
</table>

According to the physical model it is reported that an increase in adhesion energy promotes the nucleation on solid substrates. Calculated free energy of adhesion of water on an anatase surface is ΔG=−705.6 mJ/m². Low free energy on the anatase surface will facilitate faster nucleation of apatite than on a high free energy ΔG=−151.42 mJ/m² anatase/amorphous surface.
SUMMARY

The objective of this research was to modify the bioinert Ti-surface with as grown TiO₂ film for faster osseointegration by enhancing bone cell adhesion, proliferation and differentiation. Some of the general conclusions from this research are:

- Nonporous, rough microstructural topography and amorphous phases were achieved in anodized oxide films on Ti substrate with acid electrolytes H₂SO₄, HF and H₃PO₄ at 20V for 60 minutes.
- Nanoporous morphology was obtained at 20V for 240 minutes using a fourth electrolyte composition (0.1 mole/L NaF, 1.0 mole/L H₂SO₄, 0.2 mole/L citric acid). Nanotubes were 50 nm in internal diameter with average cross-sectional length of 600 nm.
- In vitro bioactivity of anodized titanium surface in SBF reveals: oxide surfaces obtained in H₂SO₄ and H₃PO₄ electrolyte show low ability for mineralization. Heat treated at 400 and 600 °C show increased precipitation in SBF. Heat treatment forms anatase phase which has low free energy of adhesion than the anodized amorphous samples, and increases ability for mineralization.
- Cell morphology with human osteoblast bone cells in nonporous and nanoporous surfaces showed the following features:
  - Nonporous surface with H₃PO₄ and HF electrolytes showed good cell attachment, colonization with extracellular matrix and nodules as an early sign of differentiation. Round up morphology, with minimum surface area of attachment and early cell death, was noticed on H₂SO₄ anodized samples.
  - Nanotube surface showed excellent bone cell attachment, proliferation and differentiation. Open nanoporous micrograph creates anchorage sites for the filopodia extensions for cell
attachment. Preferential bone cell attachment was noticed in nanoporous regions in comparison to no cells in the etched Ti surface.

- Cell adhesion, cell proliferation and cell differentiation respectively were tested with vinculin protein molecules, MTT-assays and alkaline phosphatase, showed high focal contact, high cell density and enhanced differentiation on nanoporous, HF and H₃PO₄ anodized oxide surfaces in comparison with the H₂SO₄ anodized surface and Ti-control surfaces.

- Surface properties like roughness, contact angle and surface energy changed from the nonporous to nanoporous morphology. Anodized surfaces in HF, electrolyte-C (0.1 mole/L NaF, 1.0 mole/L H₂SO₄, 0.2 mole/L citric acid) and H₃PO₄ showed high roughness, low contact angle with high wettability and enhanced the cell-material interactions.

From these results it can be concluded that the anodized titania surface is more osteoconductive than the control-Ti surface, a result that can have direct impact towards reduced healing time through faster bone cell attachment, growth and differentiation in vivo for load bearing dental and orthopedic Ti implants.
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Titanium silicide (Ti$_5$Si$_3$) synthesis under shock loading
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Titanium silicide (Ti$_3$Si$_3$) synthesis under shock loading

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Abstract

Ti–Si binary system shows exothermic reactions during the formation of several line compounds, which can be used for self-propagating high temperature synthesis (SHS) of starting powders. In this research, titanium silicide (Ti$_3$Si$_3$) was synthesized from high purity Ti and Si powders using shock waves in a 10 cm diameter and 14m long gas gun. Influences of compact density, shock velocity, milling time and filler concentrations were studied. Mullite was used as an inert ceramic filler in different wt.% and mixed with ball-milled powders. Phase analysis, microstructural analysis and microhardness measurements were done on samples that were recovered after shock loading. Results indicate that powders with a lower compact density generated higher temperatures during shock consolidation. The change in velocity generated different compressive stresses in Cu target ranging 4.4–8.6 GPa, and found to have a significant effect on the reaction kinetics. Both increasing filler material amount and decreasing milling time reduced the reaction kinetics. Though the presence of mullite decreased the reaction kinetics, it also reduced residual porosity in the compacts via forming an *in situ* intermetallic-ceramic composite.

*Keywords*: Shock synthesis of materials; SHS reaction; Titanium silicides; Ceramic fillers; Shock consolidation
Introduction

Synthesis of silicides by conventional metallurgical processing such as arc-melting, powder pressing and sintering, reaction synthesis and hot pressing methods [1,2] are quite common. In addition to the conventional techniques, several reaction synthesis schemes for example mechanical alloying [3–7], mechanically induced self-propagating reaction (MSR) [8–10] have been used to produce some of the intermetallic compounds such as Ti–Si, Mo–Si, Nb–Si binary systems. Over the past two decades synthesis of high-temperature materials through combustion reaction has been the focus of considerable attention. Highly exothermic reaction can become self-sustaining and once initiated will propagate through the reactants in the form of combustion waves. This method of material synthesis is commonly referred as self-propagating high-temperature synthesis or SHS. Self-sustaining chemical reactions can be initiated upon heating. There were two basic criteria on whether or not such reactions are possible [11]. First, the heat of the reaction must be large enough to generate a sufficiently high temperature above the melting point of the mixture, and the second, the mixing reaction time must be shorter than the time for dissipation of heat to the environment. Self propagating combustion method has been used for the synthesis of silicides of molybdenum [12, 13] and titanium [14]. Zhang et al. found that pre-heating of the elemental powder mixture was necessary for the self-sustaining mode in Mo$_3$Si, Mo$_5$Si$_3$ but not in MoSi$_2$ [12]. The temperature of the reaction for MoSi$_2$ was higher than the melting point of the diffusing species (Si). Liquid Si diffuses into Mo and yield MoSi$_2$ [13]. Trambukis et al. used a thermal explosion mode for the synthesis of titanium silicides, which involves two reactions: a solid-state diffusion reaction and a reaction involving a liquid phase [14]. It was noticed that increasing the titanium particle size and changing the heating rate affects the nature of the reaction from a solid state diffusional
process to a liquid-state reaction. Under constant heating rate experiments, a sequence of phase transformation was reported during the combustion synthesis reaction between silicon and titanium powders and was shown in Eq. (1) [14].

\[
\text{TiSi}_2 \rightarrow \text{TiSi} \rightarrow \text{Ti}_5\text{Si}_4 \rightarrow \text{Ti}_5\text{Si}_3 \quad (1)
\]

