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Bioactive Glass-Ceramic Coating of Titanium Substrates by Alkaline Hydrothermal Process

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Graduate Program in Biomedical Engineering
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Engineering Science
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BIOACTIVE GLASS-CERAMIC COATING OF TITANIUM SUBSTRATES BY ALKALINE HYDROTHERMAL PROCESS

(Thesis Format: Integrated Article)

by

Mohamed Ibrahim Osman Gebril

Graduate Program in Biomedical Engineering

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Engineering Science

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ABSTRACT

Surface modification is a well-known approach to enhance the osseointegration of titanium dental implants. In this study, a novel hydrothermal method for coating titanium surfaces with bioactive glass was developed. Our method included sol-gel synthesis of bioactive glass, followed by hydrothermal coating of titanium under different NaOH concentrations. The surface properties of coated substrates were evaluated by scanning electron microscopy, X-ray diffraction, energy dispersive X-ray spectroscopy, and surface profilometry. By varying the alkalinity of the hydrothermal process, different surface topographies, crystalline phases and chemistries could be obtained. Soaking the hydrothermally coated titanium substrates in simulated body fluid resulted in hydroxyapatite deposition, demonstrating bioactivity. All titanium surfaces were biocompatible and the topography of the coated titanium surfaces played a major role in determining the attachment of MC3T3-E1 osteoblastic cells. Our studies suggest that this novel coating method has the potential to improve the osseointegration of dental implants.

KEYWORDS

Dental implants, Titanium, Glass-ceramic, Osseointegration, Nanostructure, Sodium titanate, Sol-gel, Hydrothermal synthesis, Topography, Bioactivity, Cell attachment, Focal adhesion.

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CO-AUTHORSHIP STATEMENT

Chapter 1 " Introduction" and chapter 2 "Literature Review and Background" were written by Mohamed Gebril. Drs. Amin Rizkalla, S.J. Dixon, D.W. Hamilton and Noelle Ochotny did revisions and editing.

Chapter 3 entitled "Sol-gel Hydrothermal Bioactive Glass Coating of Titanium Substrates" was written by Mohamed Gebril with suggestions from Drs. Amin Rizkalla, S.J. Dixon, D.W. Hamilton, Roberta Flemming and Noelle Ochotny.

Experiments were designed by Dr. Amin Rizkalla. Mohamed Gebril performed all experiments. Bioactive glass synthesis, hydrothermal coating and most characterization work were conducted in Dr. Amin Rizkalla's Lab. Cell work was performed in Dr. S.J. Dixon's Lab.

Chapter 4 entitled "Summary and Conclusions" was written by Mohamed Gebril and edited by Drs. Amin Rizkalla and Noelle Ochotny.

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LIST OF ABBREVIATIONS

Al_2O_3	Aluminum oxide
BCP	Biphasic calcium phosphates
beta-TCP	Beta tricaclcium phosphate
BG	Bioactive glass
BPS	Buffered phosphate solution
BSA	Bovine serum albumin
$Ca(NO_3)_2$	Calcium nitrate
Ca^{2+}	calcium ion
CaP	Calcium phosphate
Cl^-	Chlorine ion
CPTi	Commercial pure titanium
DAPI	4', 6-diamidino-2-phenylindole
ECM	Extracellular matrix
EDX	Energy dispersive x-ray spectroscopy
FBS	Fetal bovine serum
HA	Hydroxyapatite
HCA	Hydroxycarbonate apatite
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ion
HPO_4^{2-}	hydrogen phosphate

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HVSFS	High-velocity suspension flame spraying
ICDD	International centre for diffraction data
K^+	Potassium ion
MC3T3-E1	Osteoblast-like cells
Mg^{++}	Magnesium ion
Na^+	Sodium ion
NaOH	Sodium hydroxide
P	Polished
PLD	Pulsed laser deposition
PSA	Polysialic acid
Ra	Surface roughness
RF	Radio frequency
RFGD	Radiofrequency glow discharge
SA	Sandblasted acid etched.
SBF	Simulated body fluid
SEM	Scanning electron microscopy
SiO_2	Silicon oxide
SLA	Sand-blasted large grit acid etched
TEOS	Tetraethyl orthosilicate
TEP	Triethyl phosphate
Ti	Titanium
TiO_2	Titanium oxide
Ti-OH	Titanium hydroxide

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TMOS	Tetramethoxysilane
XRD	X-ray diffraction

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CHAPTER 1: INTRODUCTION

1.1 OVERVIEW

Dental implants are medical devices placed in maxillary and mandibular bone to provide mechanical support for the replacement of lost teeth. Fabricated from titanium alloy, dental implants can be used to replace single teeth, or as anchors to support fixed bridges, removable partial or complete dentures [1]. Brånemark first presented the concept of osseointegration, in which titanium implants bond to bone without the formation of an interfacial layer [2]. Since Brånemark's initial discovery, the design and surface modifications of implants have been modified in attempts to optimize their longevity and clinical success [3] .

Brånemark and colleagues published their landmark paper on the replacement of teeth with titanium in a fully edentulous patient. Titanium and its alloys are the most widely used materials for dental implants owing to their superior properties, including mechanical strength, biocompatibility and corrosion resistance [4]. The ability of titanium to avoid corrosion is due to the formation of a titanium oxide surface layer. Titanium is a bioinert material and crucially it does not develop any chemical bond with bone. Several researchers have modified titanium implants in attempts to enhance the process of new bone formation in apposition to the implant to increase implant success rates and longevity. A wide range of surface modifications including mechanical and chemical approaches have been developed that are reviewed in Chapter 2. Increased rates of osseointegration were reported when titanium was coated with either hydroxyapatite or bioactive glass[3]. Bioactive glass is both osteoconductive and osteoinductive since it

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stimulates the recruitment of osteogenic cells as well as their subsequent differentiation and production of mineralized extracellular matrix [5]. On the other hand, calcium phosphates are biocompatible, osteoconductive material that strongly bond to bone [6, 7]. Different methods of surface coating are described in the literature including plasma spraying and dip coating. However, such techniques lead to unfavourable properties in the formed layers due to an imbalance of coating on the surface, as well as the requirement of high temperature that result in nonhomogeneous properties. Indeed, in certain cases, bioactive glass and hydroxyapatite added to implants using these techniques failed, largely due to delamination [3]. Alternate methods for forming and coating these layers are needed which is the focus of this thesis.