It has been reported self-propagating reactions can also be initiated by the passage of shock waves, which typically propagate at a speed that is much faster than those of SHS reaction fronts [15–17]. As a result the reaction will be triggered throughout the powder almost instantaneously. Large plastic deformation and high pressure in powder lead to a shock initiated chemical reaction by a mechanism different from those encountered in conventional process. Ti–Si, Mo–Si and Nb–Si binary systems were synthesized using shockwaves [3, 18–20]. MoSi\textsubscript{2} and TiSi\textsubscript{2} were synthesized by passing shock waves at a flyer velocity of 1 km/s on the mechanically pre-alloyed elemental powder mixtures of the constituent elements. The shock processed products were compared with the 50 h mechanically alloyed materials. It was reported that mechanical alloying resulted in metastable phases along with regular phases of TiSi\textsubscript{2} and MoSi\textsubscript{2}, but no metastable phase was found in the case of the shock synthesized material. The metastable phase obtained from mechanical alloying also transforms to the regular TiSi\textsubscript{2} phase, when the powder was annealed at 800 °C for 2 h. Comparing these results, it was concluded that the sample temperature reached at least 800 °C during the passage of shock waves [3]. Partial crystallization of the ball milled amorphous Ti\textsubscript{50}Si\textsubscript{50} alloy was also reported at a shock pressure of 32.6 GPa. Annealing the shock materials at 1023K for 1 h resulted in crystallization of TiSi and TiSi\textsubscript{2} phases [19]. Vecchio et al. synthesized silicides (Nb–Si and Mo–Si) using shock waves and concluded that the extent of reaction was found to be increased with shock energy, shock temperature and the energy of reaction. It was reported that silicon melting is a prerequisite in
the shock-induced reactions for Nb–Si and Mo–Si systems [20]. Shock-induced chemical reaction for Ti–Si depends on morphologies of the powder mixtures [20], shock energy and the presence of oxygen [15]. Thadani et al. [21] reported Ti–Si powder mixtures with a medium-particle size of < 45 μm experienced simultaneous plastic deformation of both Ti and Si particles; whereas fine powder particle size of 1–3 μm showed mostly agglomeration. Coarse Si powder particles of 105–149 μm showed extensive fractures due to brittleness and entrapment within the plastically deformed Ti. Based on these observations, it was concluded that medium morphology powders were the most reactive under shock loading. Krueger et al. reported that oxidation of Ti triggered the reaction for Ti₅Si₃ at a significantly lower shock energies (104 J/g) compared with the reaction under no oxygen (237 J/g with Ar but no reaction) [18]. Thadhani et al. [22] compared the shock densified Ti–Si sample with statically-pressed sample. It was observed that shock densified sample showed complete reaction at 1300 °C whereas statically pressed sample showed full reaction at 1100 °C. It was reported that Ti–Si powder mixture compacts react via defect-enhanced solid-state diffusion. Heat released due to the solid-state reaction is rapidly dissipated in the shock dense compacts but in statically-pressed compacts heat is localized and increase the temperature of the adjacent areas and the reaction continues in combustion type self sustaining [22].

Cooper et al. [23] studied the effect of initial void shape on hot spot formation. The void is approximated in the analytical model as a cylinder or a sphere with one-dimensional response that is a function only of the radius. According to their model, process of void collapse is the result of jet formation on a free surface as shock waves interacts with macroscopic hemispherical or hemicylindrical groove. Formation of long thin jets of materials is referred to be one of the contributing factors for achieving a hot spot temperature in axisymmetric model [23]. It was also
reported that both particle size and porosity of the powder has an effect on the hot spots. Increase in particle size of 5Ti + 3Si powders and decreases in initial porosity of the powders increases the shock threshold energy for reaction. Increase in particle size causes a decrease in specific area of reactants in contacts and an increase in heat sink volume per particle that leads to higher cooling rates at local hot spots [24]. Nemat-Nasser et al. studied dynamic void collapse in fcc single crystal under a uniform high strain compressive loading using thick walled cylinder method [25]. Externally applied explosives were used to collapse the thick-walled cylinder of the single crystal of copper. Complete and partial void collapses of the single crystal were observed at a detonation pressure of 4 and 2.3 GPa with an outer diameter of 60 and 50mm explosive charge. Cracks were produced in the single crystal during the unloading process by the tensile stresses that were produced by large heterogeneous plastic deformations that occur during loading regime [25]. Chen et al. described a shear assisted chemical reaction for Ti–Si system [26]. High-strain rate plastic deformation of densified Ti–Si powder mixtures, inside a mechanical threshold causes the reaction initiation at low detonation velocity explosives. Heat evolved during the intense shear localization propagates the exothermic reaction by forming liquid Si that surrounds fractured Ti particles [26].

One of the key questions still remains unanswered regarding the role of shock wave i.e., how does the reaction initiate by the passage of a shock wave? What role does shock wave plays in the synthesis of these SHS based intermetallic compounds? To answer the science behind the role of shock waves, we have added different amount of an inert ceramic filler material along with Ti and Si powders during the shock synthesis of Ti₃Si₃. Porosity varied from 40% to 60% to study how pore collapsing influences the shock synthesis. Another objective of this study was to examine the effects of ball milling time (24, 100 and 190 h) and impact velocity (0.25, 0.35 and
0.45 km/s) on the reaction kinetics. Phase analysis, microstructural analysis, microhardness measurements were done on recovered samples.

2. Experimental procedure

High purity Ti (−325 mesh i.e., >45μm, Alfa Aesar) and Si (−325 mesh, Alfa Aesar) powders were mixed in the molar ratio of Ti₅Si₃. Mechanical alloying of those powders were performed in a table top ball mill (US Stoneware, OH, USA) using zirconia milling media. Powders were milled using a ball-to-powder weight ratio as 5:1 in different time durations: 24, 100, and 190 h. Target Cu cells were compacted with four different theoretical densities i.e., 40, 45, 50, and 60 vol.% powder. Mullite (3Al₂O₃,2SiO₂), having an excellent thermal shock resistance, high thermal stability, thermal conductivity of 4.0–6.0 W/mK which is much lower in compare to Ti-21.9 and Si-148 W/m K, was used as the ceramic filler. 10, 20, 30 wt.% of mullite (MULCOA 70-30, E Minerals, USA) was added in Ti₅Si₃ and ball-milled for 3 h along with pre-alloyed and ball-milled Ti₅Si₃ powders. Four target cells were compacted with only 40% cell volume with 0, 10, 20 and 30 wt.% mullite. Impact velocities were varied from 0.25 to 0.45 km/s. Table 1 shows the experimental details.
Table 1: Different experimental conditions and related phase formations.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Porosity</th>
<th>Impact Velocity (km/s)</th>
<th>Milling time (h)</th>
<th>Phase formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-Si Powders</td>
<td>60%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃</td>
</tr>
<tr>
<td></td>
<td>55%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>0.35</td>
<td>24</td>
<td>Ti, Si</td>
</tr>
<tr>
<td>Ti-Si Powders+10wt.%Mullite</td>
<td>60%</td>
<td>0.25</td>
<td>24</td>
<td>Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>100</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>190</td>
<td>Ti₅Si₃, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.45</td>
<td>24</td>
<td>Ti₅Si₃, Mullite</td>
</tr>
<tr>
<td>Ti-Si Powders+20wt.%M</td>
<td>60%</td>
<td>0.25</td>
<td>24</td>
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</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>100</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
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<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>190</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.45</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td>Ti-Si Powders+30wt.%M</td>
<td>60%</td>
<td>0.25</td>
<td>24</td>
<td>Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.35</td>
<td>24</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
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<td>Ti₅Si₃, Ti, Si, Mullite</td>
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<tr>
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<td>190</td>
<td>Ti₅Si₃, Ti, Si, Mullite</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.45</td>
<td>24</td>
<td>Ti, Si, Mullite</td>
</tr>
</tbody>
</table>
2.1. Target and projectile

The experimental configuration used for dynamic consolidation of Ti₅Si₃ powders is illustrated in Fig. 1a. The target was made of a plate containing four cavities which had lateral dimensions of 1.85 cm×1.20 cm and 0.58 cm deep, covered by a Cu cover plate and a Cu base plate (10 cm in length) with lateral and longitudinal momentum traps (11.5 cm in length). The target cells were rectangular and placed off-center in a symmetrical arrangement to reduce tension when the release waves meet at the center. Oxygen free high conductivity (OFHC) copper was used to machine the target, and all the target parts were ground flat by lapping prior to assembly. The base plate, die plate and cover plate were bolted together outside of the impact region and momentum traps were attached with epoxy.

Fig. 1. Target parts (a) (i) side spall plate, (ii) thick base plate, (iii) cover plate, and (iv) square target plate with four powder cavities. (b) Target assembly.

The entire assembly was then attached to a circular target ring with epoxy to fit in the circular mouth of the gas gun. The target set up is shown in Fig. 1b. Similar target configuration was
used to study the consolidation of 304 stainless-steel powders [27]. The impactor was 0.49cm thick OFHC copper, attached with epoxy to a 20 cm long 6061-T6 aluminum projectile.

The main advantage of using a square target was to reduce the effect of tension in comparison with a circular target. Tensions are caused at the center of the target from the release waves once shock wave passes through the target. The momentum trap mainly removes the energy from reflected shock waves by trapping the reflected momentum into these plates after first loading. In our target design, the momentum trap falls off as it cannot sustain the tension and thus reduces the momentum of the target. Presence of lateral spall plate also reduces tension in a similar way as momentum trap. Shock wave experiments were performed in a 10 cm diameter and 14m long gas gun. Impact velocity and tilt measurements were obtained by means of a stepped circular array of shorting pins surrounding the target disk. Tilt measurements were taken to record the angle between the flat projectile head and copper containment plate surface upon impact. In these experiments, tilt angles were found to be less than 0.5 mrad. Six numbers of pins were attached at the rear end of the gun just before the target assembly for impact velocity measurements. Impact velocity in the experiment is determined by measuring the distance between the pins and time interval between pins shots by the projectile upon impact. Peak stresses at the Cu flyer plate was calculated from the longitudinal stress \( P_x \) and particle velocity \( u_p \) information using Eq. (2) [28]:

\[
P_x = 352.6 \ u_p + 132.1 \ u_p^2 \tag{2}
\]

Neglecting the powder volume, powder materials stress is calculated on Cu, as both the target and the projectile are made of OFHC Cu. For an impact velocity of 0.25 km/s

\[
P_x = -352.6(u_p - 0.25) + 132.1(u_p - 0.25)^2 \tag{3}
\]
The point of intersection of Eqs. (2) and (3) gives the stress values in Cu. At 0.25 km/s the point of intersection from the above two equation results is 4.4 GPa. Similar pressure calculations based on the above two equations shows the pressures were 6.57 and 8.6 GPa for the two impact velocities of 0.35 and 0.45 km/s, respectively. All experiments were conducted at room temperature. The consolidated powder specimens in the copper containment plate were recovered from a rag-filled catcher tank.

2.2. Phase analysis, microstructure and microhardness

A piece of the shock processed recovered sample was mounted in epoxy and polished using up to 1 micron diamond spray. Microhardness values were also obtained from polished samples. For phase analysis, shocked samples were ground and the powders were examined using X-ray diffraction. Phase analysis of the recovered samples was done using X-ray diffraction with a Philips PW 3040/00 X’pert MPD system at room temperature using Co Kα radiation with a Ni-filter. XRD studies were carried out over a 2θ range from 20° to 70° at a step size of 0.02° (2θ) and a count time 0.5 s per step. A Hitachi scanning electron microscopy (SEM) system was used (Tokyo, Japan). Microhardness measurements were taken using a Leco Vickers microhardness tester. At least 10 measurements were made on each data point on a relatively clean and polished region of the recovered samples.

3. Results

Ti–Si binary system shows several intermetallic compounds such as TiSi2 and Ti5Si3. Among them Ti5Si3 shows the highest heat of reaction, −1590 cal/cm³ [29]. Due to such high heat of reaction, once the reaction is initiated, it will propagate in the form of combustion waves. In the first generation of our experiments, the porosity was varied. Each cell with fixed volume was filled with powders such that only 40–60% of the cell volume was filled with ball milled Ti–Si
powders. In this way, the porosity was varied in four cells from 60, 55, 50 and 40, vol.%. Self-propagating reactions between Si and Ti have been initiated by the passage of shock waves with an impact velocity of 0.35 km/s on 24 h ball milled pre-alloyed Ti$_5$Si$_3$ powders. The recovered sample is shown in Fig. 2. It was found that 60% porosity powder reacted most, and the reactivity of the powder particles decreased with decreasing porosity.

![Recovered samples impacted at 0.35 km/s. (1) 40 vol.% porosity, (2) 55 vol.% porosity, (3) 50 vol.% porosity and (4) 60 vol.% porosity. Complete reaction and melting was observed in 60 vol.% porosity samples, while no reaction was observed in the 40 vol.% porosity sample. All powders were ball-milled for 24 h before impacted at 0.35 km/s.

The sample with 40 vol.% porosity showed only compaction but no reaction. X-ray diffraction shows only Ti$_5$Si$_3$ phase in 60 vol.% porosity cell, while no reaction was observed for 40 vol.% porosity.](image)
porosity sample. This is shown in Fig. 3. Presence of both Ti$_5$Si$_3$ phases and unreacted Ti and Si were found for 55% and 50% porosity samples. SEM of the recovered samples are shown in Fig. 4. Microstructure of the 60 vol.% porosity sample shows big pores with reacted mass. From these results, it was clear that not only the shock wave, but the inherent porosity in the sample has pronounced effect on the reaction kinetics. Moreover, 50 and 55 vol.% porosity samples showed that even if the reaction starts due to shock wave, the completion of the reaction depend on powder particles properties.