1.2 OBJECTIVES OF THE THESIS

Taking into account the limitations described in formation of homogenous layers of hydroxyapatite and bioactive glass on titanium the overall focus of this study is to synthesize a bioactive and osteoconductive nanowire coating onto titanium substrates by a two stage sol-gel-hydrothermal process, and to develop a novel sol-gel hydrothermal coating method onto titanium (Ti) implants. We also examine the effects of reaction pH and time under hydrothermal conditions on the surface topography and chemistry of the coatings and assess attachment of osteoblast like-cells to the bioactive glass coating.

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1.3 HYPOTHESIS

We hypothesize that the hydrothermal conditions and pH levels of the reaction will modulate the physical, chemical and biological properties of the coatings as well as enhance cell attachment on the surface of the coated titanium.

1.4 THESIS OUTLINE

As highlighted above, the objectives and hypothesis of this study focuses on the development of the bioactive glass coating on the surface of titanium through a two-step sol-gel hydrothermal process. The literature review is presented in chapter 2. Chapter 3 includes sol-gel synthesis of the bioactive glass and hydrothermal treatment process along with the different characterization techniques to validate the success of the developed approach. Chapter 4 provides a conclusion of this study and suggestions for future work.

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CHAPTER 2: LITERATURE REVIEW AND BACKGROUND

2.1 BONE ANATOMY AND PHYSIOLOGY

2.1.1 STRUCTURE AND FUNCTION OF BONE

Bone is a well-organized tissue that is composed of cells and extracellular matrix (ECM). The ECM contains organic and inorganic components. The organic and inorganic components provide bone's strength and flexibility. The organic component represents 40% of bone and is mainly composed of collagen type I and also includes glycoproteins, peptides, lipid materials, and adsorbed serum proteins [1]. Inorganic ECM is mainly composed of hydroxyapatite (HA), calcium phosphate, calcium carbonate, calcium fluoride and magnesium fluoride. Bone compressive and shear strength as well as hardness are enhanced by the carbonated HA complex [2]. Organic-inorganic structure of bone is formed as a result of nucleation of HA along the collagen fibers [3] The unique structure of bone explains its mechanical properties as well as its resistance towards different compressive and tensile forces applied during daily activities [2, 3].

Regarding the macroscopic structure of bone, there are two different types of bone that can be differentiated by the degree of macroporosity (Figure 2.1) [3, 4]. Cancellous also known as spongy or trabecular bone is the less dense type of bone. It has more than double the porosity of compact bone, and is rich in blood vessels, bone marrow and connective tissues. Cancellous bone plays a major role in hematopoiesis [4]. On the other hand, compact bone also known as cortical bone is the main structural element of the skeletal system. Cortical bone represents 80-85 vol % of total bone in the body. Compact

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bone has a superior ability to withstand the stress upon bone 20 times more than cancellous bone [2].

Bone cells are supplied with oxygen and nutrition as well as removal of cellular byproducts through a network of blood vessels. In cortical bone, nerves and blood vessels run through Haversian and Volkmann's canals.

2.1.2 FUNCTIONS OF BONE[5]

1. Protection of internal organs and the central nervous system
2. Reservoir of inorganic ions
3. Hematopoiesis
4. Mechanical support of soft tissues
5. Enable motion by providing articulations and attachments for muscles

2.1.3 BONE CELLS AND REMODELING

Bone is a living tissue that undergoes continuous cycles of deposition and resorption [6]. Understanding the unique properties of bone as well as the action of bone cells is essential for the development of new biologically relevant biomaterials. The process of bone remodeling involves various types of bone cells in order to carry out bone resorption and formation. Osteoclasts resorb old or damaged bone and osteoblasts form new bone. In a healthy adult, there is a balance between resorption and deposition to maintain the skeletal system. New bone is originated in form of lamellae or concentric sheets. A Haversian canal is present in the centre of the new bone. As bone is deposited

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by osteoblasts forming new lamellae, blood and nutrients are supplied through the Haversian canal to older lamellae.

2.5.1.1 OSTEOBLASTS

Osteoblasts are differentiated cells that originate from mesenchymal stem cells, followed by proliferation and differentiation into pre-osteoblasts and then maturation. Osteoblast development occurs in three main stages [7, 8]:

1. Cell proliferation
2. Matrix production and maturation
3. Mineralization

Osteoblasts synthesize and secrete different proteins including type I collagen. This is followed by mineralization of the matrix [2]. Some osteoblasts become encased within the bone matrix they have formed and terminally differentiate into osteocytes

2.5.1.2 OSTEOCYTES

Osteocytes have less metabolic activity than osteoprogenitor or osteoblastic cells. Osteocytes respond to mechanical stimulation and can send signals to osteoblasts and osteoclasts to promote bone formation and bone resorption.They play a role in the process of maintaining local bone The processes that connect adjacent osteocytes permit communication among cells as well as delivery of nutrients [1].

2.5.1.3 OSTEOCLASTS

Osteoclasts are bone resorbing cells. Hydrochloric acid and proteolytic enzymes are released by osteoclasts. The low pH results in dissolution of the hydroxyapatite crystals

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of the extracellular bone matrix. Thereafter, the organic matrix is degraded by the action of hydrolytic enzymes. Degradation products are then removed and released into the extracellular space [9].