**Fig. 3.** X-ray diffraction results for as received Si and Ti-powders. Recovered shocked samples are shown in: (a) 60 vol.% porosity shows Ti$_5$Si$_3$ phase in which peaks are indexed as 1: (21 30), 2: (10 12), 3: (21 30), 4: (30 30), 5: (11 22). (b) 40 vol.% porosity unreacted (1) (2) (3) (4) (5) (a) 60 % porosity reacted (b) 40 % porosity unreacted Ti Si

SEM of the recovered samples are shown in Fig. 4. Microstructure of the 60 vol.% porosity sample shows big pores with reacted mass. From these results, it was clear that not only the shock wave, but the inherent porosity in the sample has pronounced effect on the reaction.
kinetics. Moreover, 50 and 55 vol.% porosity samples showed that even if the reaction starts due to shock wave, the completion of the reaction depend on powder particles properties.

Fig. 4. Microstructure for (a) 60% and (b) 50% porosity recovered samples that were impacted at 0.35 km/s. Powders were ball-milled for 24 h prior to shock loading. (c) 40 vol.% porosity recovered sample impacted at 0.35 km/s. Only Ti and Si peaks were detected for this sample.

3.1. Influence of inert filler

To investigate the role of shock waves on Ti₅Si₃ synthesis, mullite (3Al₂O₃, 2SiO₂) ceramic powder was added as an inert filler. The powder porosity in all cavities was kept constant at 60 vol.% due to its high reactivity and only composition in each cavity was varied. 0, 10, 20 and 30 wt.% of mullite was added with pre-alloyed ball-milled Ti₅Si₃ powders and placed in four copper target cavities. The target was impacted at 0.35 km/s. X-ray diffraction of the recovered samples showed a decrease in the reaction kinetics with increasing the mullite content as shown in Fig. 5. The 10 wt.% mullite addition showed a partial reaction because the diffraction peaks of Ti₅Si₃, mullite and unreacted phases of Ti and Si were found. Only unreacted phases of Ti, Si and mullite peaks were observed in the 30 vol.% mullite sample. Typical microstructures of the
recovered samples are shown in Fig. 6. Improved microstructure of the recovered samples was observed with 10 wt.% mullite addition in comparison to 0 wt.% mullite.

![Cobalt alloy X-ray diffraction results for recovered shocked sample with (a) 0 wt.% (b) 10 wt.% (c) 20 wt.% and (d) 30 wt.% mullite impacted at 0.35 km/s. Results show Ti₅Si₃ and mullite peaks for 0 wt.% (a), and unreacted peaks of Ti and Si along with mullite in 30 wt.% mullite (d).](image)

**Fig. 5.** X-ray diffraction results for recovered shocked sample with (a) 0 wt.% (b) 10 wt.% (c) 20 wt.% and (d) 30 wt.% mullite impacted at 0.35 km/s. Results show Ti₅Si₃ and mullite peaks for 0 wt.% (a), and unreacted peaks of Ti and Si along with mullite in 30 wt.% mullite (d).

Although the initial porosity for all the four samples were 60 vol.%, the final microstructure shows a difference in porosity as a function of mullite content. Hardness values increased with the addition of mullite and are shown in Fig. 7. Fully reacted system in the 10 wt.% mullite sample showed highest hardness value of 1.4 GPa. The increase in hardness was due to in situ formation of an intermetallic-ceramic composite of Ti₅Si₃ and mullite. Hardness values were lower for composites that in, which the reaction did not complete.
ullite impacted at 0.35 km/s. Powders were ball milled for 24 h before shock loading. Microstructure of recovered samples with (c) 30% mullite impacted at 0.35 km/s.

Fig. 6. Microstructure of recovered shocked samples for (a) 0 wt.% mullite and (b) 20 wt.% mullite impacted at 0.35 km/s. Powders were ball milled for 24 h before shock loading. Microstructure of recovered samples with (c) 30% mullite impacted at 0.35 km/s.

Fig. 7. Microhardness values of shock compacted samples show an increase due to the addition of mullite in Ti$_5$Si$_3$ at a constant velocity (0.35 km/s).
Fig. 8. X-ray diffraction results for ball milled and shock recovered powders of 10 wt.% mullite in Ti$_5$Si$_3$ for (a) 24 h, (b) 100 h, and (c) 190 h. All the tests were performed at an impact velocity 0.35 km/s.

3.2. Influence of milling time and impact velocity

To understand the influence of milling time, Ti and Si powders were ball-milled for 24, 100 and 190 h. Each of these ball-milled powders was mixed with 10, 20 and 30 wt.% percent mullite as the inert ceramic filler and compacted in the four cells of the target. Impact velocity of 0.35 km/s and 60 vol.% porosity in each target cells were kept constant. Only milling time of the powders was varied. Fig. 8 shows the effect of milling time for 10 vol.% mullite samples. X-ray diffraction result shows unreacted phases of Ti and Si for 24 h ball-milled shocked sample, while
complete reaction was observed for the 190 h sample. It was clear that increasing milling time of powders increased the reaction kinetics, when both the porosity and the impact velocity kept constant.

To confirm that Ti$_5$Si$_3$ formation did not happen during ball milling, X-ray diffraction was done on ball-milled powder. Both the 24 and 190 h ball milled powder show only unreacted phases of Ti and Si and the corresponding diffraction spectra are shown in Fig. 9.

![XRD traces of pre-shocked Ti and Si powders and their mixtures for different ball milling time.](image)

**Fig. 9.** XRD traces of pre-shocked Ti and Si powders and their mixtures for different ball milling time. (a) As-received Si powder, (b) as-received Ti-powder, (c) 24 h ball-milled Ti and Si powder mixture, (d) 190 h ball-milled Ti and Si powder mixture. All ball-milled powders show only Ti and Si phases.
SEM images of the preshocked ball-milled powders are shown in Fig. 10. The 190 h ball milled powder shows significant agglomeration, compared with the 24 h ball-milled powder prior to shock compaction.

![SEM images of preshocked ball-milled powders](image)

**Fig. 10.** SEM of pre-shocked powder at different ball milled times (a) 24 h and (b) 100 h. SEM of pre-shocked (c) 190 h ball-milled powder showing extensive agglomeration with increasing milling time.

Effects of shock loading on reaction kinetics were studied at three different impact velocities of 0.25, 0.35 and 0.45 km/s. A 60 vol.% porosity in each cell and 24 h ball-milling time of the pre-alloyed powders were kept constant. Four cells were compacted with 0, 10, 20 and 30 wt.% mullite. There was no reaction in any cell at an impact velocity of 0.25 km/s. Partial reaction was observed at an impact velocity of 0.35 km/s, but a complete reaction was observed when the impact velocity was 0.45 km/s. Microhardness values increased from 0.974 to 1.4 GPa for 10 wt.% mullite composition in partially reacted and completely reacted system.
4. Discussion

Our results show that shock wave can initiate an SHS type reaction, but the completion of that reaction depends on several factors such as the stored energy in the powder and the impact velocity. It is clear from the results that porosity is an important factor that controls the reaction kinetics. The high impact pressures produce hotspots with void collapsing, which is significant mechanism of surface heating at the boundaries as reported by Cooper et al. [23]. In their computational model of void collapse is based on two-dimensional plain strain where the spherical void approximated as a cylindrical void. Jetting is considered as a prominent feature of collapse mechanism in the model. Long thin jets of materials were the main contributing factors for high temperatures and hot spots. According to the model, the maximum velocity occurs just before the collapse in case of cylindrical voids but the triangular model exhibits higher velocity after collapse. In the triangular void model jetting initiates at the apexes of the void and then coalesces into a single jet just before collapse [23]. Based on the model proposed by Cooper et al. a similar model has been proposed to understand the influence of porosity. The main goal of our research is to understand the role of shock wave. Fig. 11 shows a schematic of our model. During shock synthesis, as the shock wave propagates through a porous media, the pore collapsed due to high stresses. As a result heat is released and local areas of hotspots are created. If the temperatures of the hotspots are higher than the temperature necessary to initiate the SHS reaction, Ti$_5$Si$_3$ formation will initiate. If a sample has a high porosity, it will create multiple areas with high localized temperature, which will initiate the reaction at multiple points. Ti$_5$Si$_3$ formation will not occur if the porosity in the sample is not high enough to raise the initiation temperature for the reaction. When all other parameters are constant, the 60 vol.% porosity
sample showed complete reaction, while the sample with a 40 vol.\% porosity didn’t show any reaction.

![Diagram](image)

**Fig. 11.** Model to explain formation of Ti$_5$Si$_3$ by shock waves. (i) Pre-shocked powder compaction with porosity in target cells (ii) Pore collapsing after shock wave (shown with arrows) passes through the powder. Hotspots are the regions formed by pore collapsing, shown with white spots initiates the reaction.

From these results, it can be concluded that it is not the shock wave, but the generation of high temperature due to shock wave via pore collapsing is the most important factor that controls the reaction kinetics.

To validate our hypothesis even further, we decided to use inert ceramic filler material that will act as a heat-sink. We used mullite ceramic with ball-milled Ti and Si powders. It was found that increase in the amount of mullite content decreased the reaction kinetics at a constant porosity of 60 vol.\% and impact velocity of 0.35 km/s. **Fig. 12** shows another schematic model with mullite filler materials. Mullite is uniformly distributed in the Ti–Si particles matrix. As shock wave passes through the powder mixture, pores collapse produced regions of local hotspots. Less number of hot spots are generated due to the presence of inert mullite filler particles as mullite acts as a heat sink and reduces local temperature. Since the amount of mullite is less than the porosity, some parts of the recovered sample may react and form Ti$_5$Si$_3$. For example, partial
reaction was observed in the sample with the addition of 10 wt.% mullite at 60 vol.% porosity at an impact velocity of 0.35 km/s.

\[ \text{Fig. 12. Model to explain effect of inert ceramic filler on the synthesis of Ti}_5\text{Si}_3 \text{ by shock waves. (i) Pre-shocked powder compaction with 60 vol.}% \text{ porosity in target cells. (ii) Decrease in hotspots with increase in mullite percentage as shock wave passes through the powder.} \]

Mullite not only absorbs the heat of the reaction during pore collapsing, but also reduces the Ti and Si particles contact surface. As a result, as the mullite content increased, the reaction kinetics decreased. These results clearly show that the role of shock wave is like a torch to ignite a reaction, but the starting materials primarily control the actual reaction rate.

We have also noticed that increasing the ball milling time from 24 to 190 h increased the powder particle deformation, which increased the stored energy in the system. The second important factor we addressed is the effect of milling time on Ti$_5$Si$_3$ formation. Ball milled powders were heat treated at different temperatures from 200 to 600 °C in furnace and then the phase analysis was done using XRD. We noticed unreacted peaks of Ti and Si in 24 h ball milled powder even in the sample using a heat treatment at 500 °C. A complete reactant was obtained only in the sample that was heat treated at 600 °C. X-ray diffraction on the 600 °C heat treated mass show fully reacted Ti$_5$Si$_3$ for 24 h ball-milled powder. In the case of 100 and 190 h ball milled powders, the formation of Ti$_5$Si$_3$ peaks was noticed at 500 and 400 °C heat treated samples,
respectively. From these heat treatment studies, it can be concluded that initiation temperature for the Ti$_5$Si$_3$ reaction decreases with the increase in milling time. For the case of 24 h ball-milled samples, at least 600 °C temperature was necessary to initiate the reaction under shock wave, while the same for 190 h samples was 400 °C. Similar observations were also reported by other researchers [15]. The reason for the decrease of initiation temperature is because of a higher stored energy obtained during milling of the powders. It is also important to note that once a SHS reaction starts, the heat release from the reactants helps to raise the temperature further and continue the reaction. We hypothesize that mullite added to samples acts only in the reaction state, and not the propagation part.

The increase of impact velocity from 0.35 to 0.45 km/s increased the shock-generated compressive stresses from 6.57 to 8.6 GPa. With an increase in the generated stress, even larger pores will be collapsed with generation of higher temperature. As a result, the reaction in the 10% mullite composition becomes complete under a shock impact at 0.45 km/s impact velocity due to this higher generated stress while a similar reaction only partially occurred under a 0.35 km/s impact.

5. Conclusions

Ti$_5$Si$_3$ was synthesized by shockwave from high purity Ti and Si powders. Lower compact density produced higher reactivity due to generation of higher temperature via pore collapsing in the sample. Presence of mullite as an inert filler decreased the reaction kinetics and also reduced the residual porosity. Fully reacted system with the filler showed higher Vickers microhardness values. Longer milling time decreased the initiation temperature for the reaction due to higher stored energy in the powders in the form of agglomeration.
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References


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Application of fused deposition in controlled microstructure metal-ceramic composites
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Abstract

Purpose – Al-alumina interconnected phase composites were processed using the direct fused deposition process. These materials with tailored microstructures can find applications as structural materials with gradient properties.

Design/methodology/approach – In this process, feedstock material with fused silica as a starting material was compounded at a high shear mixer and then extruded as a filament using a single screw extruder. Extruded filaments were used with a commercial fused deposition modeler, FDM 1650, to process controlled porosity green ceramic structures. Porous green ceramic preforms were subjected to binder removal and sintering cycles in furnace air. Controlled porosity sintered ceramic structures were infiltrated with Al 5052 metal by pressureless reactive metal infiltration to form an in situ Al-alumina structured composite.