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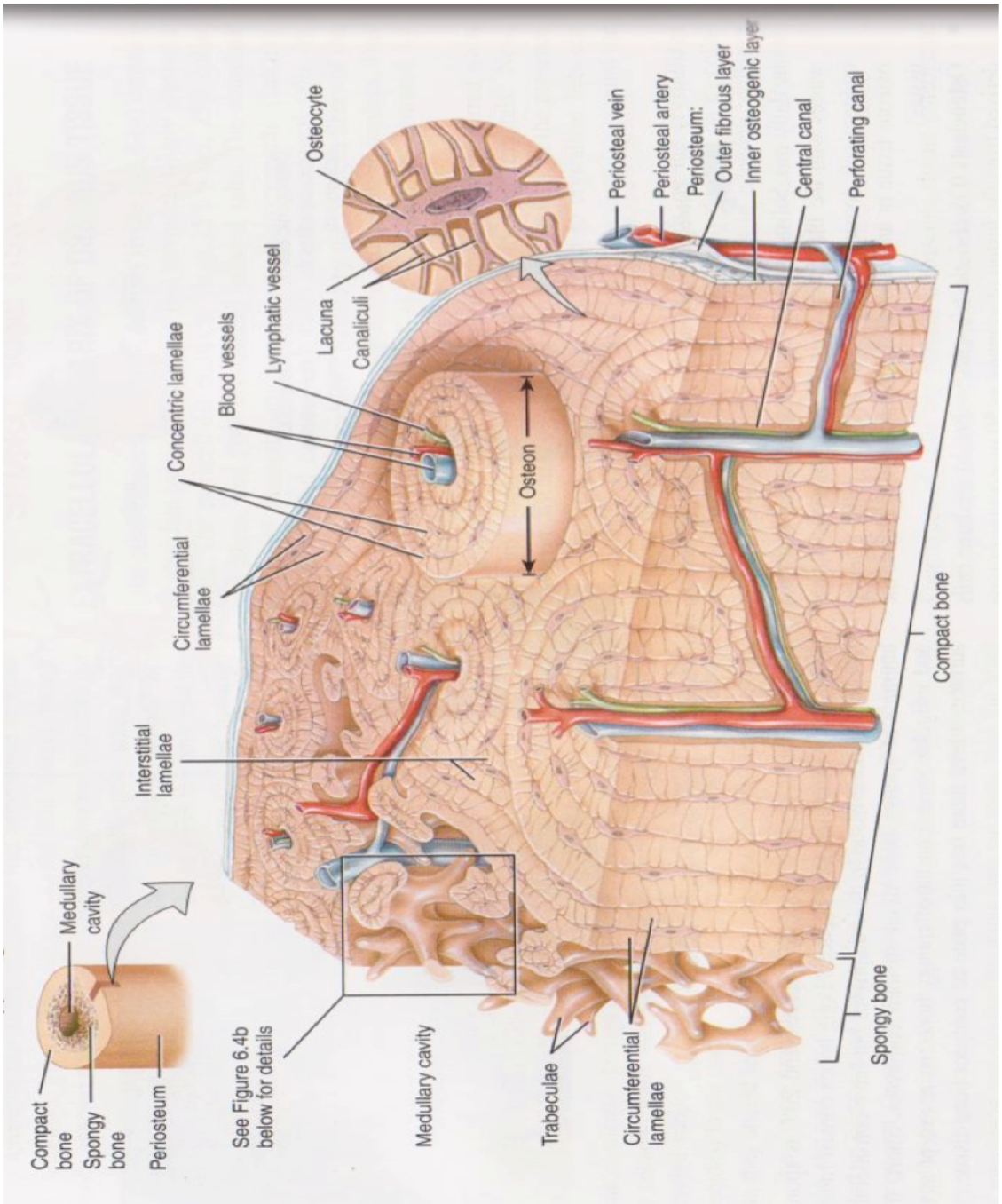

Figure 2-1 **:** The diaphysis of a long bone, from spongy bone tissue and the medullary cavity on the left. Compact bone tissue in the middle. The periosteum is on the right. Osteocyte is shown at the top right. Reproduced from Anatomy and Physiology: From Science to Life, 2nd edition with permission of John Wiley & Sons, Inc. Appendix A [10].

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2.2 DENTAL IMPLANTS

2.2.1 OVERVIEW

Dental implants are medical devices that are placed within maxilla and/or mandible in order to provide mechanical support to different prosthetic appliances used such as a single crown or bridge, or a partial or complete denture [11]. Different materials, designs and surface modifications of dental implants are developed because of their increased clinical importance as a solution for patients with either esthetic or functioning issues.

Historical studies reported that missing teeth replacement was introduced early in the history by using either homologous or alloplastic materials. These materials might provoke systemic responses [11]. In the 19th century, different studies demonstrated the use of rubber, gold and porcelain for teeth replacements. In 1939, Stock developed a threaded vitallium dental implant [11]. While studying bone healing in a rabbit model, a great turning point occurred in 1952, when a Swedish orthopedic surgeon Branemark. discovered a titanium cylinder placed in the tibia of a rabbit could not be removed [12]. The retentive titanium presented the concept of osseointegration. In 1962, Brånemark and his team treated the first edentulous patient [13].

2.2.2. INDICATIONS AND CONTRAINDICATIONS FOR DENTAL IMPLANTS

Dental implants are used for replacement of single, partial and complete loss of teeth. They are contraindicated in case of unfavorable occlusal function, macroglossia, which is known as enlarged tongue in completely edentulous patient, systemic disorders such as uncontrolled diabetes and patients receiving radiotherapy due to the inhibition of bone formation around implants and altered bone quality [12, 14].

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2.3 OSSEOINTEGRATION

It is known as direct functional and structural bond formation between bone tissue and artificial implant surface [15].

Osseointegration refers to the irreversible biological stability of implant in bone tissue. It is reported to be as a result to the absence of negative local or systemic reactions towards the implant. Studies exploring the mechanism of osseointegration from have been conducted [16]. A 5-year clinical study using screw-shaped titanium implants showed that proper healing, operator skills as well as stress distribution are very important for the successful bone formation [17].

2.3.1 FACTORS AFFECTING OSSEOINTEGRATION

Implant material, design and surface topography are responsible for enhancing osseointegration [18]. Moreover, healthy bone and pharmacological agents such as bisphosphonates enhance osseointegration. The effect of bisphosphonates is debatable. Other studies reported that its usage inhibits bone formation since it alters bone formation cycle by activation of osteoblasts and inhibition of osteoclastic activity [19, 20]. In contrast, there are several factors that inhibit osseointegration such as inadequate implant stability, excessive occlusal stresses, radiation therapy, pharmacological agents such as NSAIDs, osteoporosis, rheumatoid arthritis, smoking and alcoholism [19, 21-24].

2.3.2 PERI-IMPLANT HEALING

After surgical placement of the implant within the maxilla or the mandible within less than 1 mm distance around the implant, an initial phase of blood coagulation is formed.

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The blood clot forms a fibrin matrix, induces foreign body reaction and increases phospholipid hydrolysis and intracellular calcium. Then, osteoconduction occurs in which osteogenic cells proliferate at the implant surface. *De novo* bone formation takes place as osteogenic cells differentiate into osteoblasts. Woven bone is produced then remodeled and replaced by mature lamellar bone [16, 25, 26].

Osteoconduction is defined as stimulation of undifferentiated osteogenic cells proliferation and their migration to the implant surface. Next is osteoinduction, which is defined as osteogenic cells differentiation into osteoblasts that form new bone. Finally, peri-implant osteogenesis is the process that ultimately results in the biological fixation required for implant anchorage.

Primary stability of a dental implant is very important for osseointegration; it depends on the implant design, accuracy of surgical placement as well as implant location. Inadequate stability enhances fibrous tissue formation, that inhibit osseointegration, resulting in loosening of the implant and its eventual failure [16, 26].

Peri-implant osteogenesis may fail for a number of reasons, including: 1. impaired vascularization; 2. inadequate source of osteogenic cells; 3. hyperactive osteoclasts; 4. abnormal cell proliferation; or 5. abnormal local or systemic inflammatory responses [27].