Findings – The main advantage for this approach is to control distribution of both metal and ceramic phases in the composite. During metal infiltration good bonding was observed between the metal and the ceramic phases. Composites were tested under both quasi-static and dynamic shock loading to evaluate their mechanical properties. Compression strength of these composites was 689 ± 95 MPa.

Originality/value – This paper describes application of the direct fused deposition process for fabrication of ceramic/metal composites where both macrostructure as well as microstructure can be controlled simultaneously. The paper also focuses on one of the potential application area for 5052-Al alloy.
1. Introduction

Structured metal-ceramic composites were fabricated using fused silica ceramic preforms and Al metal. Although ceramic materials possess high strength, high corrosion resistance and excellent high temperature creep resistance, which makes them suitable for high temperature applications, they suffer from low ductility. Addition of metals into ceramics improves the toughness of ceramic-based composites. Several thermodynamically favorable reaction-based processes have been used to fabricate metal-ceramic composite parts including direct metal oxidation (Breval et al., 1990; Newkirk et al., 1987; Antolin et al., 1992), chemical vapor infiltration (Besmann et al., 1991), and liquid-solid displacement reactions (Breslin et al., 1994). Among these numerous processing techniques, the most relevant to our work involves liquid metal infiltrated ceramic composites. In these composites, liquid metal is infiltrated into a porous ceramic preform. Liquid metal penetrates through the open cell porous structure and fills the voids, which can be tailored via controlling pore size, pore volume and pore-pore interconnectivity. The resultant composite is typically a near net shape structure with tailored microstructure. The metal infiltration can be achieved via pressure-assisted or pressureless. In the case of pressure-assisted metal infiltration, a positive pressure forces liquid metal to penetrate fine interconnected pores of the ceramic preform. But for pressureless infiltration, thermodynamic forces cause liquid metal infiltration into the preform, where wetting of the ceramic by the metal is one of the most important issues. Pressureless metal infiltration can also be classified in two categories, non-reactive and reactive (Soundararajan et al., 2001; Loehman et al., 1996; Saiz and Tomsia, 1998; Bandyopadhyay, 1999). In non-reactive metal infiltration, no reaction occurs between the preform and the infiltrant, whereas in reactive metal infiltration, there is a reaction. The important issues in reactive metal infiltration are:
• choice of metal;
• reaction between the metal and the ceramic; and
• reaction parameters such as time, temperature and environment.

During the past two decades Al was used frequently as a metal of choice for the infiltrant due to its light-weight, low cost, low melting point and wide availability. Mullite-Al (Soundararajan et al., 2001; Bandyopadhyay, 1999; Saiz et al., 1996), Al2O3-Al (Loehman et al., 1996; Swaminathan et al., 2002), Al2O3-Al-AlN (Nagendra et al., 1999), and SiO2-Al (Fahrenholtz et al., 1998) composites have been processed via pressureless reactive metal infiltration. In all of the above composites, penetration occurs by a diffusion process (Loehman et al., 1996) and the driving force for the in situ reaction is the change in negative Gibbs free energy. The reaction between silica and molten Al were studied by Standage and Gani (1967) and observed that the reaction did not start immediately but proceeded at a constant rate after it began. Penetration of Al metal into silica between 700 and 1,000 °C was reported by Brondyke (1953) and mentioned that there was dissolution of Si in the molten Al. It was concluded that penetration was controlled by the newly formed Al2O3 layer at the interface.

In this research, the fused deposition of ceramics (FDC) process (Agarwala et al., 1996; Bandyopadhyay et al., 1997; Bandyopadhyay et al., 1998) was employed to fabricate 3D interconnected porosity honeycomb silica ceramic parts followed by pressureless metal infiltration to produce metal-ceramic composites. The advantage of using FDC over other processes is that pore size, pore volume, and pore-pore connectivity can be controlled to form green ceramic parts directly from a computer-generated file. Moreover, both the micro- and the macro-structures can be controlled simultaneously. Processing of these Al-Al2O3 composites and their mechanical properties under quasi-static and dynamic loading are reported here.
2. Experimental procedure

2.1 Processing

Feedstock material used for the FDC had 55 vol.% of fused SiO₂ loaded in polypropylene (PP) based-thermoplastic binder. Apart from commercial PP, the binder also contains vestowax and tackifier to modify the strength and viscosity of the binder. The binder system was developed at WSU and composed of 44 vol.% PP, 13.9 vol.% elastomer, 18.7 vol.% plasticizer, 7.8 vol.% tackifier and 15.6 vol.% wax. A complete design of experiments was conducted to develop this PP-based binder for FDC feedstocks. The fused silica feedstock used for FDC was comprised of 56 vol.% fused silica and the rest was PP-based binder system. Feedstock materials were compounded in a high-shear mixer (Polylab, Haake, Hamburg, Germany) at 180 °C for 1 h. Extrusion was carried out with a single screw extruder attached with the Haake-Polylab system. Silica mixtures were extruded at a screw speed of 7 rpm and the temperature profiles were 170°C for zone 1, 172 °C for zone 2, 174 °C for zone 3 and 168°C for die zone or zone 4. The rod die was 1.78 mm in diameter. A horizontal conveyor belt was attached to the extruder for continuous extrusion of filament and subsequent spooling. Extruded filament was used with a commercial fused deposition modeler 1650 (Stratasys, Eden Prairie, MN) as a feedstock. Set-point temperature for building parts from the silica filament was established at 235 and 237 °C for main and support materials, respectively. Envelope or surrounding temperature was set at 48 °C and a material flow rate of 120 percent was established for silica filaments. FDM machines require several presets material dependent values such as preflow, start-flow, main flow, roll back and head speed. These values are preset in Stratasys’ Quickslice software for commercial filament materials such as investment casting wax and acrylonitrile butadiene styrene. Commercially available filaments have different material characteristics such as viscosity,
melting points, and drag coefficient compared to the silica filaments developed for our work. New sets of material-dependent values had to be established for the silica filaments to improve the quality of parts fabricated from the silica filament. New preset values established for the silica filaments are given in Table I. The filament was fed through a liquifier tip of 0.625 mm diameter and parts were built according to desired CAD files. The semi-molten filament was deposited in x and y directions forming a complete cross-section of the part, before being lowered to 0.35mm for the next layer deposition on top of the previous layer. This process continues until the part manufacturing was done. Parts used for quasi-static and dynamic experiments were 25 mm diameter and 25mm long cylinders with road width (width of deposited filament) of 0.625mm and road gap (distance between ordered deposits of filament) of 1.625 mm, with a 90° offset between each layer. We have also produced other shapes to show the flexibility of the process.