2.4 BIOACTIVITY OF DENTAL IMPLANTS

Bioactive materials are used to replace, as well as to reconstruct bony defects. Bioactive glass bonds directly to the surface of bone through production of an apatite layer. To evaluate the bioactivity of a material, *in vitro* testing is done to ensure formation of the

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HA layer. First, immersion of samples in simulated body fluid (SBF) is done followed by evaluation. SBF was developed by Kokubo *et al*. [28] and Hench *et al.* [29]. It resembles human blood plasma composition, except for less HCO_3^- and more Cl^-.

Table 2-1. Ionic composition of SBF and blood plasma

Ion	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	0.5	142.0	5.0	1.5	2.5	147.8	4.2	1.0
Plasma	0.5	142.0	5.0	1.5	2.5	103.0	27.0	1.0

The production of an apatite layer indicates the bonding ability of the material to bone. The main stages of apatite formation on a bioactive glass include ion exchange of Na^+ and K^+ with H^+ or H_3O^- from the solution. $SiOH_4$ is released to the solution. The silicon-to-oxygen bonds break down, forming silanol on the materials surface. Thereafter, condensation and re-polymerization of the SiO_2-rich layer on the surface occur. Ca and P_i ions deposit on the surface forming a layer of amorphous calcium phosphate. At the end, amorphous calcium phosphate undergoes crystallization to form hydroxycarbonate apatite HCA [30, 31] .

The mechanism of bioactivity is very important, especially the last two steps. Knowing that the bioactivity test is time-dependent, the longer the time needed for the material to form HCA indicates poorer bioactivity [30] .

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2.5 DENTAL IMPLANT MATERIALS

2.5.1 METALS

2.5.1.1 STAINLESS STEEL

Temporary orthopedic devices such as screws and plates are formed of 316L Stainless steel is used for. However it is strong, easy to machine and inexpensive, it is not used as dental implant material because of high corrosion as well as inability to osseointegrate compared to titanium [18, 32].

2.5.1.2 COBALT-BASE ALLOYS

These alloys are produced of chromium and cobalt. They are characterized by high corrosion resistance due to formation of chromium oxide layer, but these alloys are inferior to titanium. They are mostly used in total joint replacements [18, 33].

2.5.1.3 TITANIUM AND TITANIUM ALLOYS

Titanium and its alloys have excellent biocompatibility and superior mechanical properties. Titanium is characterized by forming a passive protective oxide layer that enhances its corrosion resistance in physiological environment. Titanium is used in either 99.75% pure form known as commercial pure titanium or as Ti-6Al-4V alloy with 6% aluminum and 4% vanadium. Owing to superior mechanical properties as well as corrosion resistance, commercial pure titanium (CPTi) has been widely used. Different composition of carbon, hydrogen, iron, nitrogen and oxygen results in four grades of CPTi. The different composition of each grade of titanium has a significant influence on their mechanical properties. Currently grade 4 CPTi as well as titanium alloy is widely used for medical devices [11, 34, 35] .

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Once titanium is in contact with body fluids, a thin passive oxide layer is formed on the surface of the implant. Different oxides are formed including TiO, TiO_2 and Ti_2O_3 but TiO_2 is the most stable oxide layer[36]. Biocompatibility, high corrosion resistance and excellent osseointegration are greatly influenced by the oxide layer [36].

Different surface treatments were introduced to increase osseointegration by stimulating proliferation of osteoblasts on the implant surface. These include: acid etching [37], sand blasting [37], alkaline heat treatment [37], calcium phosphate coating, plasma spraying, dip coating [38], and bioactive glass coating.

2.6 SURFACE MODIFICATIONS OF TITANIUM

2.6.1 MECHANICAL METHODS

Mechanical approaches for surface modifications include removal, shaping, or treatment of titanium surfaces through the application of physical forces or removal of surface material by cutting or abrasive action.

2.6.1.1 SURFACE POLISHING

In polishing, a smooth surface finish is formed using fine abrasive grades accompanied by lubrication [39]. Alumina, diamond and SiC are the most common used polishing media. The finest polishing grades can produce surfaces with roughness value Ra of 0.1 μm or less [40]. However, polishing is usually used as an intermediate step prior to other surface treatments; polishing as a final surface modification develops mechanical surface stresses and alters the chemical composition of the titanium surface [39]. The main impact of polishing is removal of the native surface layer, descaling and smoothening of the Ti surface.

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2.6.1.2 SURFACE BLASTING

Surface blasting is the application of particles under high velocity to surfaces. It is mainly used for cleaning, descaling, removal of the native surface layer, increasing surface roughness and enhancing adhesion of bonded materials [41-43]. Various ceramics including alumina, silica, titania of different particle sizes are used for surface blasting of titanium. Particle size is the most critical parameter controlling the process of blasting and its impact on the surface topography. For example, a surface roughness of 0.5-1.5 μm R_a value is produced using alumina particles of 25-75 μm. On the other hand, the use of alumina particles of 200-600 μm resulted in Ra of 2-6 μm [43, 44]. Moreover, smooth rounded surfaces are formed by gentle shot peening. Chemical treatment of the blasted surface is recommended to remove the particles embedded during blasting. The ability of the alumina and silica particles to alter the chemical composition of titanium surfaces encouraged the use of blasting prior to the application of hydroxyapatite on the surface of titanium [45]. A thin oxide layer of less than 10 nm of TiO_2 is expected on the blasted surfaces accompanied by traces of blasting medium [39].

2.6.2 CHEMICAL METHODS

Chemical methods reported for surface modification of titanium include the chemical reaction taking place between chemicals and the surface of titanium.

2.6.2.1 SOLVENT CLEANING

Solvent cleaning is mainly used to remove oils, fatty surface and greased contaminates that remain after manufacturing. Alcohols, ketones and chlorinated carbons are common examples of organic solvents used. However, the solvent is not intended to react with the oxide layer on the surface of titanium but surface analysis revealed that some carbons

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remain as residues on the surface [46, 47]. This is due to the reaction occurring between the organic solvents and titanium oxide layer.

2.6.2.2 WET CHEMICAL ETCHING

Wet chemical etching is a process that depends mainly on the reaction between certain chemicals and the titanium oxide layer. Removal of the native oxide layer and increasing surface roughness are the ultimate goals of the etching process

2.6.2.2.1 Acid Etching

Acid etching removes oxide scales and forms clean surfaces. The surface topography of acid etched titanium is affected mainly by the pretreatment condition of titanium. Mild treatments did not have any effect on the surface condition [39].In contrast, significant differences in the surface topography are observed when titanium surfaces are extensively etched. In case of alloyed titanium, differences in etching alpha and beta phases result in surface topography in which beta phase is protruding from alpha [41]. Blasted surfaces prior to etching show higher surface roughness [41, 48]. In general etching produces a surface roughness varying from 0.1 μm or more according to the pretreatment topography [49, 50]. A thin oxide layer of less than 10 nm is formed on the surface of titanium as a result of etching. The oxide layer grows slowly over a year from 3 nm to 6 nm [51].