Once green porous ceramic parts were processed, parts were then subjected to a binder removal and sintering cycle in a muffle furnace. Green parts were packed in fine Al₂O₃ (A16SG, ALCOA) setter powder to wick out binder materials. The entire assembly was heated to 1,300 °C using a stepped time-temperature cycle of 2 °C/min and then soaked for three hours at 1,300 °C before cooled back to room temperature at 2 °C/min. Sintered samples were taken out of the alumina setter bed and cleaned in compressed air, before metal infiltration. Figure 1 shows a schematic process flow chart. Figure 2(a) shows as processed or green parts after FDC with different shapes, and Figure 2(b) shows porous SiO₂ structures after binder removal and sintering.
2.2 *Metal infiltration*

Controlled porosity sintered ceramic parts were placed in an alumina crucible with small coupons of Al-5052 for pressureless reactive metal infiltration at 1,150 °C for 1 h in a muffle furnace (Thermolyne, Iowa). Metal infiltration was carried out at 1,150 °C in ambient atmosphere because it was found that the highest reaction rate can be achieved at that temperature (Soundararajan et al., 2001). The equilibrium reaction between silica and Al is (Loehman et al., 1996):

\[
4\text{Al} + 3\text{SiO}_2 = 2\text{Al}_2\text{O}_3 + 3\text{Si} \quad (\Delta G^0 = -525 \text{ kJ/mol at 1,500 °C}) \tag{1}
\]

In this substitutive reaction, Al substitutes for Si and forms \(\text{Al}_2\text{O}_3\); and the displaced Si moves to the molten Al melt. The substitution reaction is favored because of higher stability of \(\text{Al}_2\text{O}_3\) compared to \(\text{SiO}_2\) due to a lower Gibbs free energy at the reaction temperature. The crucible was cooled to 750 °C and the metal infiltrated samples were taken out of the crucible. A final polishing or surface finish operation was required before testing these samples. Figure 2(c) shows an optical image of \(\text{Al}_2\text{O}_3\)-Al metal-ceramic composites processed from porous fused silica preforms.

<table>
<thead>
<tr>
<th>Material dependent parameters</th>
<th>Values for SiO(_2) filament</th>
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<td>Pre-flow</td>
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<td>Head speed</td>
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Table I Material-dependent parameters for the FDC of silica filaments
Fused Silica powder and polypropylene based thermoplastic binder → High shear mixing at 180 °C for 1h

Grinding of compounded mix

1.78 mm diameter filament extrusion using single screw extrusion

Fused deposition of ceramics of extruded filament at 210 °C using FDM 1650

Binder Removal and Sintering

**Controlled porosity silica ceramic performs**

Infiltration of Al 5052 @ 1150°C for 1h into silica ceramic performs

Structured Al-Al₂O₃ composites

**Figure 1** Process flow chart for the fabrication of structured Al-Al₂O₃ metal-ceramic composites using the FDC process.
Figure 2 (a) Green silica parts via fused deposition of ceramics (FDC) process, (b) FDC processed controlled porosity parts after binder removal and sintering in furnace air at 1300 °C,
(c) Structured Al-Al$_2$O$_3$ metal ceramic composites after infiltration of Al 5052 metal into controlled porosity silica performs.

2.3 X-ray diffraction

Phase analysis was carried out using a Philips PW 3040/00 X’pert MPD system at room temperature with Co-Ka radiation and a Ni-filter. XRD studies were carried out over the 20 range of 20-70 degrees at a step size of 0.02 (20) and a count time 0.5 s per step.

2.4 Quasi-static compression

Uniaxial compression testing was done with seven samples with the same volume fraction of metal. Cylindrical samples of 6mm diameter and 12mm long were used for these tests. Tests were conducted using an Instron servo-hydraulic machine under stroke control mode with a stroke rate of 1 mm/min.

2.5 Dynamic shock loading of samples

Shock wave experiments were performed on these Al$_2$O$_3$-Al composite samples in a 75 mm diameter 1.57 m long gas gun with a 50 mm long cylindrical projectile made of Al6061-T6. Impact velocity and tilt measurements were obtained by means of a stepped circular array of shorting pins surrounding the target disk. As the projectile hits each pin, the known distance between pins, measured by the traveling microscope, can be used to calculate velocity from the time interval between the shorting pins from the oscilloscope. The Al$_2$O$_3$-Al samples were cut into truncated cones of 25 mm diameter at the base with sides sloping inward at 88 from the perpendicular to a thickness of 6.25 mm. Samples were truncated for easier release during shock loading and reduce post-shock damage. Samples were mounted in a Al 6061-T6 guard ring of outer diameter 56 mm and inner diameter 25 mm to reduce the long-term radial release and also to reduce the additional plastic work produced in the core of the disk after the shock wave passes through the material (Johnson et al., 1994; Stevens and Jones, 1972). The sample assembly was
then fixed in an Al target ring with epoxy. The experimental set-up is shown in Figure 3(a) and (b).

**Figure 3** (a) Schematic of projectile and target configuration for dynamic impact tests for structured Al-alumina composites, (b) Front view of 76 mm diameter actual target assembly showing the sample at the center mounted in a 6061 Al ring
Further details of the optimization of this target set-up can be found in Atisivan et al. (2001). Three composite samples were impacted at velocities of 109, 121 and 135 m/s to understand the dynamic response of these materials. There were two reasons for choosing those impact velocities. Peak velocity of 150 m/s can be achieved from the 75 mm diameter gun. Also, the Hugoniot elastic limit (HEL) for the Al (6061) is 0.7 GPa (Huang and Asay, 2005). Our aim was to conduct all the experiments such that Al as the projectile and the metallic Al phase in the composite were within the elastic region of Al. Based on the above two limitations the impact velocity was chosen above 100 m/s and below 150 m/s.