There are two main methods of acid etching. First is by using a mixture of nitric acid and hydrofluoric acid in a ratio of 10:1. In one method, hydrofluoric acid reacts with the surface titanium oxide forming titanium fluorides and free hydrogen. The 10:1 ratio must be stabilized to avoid the formation of free hydrogen that alters the surface characteristics

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of titanium [52]. In the other method, a mixture of 1:1 hydrochloric acid and sulphuric acid is used. The degree of pickling or etching is mainly affected by the temperature, acid concentration and treatment time that vary from one minute to one hour [41, 48, 53] .

2.6.2.2.2 Alkaline Etching

Alkaline treatment of titanium is performed using 5M NaOH for 24 hours at 60 °C [54, 55]. It produces a sodium titanate gel layer of 1 μm thickness as well as surface porosities. Alkaline etching is used mainly as a pretreatment to gel-derived apatite coating [53]. In some cases, the use of alkaline etching after acid etching produces a high surface roughness [53].

2.6.3 PASSIVATION TREATMENTS

The main purpose of passivation treatments of titanium is the formation of a stable oxide layer to prevent ion release [39]. There are two main approaches regarding passivation, heat treatment in air or immersion in a strong oxidizing agent.

2.6.3.1 NITRIC ACID PASSIVATION

It is mainly formed using HNO_3 solution in which titanium is immersed for 30 minutes at room temperature followed by rinsing and drying in order to neutralize the surface [56]. Usually passivation is the last step in surface modification of titanium. Although nitric acid passivation was reported to have no effect on the titanium oxide layer, higher ion release is observed from passivated titanium alloys [57, 58].

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2.6.3.2 HEAT TREATMENT

It is used as an alternative treatment method to passivation. It has no direct effect on the surface topography but it has an influence on the oxide scale layer. Heat treatment at 400 °C produces an oxide layer of 30 nm [59].

2.6.4 OTHER CHEMICAL SURFACE TREATMENTS

In addition to the previously explained methods, there are other approaches that are reported in the literature regarding the surface modification of titanium. These methods include apatite-coating techniques that will be explained in the following section.

2.6.4.1 HYDROGEN PEROXIDE TREATMENT

The reaction between the hydrogen peroxide and the titanium oxide produces Ti gels. The biocompatibility of the titanium upon using hydrogen peroxide is explained by the reaction between the hydrogen peroxide and titanium. The hydrogen peroxide is formed during inflammatory reaction [60, 61] .

2.7 BIO-CERAMICS AND BIOACTIVE GLASS COATINGS

2.7.1 CALCIUM PHOSPHATES

Calcium phosphates are a biocompatible, osteoconductive material that bonds strongly to bone [62, 63]. HA is widely used for dental owing to its similar composition to that of the mineral phase of bone and teeth. Different methods are being used for the production of HA such as wet chemical methods [64], solid-state reactions [65], hydrolysis methods [65] and sol-gel chemistry. Considering hydrolysis, acid calcium phosphates such as anhydrous dicalcium phosphate, octacalcium and dicalcium phosphate dihydrate phosphate, are used to produce HA [66] . Wet precipitation is a process in which calcium

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deficient apatite precipitate is formed under alkaline conditions through either reaction between calcium nitrate and ammonium phosphate or drop-wise precipitation of phosphoric acid to a suspension of calcium hydroxide [66, 67]. Sol-gel chemistry involves hydrolysis of metal alkoxides and calcium salts followed by polycondensation. Homogenous as well as controlled composition of the final product are the main characteristics of the sol-gel process. However, calcination at high temperature is required in order to produce crystalline HA. Calcination results in production of secondary phases of HA such as granular particle shapes and beta-tricalcium phosphate (TCP) [68, 69].

In contrast, exposing calcium and phosphorus precursors to high pressure through hydrothermal process produces crystalline HA [70]. A recent study reports the synthesis of HA nanowire through a combination of solvothermal processes and sol-gel chemistry [71].

Synthesis of HA bone scaffold is challenging owing to various degradability levels between different forms of HA. For example crystalline HA is poorly degradable while the fragility of the amorphous HA limits its use [72].

Crystalline HA has a limited osteoconductivity and bioactivity owing to its chemical stability in body fluids. In contrast, amorphous HA has a high dissolution rate that initiates an immune system response [72]. Biphasic calcium phosphates (BCP), formed of a combination of beta-TCP and HA, was developed to assure proper functioning of the HA [73]. Different studies reported the excellent effect of HA and its ability to bond directly to bone [74]. Moreover, superior implant stability was reported after the clinical

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application of the calcium phosphate coatings for total joint arthroplasty due to the enhanced osseointegration levels [75].

2.7.2 BIOACTIVE GLASS

In 1971, Hench and his colleges developed a specific glass formulation, known as bioactive glass in system Na_2O- Cao-SiO_2-P_2O_5, which does not initiate formation of fibrotic tissue [76]. Bioactive glasses are silicate-based glasses that are amorphous and biologically active. They are able to produce a strong bond when they are in contact with body fluids through formation of a bone-like HA layer [77, 78]. A series of reactions take place as a bonding mechanism of silicate glasses to bone [30]. Ions released from the bioactive glass produce a silica gel layer that stimulates favorable intracellular and extracellular responses to enhance bone synthesis. An amorphous calcium phosphate layer is formed due to ion exchange between body fluids and bioactive glass followed by crystallization to form carbonated HA.

Melt-derived bioactive glass was first introduced, in which melts of SiO_2 and P_2O_5 network formers and CaO and Na_2O network modifiers are quenched [76]. In the beginning of 1900s, sol-gel produced bioactive glass replaced the melt-derived. The main advantages of sol-gel bioactive glass include mixing at the molecular level that provides better control over chemical homogeneity and composition of the produced glass, low temperature process and high surface area of the bioactive glass that enhances the degradability of the glass.

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In sol-gel chemistry a series of steps take place in order to form bioactive glass including hydrolysis and polycondensation of metal alkoxide followed by gelation, aging, drying and densification [79]. A metal alkoxide M-ORx in which an oxygen linkage O bonds a metallic ion M to a functional group R. Tetraethoxysilane (TEOS) as well as tetramethoxysilane (TMOS) are the most commonly used metal precursor owing to their high reactivity to water.