3. Results and discussion

3.1 Composites microstructure

Volume fraction of metal in the final composite depends on the volume fraction porosity ($\phi_{th}$) in the sintered silica preform. All samples were fabricated with a uniform porosity distribution and the volume fraction porosity of the ceramic preform is calculated to be 24 percent based on Equation 2 (Hattiangadi and Bandyopadhyay, 2000):

$$\phi_{th} = \pi \cdot a \cdot L / 4 \cdot w^2$$

where $w$ is the road gap, $L$ is the path length of material deposition by the FDC process and $a$ is the road width of the deposited layer. This 24 percent was designed porosity into the structures. Silica performs also had, ~7 percent residual porosity due to incomplete sintering at 1,300 °C for 3 h. The volume change, $\Delta V$, due to the substitutional reaction during formation of alumina from silica as stated in Equation (1) can be calculated from the molar volumes, $V_m$, of reactants and products. In the Al/silica system, the relevant molar volume values in cm$^3$/mol are as follows: SiO$_2$ (26.43), Al (9.99), Al$_2$O$_3$ (25.62) and Si (12.00). The predicted volume change after Al
penetration, $\Delta V$, is 33.57 cm$^3$/vol and the fractional volume change per mole of SiO$_2$ is 0.106 based on Equation 3.

$$\Delta V/3V_m(\text{SiO}_2) = \frac{2V_m(\text{Al}_2\text{O}_3) + 3V_m(\text{Si}) - 3V_m(\text{SiO}_2)}{3V_m(\text{SiO}_2)}$$ (3)

Figure 4 (a) Microstructure of the as processed Al-alumina composite samples. C indicates alumina ceramic phase and M indicates Al metal. Also i and ii are in the areas chosen for further EDS analysis. (b) High magnification micrograph shows area (i) in Figure 4a.

It is clear that there is an increase in volume change due to alumina formation, which results in a porous microstructure in the sample as shown in Figure 4. Apparent density of the composite samples was measured as 2.56 g/cm$^3$. Figure 4(a) and (b) also shows that there is no gap between the metal and the ceramic-phase at the inter-phase, which indicates good bonding between the two phases. Some microcracking was observed in the ceramic phase, which is due to the volume change upon transformation from silica to $\alpha$-alumina.
Energy dispersive spectroscopy (EDS) analysis was conducted on the polished surfaces of the composite and shown in Figure 5. EDS analysis could not detect any Si in the metal as well as in the ceramic phase, but small amounts of Si was detected at the interface.

![EDS Analysis Graph](image)

**Figure 5** EDS analysis of the interface marked as area (i) and at the ceramic portion marked as area (ii) in Figure 4.

To further confirm the formation of a-alumina from silica, an X-ray diffraction study was conducted on thin cross-sections of composite samples. It can be observed from Figure 6 that only $\alpha$-Al$_2$O$_3$ and Al peaks are present. No unreacted SiO$_2$ peaks were found in the XRD results. Based on EDS and XRD results, it was clear that complete phase transformation, from silica to a-alumina, could be achieved during metal infiltration at 1,150°C, which formed an interpenetrating phase Al-Al$_2$O$_3$ composite.
Microstructures of the Al₂O₃-Al composite obtained from reaction between Al and SiO₂ ceramics by reactive metal infiltration shows both ceramic and metal phases. In FDC based fabrication, the microstructure can be tailored based on the tool-path and the part geometry, where ceramic width and the spacing between the ceramic roads will control the overall microstructure.

3.2 Mechanical properties

It was important to understand the failure criteria for the composite material processed via FDM. To better understand the failure analysis, it is important to know the threshold criteria for crack initiation and propagation under static and dynamic loading conditions. Uniaxial compression tests based on seven cylindrical samples of Al₂O₃-Al composites showed a compressive strength of 689 ± 95 MPa. Samples were sectioned to observe failure behavior due to quasi-static compression loading. Extensive cracking was observed along the ceramic phase of the
composites as shown in Figure 7(a). No cracking in the metal phase was observed. Crack deflection was also not seen in these samples. Damage was primarily concentrated along the brittle ceramic phase and was arrested at the metal-ceramic interface.

![Figure 7](image_url)

**Figure 7** (a) SEM micrograph showing failure in the ceramic phase under quasi-static compression loading. Arrow indicates direction of compression loading. (b) Micrograph after impact under dynamic shock loading at 135 m/s showing large crack along the ceramic phase. In both cases, cracking was only evident in the ceramic phases. C indicates alumina ceramic phase and M indicates Al metal.

Microhardness measurements on composites were taken using a Vickers indenter. All samples were polished, using 1mm diamond paste, and then microhardness measurements were taken on the polished surfaces of the metal, the ceramic and the metal-ceramic interface. Microhardness values were taken in an average of five different places on each phase and each of these indentations were far apart from each other. Loads of 100 gm (<1N) and 1 kg (<10 N) were applied on the metal and ceramic phases, respectively. Indentation diagonals for the metal and
the ceramic phases were 54.2 ±1.02 and 113.35 ± 0.76 mm, respectively. Since, ceramic is harder than metal, ceramic phases should show lower values for indentation diagonals than metal phases under the same applied load. But in this case the applied loads are different for the ceramic than the metal, which resulted in lower indentation diagonals for the metal phase. Microhardness values were 618 ± 24 MPa for the metal phase and 1,411 ± 19 MPa for the ceramic phase. Reported microhardness value in the alumina ceramic phase varies between 8 and 12 GPa (Loehman et al., 1996), which is significantly higher than our measured values. It is believed that the presence of micropores and cracks due to the substitution reaction and related volume change reduced the microhardness values in the as processed composite. The highest hardness value was obtained at the metal-ceramic interfaces, 3,557 ± 45 MPa, which indicates presence of some other harder phases such as spinels, but we could not detect any other phase using XRD measurements, though the EDS result indicated the presence of some Si at the interface.

Samples were also tested under dynamic loading to understand the deformation behavior at high strain rate. Plate impact dynamic recovery experiments were carried out at three different impact velocities, 109, 121 and 135 m/s. In all three cases, single piece samples were recovered. Samples tested at impact velocities 109 and 121 m/s did not show any cracking during cross-sectional observation of cut and polished samples under SEM, but similar examination of a 135 m/s sample show extensive cracking at the ceramic phase, as shown in Figure 7(b). Single piece sample recovery showed that though there was extensive damage in the ceramic phase of the composite, no catastrophic failure occurred due to the presence of the interconnected ductile metal phase. Cracks were arrested at the ceramic/metal interface. Cracking was more pronounced in the ceramic phase compare to the metal phase of the composite. Dynamic data also showed
that there is a threshold for crack initiation in ceramic phases of the composite samples, which was between 121 and 135 m/s for these composites. No significant difference was observed in cracking behavior between the quasi-static and the dynamic loading. In both cases, failure was primarily in the ceramic phase.

4. Conclusions

Controlled porosity silica preforms were fabricated via the FDC process. Porous silica preforms were infiltrated at 1,150°C for 1 h with Al 5052. XRD and EDS results indicate complete transformation of SiO₂ to α-alumina, and formation of an in situ Al/Al₂O₃ composite. Microstructure of the as processed composite reveals good interfacial bonding between the metal and the ceramic phases. During mechanical testing, cracking was observed in the ceramic phase of the composite under quasi-static as well as dynamic loading but no cracking was seen in the metals or at the interface. Al-alumina composites show a compressive strength of 689 ± 95 MPa. Dynamic deformation shows that the onset of failure occurred by crack propagation in the ceramic phase at an impact velocity between 121 and 135 m/s.

References