Understanding the mechanism of hydrolysis and condensation reactions is essential to investigate the reaction parameters. There are many factors affecting it including temperature, nature and concentration of the electrolyte, nature of solvent and type of alkoxide precursor. Hydrolysis reaction depends specifically on the electrolyte concentration, the form of alkoxide group, (the bulkier the alkoxide group the lower rate of hydrolysis), and R- ratio of water to TEOS [30, 79].

Regarding gelation, a 3-D network of polycondensed particles produces the gel. The gelation process is influenced by the extent of cross-linking of particles and the particle size. In aging, viscosity of the solution increases and pore size decreases owing to further polycondensation [79, 80]. Sufficient strength of gel is essential in order to avoid cracking during drying. Considering drying, cracking is observed owing to an increase in the capillary pressure especially in small pores less than 20 nm due to removal of liquid from the gel [81]. Controlling the rate of hydrolysis and condensation to form monodispersed pore sizes prevents cracking of gels during drying [80-82]. Knowing that glass is normally an amorphous material, it needs to be sintered or heated to get rid of pores and to crystallize it.

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Due to the inferior mechanical properties of bioactive glass, it has limited applications. In the apatite containing glass, it was reported that the bonding of the glass to the bone is achieved through the bond between bone apatite and glass-ceramic. In the processing of bioactive glass, two main approaches were described by researchers including melt-derived and sol-gel derived bioactive glass. Melt-derived bioactive glass is characterized by lower dissolution rate as well as lower rate of apatite layer formation on the surface of the glass. In contrast, sol-gel derived glass showed higher dissolution rate, higher rate of HA formation and higher crystallinity compared to melt-derived bioactive glass [80]. The sol-gel bioactive glass shows continuous release of silica ions and break down of the glass network giving calcium and phosphorus that enhances the process of hydroxyl carbonate apatite formation.

2.8 PRODUCTION OF COATINGS ON TITANIUM SURFACE

The production of surface coating on the surface of titanium, mostly hydroxyapatite or calcium phosphate coating, encouraged a lot of researchers to attempt to enhance the biological properties of titanium, combining the superior mechanical properties of titanium with the excellent bioactivity of calcium phosphate or bioactive glass. These coatings would enhance the process of new bone formation accordingly increase the success rate of the implant.

2.8.1 SOL-GEL

Using sol-gel method, HA coatings are developed on the surface of titanium[68]. Titanium substrates are dipped in calcium, mostly nitrate salts and phosphorus gels, for a certain period of time. The coatings produced are characterized by high porosity, low density and inadequate adhesion ability to the surfaces. Porosity has an advantageous

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influence since it increases the surface area in contact with tissue fluids. In order to enhance adhesion as well as density, coatings are sintered. Various forms of calcium phosphates are formed as a result of different sintering temperatures [83, 84].

Dip coating method is used to apply two layers of coating[85]. After primarily deposition of calcium and nitrate solution, the film is dried at 200 °C. Thereafter, second deposition is performed followed by drying at 750 °C. Higher corrosion resistance as well as higher adhesion ability of the coating is observed due to the formation of an intermediate layer of TiO_2 between HA and titanium. The optimal oxide layer is 200 nm. If its thickness is less than 200 nm, lower corrosion resistance is observed. In addition, an oxide layer of more than 200 nm reduces the adhesion ability between the coating and titanium due to the thermal mismatch.

Different developments of the mentioned technique were investigated [85]. For example a $CaTiO_2$ was applied as an intermediate layer to enhance adhesion of HA to the titanium surface. Altering the precursors, such as using HA/ethanol solution, was investigated in order to develop a coating with higher roughness, porosity, homogeneity and bonding to the surface [86].

Owing to the long processing time as well as post-sintering limitations, the use of sol-gel coatings in the industry is limited.

2.8.2 PLASMA SPRAYING

Plasma spraying is a commonly used technique for surface modification of implants by applying HA on the surfaces of titanium and its alloys. During plasma spraying, a

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complex of thermal changes involving powder particles, the plasma zone as well as the substrate are observed.

Regarding the mechanism of plasma spraying technique, particles are exposed to very high heating temperature for few seconds in the 10,000 °C jet. However particles undergo different melting rates, some particles do not melt due to the limited time in the plasma zone. Thereafter HA droplets are impacted on the surface of titanium [87-90].

Limitations of the plasma spraying technique include, nonhomogeneous coating due to the formation of a mixture of crystalline and amorphous HA as a result of quick exposure to high temperature, low Ca/P ratio as a result of the reaction due to the high temperature applied and formation of rough surfaces [87, 91]. Although surface roughness is considered an enhancing factor of the process of implant to bone bonding, the roughness should be in a certain range considering that extremely rough surfaces are not favorable. Moreover, it is reported that plasma sprayed coatings exhibit different bond strengths to the implant surface. This results in the formation of microcracks, nonuniform coating and limited delamination resistance [92, 93].

2.8.3 ION-BEAM METHODS

In ion beam method, calcium and phosphorus ions are embedded on the surface of the substrate. Thereafter, the substrates are exposed to SBF in order to form a titanium hydroxide layer that acts as bonding sites for HA. It is characterized by strong adhesive bonding of the coating to the surface of the substrate. The main limitations of this technique include formation of amorphous coatings on the surface of the substrate as well as expensive cost [94].

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2.8.4 LASER METHODS

It is known that plasma sprayed HA showed nonhomogeneous coating with high levels of thickness. Moreover the coating is mechanically bonded to the surface of the substrate that is poorly accepted for clinical application. In contrast, pulsed laser deposition PLD is able to form a thin crystalline coating with acceptable adhesive bonding to the substrate surface. Regardless the advantages of the PLD method, the expensive cost of the process as well as the machine itself may limit its use [95, 96].

2.8.5 RF SPUTTERING

In this technique, RF-magnetron sputtering from calcium phosphate glass targets, followed by post-annealing, is used to produce a thin apatite layer on the substrate. The production of calcium phosphate occurs as a result of sputtering in which phosphorous oxide is lost. The higher Ca/P ratio of the target glass as well as the higher post-annealing temperature, the more crystalline phases formed. Lately, sputtered HA showed excellent results regarding bone formation. The length of the process as well as the expensive cost of RF sputtering limit its application [87, 97].

2.8.6 HYDROTHERMAL TREATMENT

Hydrothermal treatment is performed on already existing coating in order to form a targeted phase [38, 98-100]. Plasma spraying, anodic oxidation or any reported techniques can be used. Thereafter the substrates are hydrothermally treated in water or SBF aqueous medium in order to produce HA. A thin layer of HA is formed in case of hydrothermally treated anodic oxide film that has calcium and phosphorus. Compared to the anodized oxide surface, higher concentration of calcium and phosphorus ions is observed on the surfaces due to the release of calcium and phosphorus ions during

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hydrothermal treatment. Although hydrothermal treatment enhances the HA formation, it alters the stability of the oxide layer bonding to the surfaces [98, 99, 101]. The hydrothermal condition including reaction pH, temperature, pressure and reaction time are the main factors affecting the process of HA formation [93]. Increase in temperature and pH accelerates the release of calcium and phosphorus ions from the original coating and eventually increases the HA production.

2.8.7 ELECTROCHEMICAL CATHODIC DEPOSITION

CaP is deposited on the surface of the substrate using cathodic deposition under ambient temperature [102, 103]. Good shape conformity, room temperature process, uniform coating thickness as well as short processing times are the main advantages of this technique. The major disadvantages of this method include the development of stresses in the coatings and inadequate bonding to the surfaces[104, 105].

2.8.8 THERMAL SUBSTRATE METHOD

In this method, an aqueous solution of calcium and phosphorus is used to produce CaP coating on titanium surfaces. The immersed substrates are exposed to high temperature of 1008 °C known as Joule heating. Thereafter CaP coating is produced. HA, which is the major component of the coating, increases as a result of elevated reaction time and temperature. Heating the aqueous solution containing calcium and phosphorous ions under accurate conditions of pH and temperature has a critical effect on the production of HA because it is less soluble under higher temperature. The diversity of crystallinities produced is considered as the main disadvantage of this technique that limits its use [106].

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2.9 RATIONAL OF THE STUDY

Considering the drawbacks of methods of surface coating applications reported in the literature, the target of this study is to synthesize a bioactive and osteoconductive nanowire coating onto titanium substrates by a two stage sol-gel-hydrothermal process to develop a novel sol-gel hydrothermal coating method onto Ti implants and to study the effect of reaction pH and time under hydrothermal conditions on surface topography, chemistry and osteoblast like-cell attachment to the bioactive glass coating. We developed bioactive glass coating on the surface of titanium through various hydrothermal conditions. We hypothesize that pH levels of the reactions will modulate physical, chemical and biological properties of the coatings as well as enhancement of cell attachment on the surface of the coated titanium.

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CHAPTER 3: SOL-GEL HYDROTHERMAL COATING OF TITANIUM SUBSTRATES WITH BIOACTIVE GLASS

3.1 INTRODUCTION

Dental and cranio-maxillofacial implants are used to restore and reconstruct function and form to the edentulous or partially edentulous jaws and the cranio-maxillofacial skeleton of the patient. Implants are also used to replace maxillofacial structures [1-3]. Thus, implants not only play a significant role in restoring lost masticatory function but also promote self-esteem, especially in patients with orofacial defects [4].

Titanium (Ti) and its alloys are widely used implant materials for both dental and orthopaedic applications owing to their biocompatibility, superior mechanical properties and high corrosion resistance in body fluids [5, 6]. Although Ti offers outstanding characteristics, it is a bioinert material that does not form chemical bonds with the bone surface. Consequently, titanium does not enhance the process of osseointegration or new bone formation. Different surface treatments and modifications have been introduced to improve osseointegration of Ti. Porous structure production on Ti-based materials showed promising effects to provide nucleation points for calcium phosphate precipitation and bone formation [6]. Different studies report that the nanostructured surface of Ti improves its bioactivity and biocompatibility [7, 8].

Ti naturally forms a thin oxide layer in air which is usually only a few nanometers thick [4]. The structure as well as the chemical stability of the oxide layer support biocompatibility, bioinertness and corrosion resistance of titanium and titanium alloys [9]. Ti implants readily adsorb proteins (i.e. albumin, fibronectin and fibrinogen) from biological fluids on placement in bone. Subsequently, macrophages as well as neutrophils

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are found near the implant, then production of foreign body giant cells from activated macrophages. Healing next involves the migration of osteogenic cells to the implant site that can differentiate into osteoblasts, that form new bone [10, 11]. Despite, a thin non-mineral layer generally separates the bone-Ti interface; osseointegration is referred to mechanical interlocking of Ti and bone. Although Ti implants demonstrate a good clinical success rate, the treatment is offered to patients after careful selection, which precludes a number of patient groups, including those suffering from diabetes, osteoporosis, bone disorders or cardiovascular conditions. Therefore, the modification of Ti implant surfaces is being explored as a way to promote osseointegration, faster healing times contact and device longevity *in vivo*. To make Ti bond to bone in patients with poor bone quality or quantity [12], mechanical, chemical and physical methods have been introduced to promote the bioactivity of Ti [4, 9, 13, 14].

Bioactive glasses are able to form a mechanical bond with bone. These ceramics are characterized by biocompatibility and osteoconductive properties similar to those of hydroxyapatite. In physiological conditions, bioactive glasses form a hydroxycarbonate apatite layer, which is recognized as bone-like tissue. Production of an apatite layer is preceded by surface dissolution of the glass network, resulting in silica-rich gel layer production that is essential for biocompatibility [15]. Bioactive glasses are particularly attractive, especially when compared to conventional hydroxyapatite ceramics, because they have osteoinductive properties and stimulate new bone growth [16].

Coating metal surfaces with 45S5 Bioglass® is a challenging process. Plasma spray methods usually do not succeed due to rapid dissolution in body fluids when implanted and weak glass/metal interface. High thermal expansion coefficients of Bioglass®

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compared to those of Ti alloys limit using these glasses for coatings, due to cracking of the glass under thermal stress [17]. Moreover, coating metal surfaces using an enameling technique is not successful due to the glass crystallizing, which results in lack of adhesion to the substratum [18]. There has been some success in coating tailored bioactive glasses on Ti substrates using the SiO_2–Na_2O–K_2O–CaO–MgO–P_2O_5 system [19].

Considering the difficulties in producing homogenous layers of hydroxyapatite and bioactive glass on Ti, the overall focus of this study was to synthesize a bioactive and osteoconductive nanowire coating onto Ti substrata and to develop a novel sol-gel hydrothermal coating method onto Ti implants. We also characterized the effects of reaction pH and time under hydrothermal conditions on the surface chemistry as well as the surface topography of the coatings and used osteoblast like-cells to assess biocompatibility and cell attachment. We hypothesized that, by varying the hydrothermal conditions and pH levels of the reaction, we would modulate the physical, chemical and biological characteristics of the coatings.

3.2 MATERIALS AND METHODS

Tetraethyl orthosilicate 98% (TEOS), calcium nitrate and triethyl phosphate 99.8 % (TEP) were purchased from Sigma-Aldrich (Milwaukee, WI). Hydrochloric acid (HCl) was purchased from Caledon laboratory (Georgetown, ON). Ethanol (ethyl alcohol 95%) was obtained from a solvent purification system.

3.2.1 BIOACTIVE GLASS SYNTHESIS

Bioactive glass (70% SiO_2, 26% CaO and 4% P_2O_5) was synthesized by sol-gel chemistry. Tetraethyl orthosilicate (TEOS), calcium nitrate $Ca(NO_3)_2 6H_2O$ and triethyl

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phosphate (TEP) were used as precursors for silica, calcium and phosphorous respectively. The sol-gel process occurred through the hydrolysis and polycondensation of the TEOS with ethanol and water as byproducts as shown in equation 1. TEOS was mixed with distilled water in a 1:8 ratio. 5 M HCl was added to initiate the hydrolysis reaction. After complete dissolving of the TEOS for 30 minutes, calcium nitrate was added. After 30 minutes, TEP was added and then the mixture was stirred for 3 hours at room temperature. The end product was left at room temperature for 48 h for gelation to occur followed by vacuum drying at 50˚**C** to obtain the final glass. The glass was then ground using a mortar and pestle.

3.2.2 AQUEOUS SOLUTION PREPARATION

Various concentrations of NaOH solution (0.25, 0.5, 1 and 2 M) were prepared in distilled water. The pH levels of the different NaOH concentrations were 13.4, 13.7, 14 and 14.3, respectively.

3.2.3 SURFACE PREPARATION OF TITANIUM

Grade 2 Titanium rods were purchased from Online Metals. Disc specimens of 1.5 mm thickness and 15 mm diameter were polished using a BUEHLER MetaServ grinder, followed by ultrasonic cleaning with 95% ethanol for 5 minutes. The substrates were then sandblasted using 50 μm Al_2O_3 particles, followed by ultrasonic cleaning for 5 min with distilled water. Thereafter, substrates were acid etched using a mixture of 1 mL sulfuric acid and 1 mL hydrochloric acid concentrations for each substrate for 15 minutes at 80˚**C**. Then washing with deionized water using ultrasonic agitation.

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3.2.4 HYDROTHERMAL COATING

Prior to hydrothermal coating, 0.5 g of the synthesized bioactive glass (BG) powder was first dispersed in 30 mL of NaOH solution at four levels of molarity (0.25 M, 0.5 M, 1 M and 2 M). Thereafter, glass slurry and three Ti substrates were placed into a 125 mL Teflon-lined acid digestion bomb (Parr Instrumentation Company, Moline, IL) and heated to 170˚**C** using a band heater for 24 h. The temperature of the hydrothermal treatment was controlled using a thermocouple and a temperature and process controller (iSeries, Omega, Stamford, CT). Following completion of the hydrothermal reaction, the acid digestion bomb was brought to room temperature using a fan. Coated substrates were carefully removed and washed gently with deionized water.

3.2.5 PHYSICAL, CHEMICAL AND BIOLOGICAL CHARACTERIZATION

3.2.5.1 SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDX)

EDX was used to evaluate the elemental distribution and chemical composition of the coated substrates. Surface morphology of the coated Ti substrates was visualized using LEO (Zeiss) 1540XB FIB/SEM. Prior to imaging, osmium metal was sputtered on the surface of the Ti substrates.

3.2.5.2 X-RAY DIFFRACTION (XRD)

XRD of the coated specimens was conducted using a Bruker D2 PHASER desktop X-ray diffractometer operating at Voltage 30 kV, current 10 mA and Cu K_α target, λ= 1.54184 Å and detected with a Lynxeye detector. The step scan was 0.2 degree/min. Polished Ti substrates were used as control. XRD evaluation was conducted in the 2θ range from 10 to 60˚ using Bragg's Law $\lambda = 2d \sin\theta$ where λ is the X-ray wavelength (Å), d is the

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interplanar spacing (Å) and θ is the diffraction angle (degrees).

3.2.5.3 SURFACE ROUGHNESS

The micro-roughness (Ra scale) (Surface Area=176.6 mm^2) of the uncoated and coated Ti substrates was detected using a Surftest SJ-210 mechanical stylus profilometer surface roughness tester (Mitutoyo, Kanagawa, Japan). The Ra values were detected according to accepted standards (ISO 4287:1997) with a cutoff length, λ_c = 0.8 mm and evaluation length of 4 mm.

3.2.5.4 BIOACTIVITY ASSAY

Ti substrates including polished (control), sand-blasted and acid etched (SA) as well as substrates treated hydrothermally in 0.25, 0.5, 1 and 2 M NaOH were immersed in simulated body fluid (SBF) [20, 21] (20 ml, pH 7.25) and placed in an incubator (Branstead/Lab-Line) at 37°C and 120 rpm for 7 days. Apatite-like layer formation on the substrates was evaluated using SEM. XRD patterns were collected for each specimen to identify the crystalline apatite peaks.

3.2.5.5 CELL INTERACTION

The substrates were prepared for cell culture as follows. A PDC-32G plasma cleaner (Harrick Plasma, Ithaca, NY) was used to sterilize all substrates in low-temperature, radio-frequency glow discharge (RFGD) argon plasma. In order to produce a full vacuum, the samples were flushed twice with argon for 30 seconds then rested for 3 minutes. Thereafter, the RF valve as well as argon tank were turned on. Finally, the vacuum was stopped and substrates were placed in a sterile culture dishes.

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MC3T3-E1 osteoblast-like cells were plated on the substrates in alpha-modified minimum essential medium supplemented with 10% FBS (fetal bovine serum) and antibiotic solution (10,000 U/ml penicillin, 10,000 mg/mL streptomycin, and 25 mg/mL amphotericin B) at a density of 7,000 cells/cm^2. Samples were incubated for 24 hours at 37°C in a humidified atmosphere of 5 % CO_2. Cells were then fixed with 4% paraformaldehyde for 10 min at room temperature and rinsed 3 times with phosphate-buffered saline (PBS). Cells were permeabilized with 0.1% Triton-X100 for 10 min then washed with PBS three times. Cells were blocked with BSA in PBS. Next, cells were incubated with vinculin antibody (Sigma) (1:1000) in in PBS+BSA at 4^{o}C overnight followed by washing three times with PBS. The secondary antibody (Alexa-Fluor 488) along with rhodamine phalloidin (1:1000) in PBS were added for 60 minutes at room temperature followed by washing three times in PBS. Cells were sealed with ProLong mounting media containing DAPI (4', 6-diamidino-2-phenylindole).

An Axio Imager.M.2m fluorescence microscope (Carl Zeiss, Jena, Germany) and Zen 2011 software were used to view cell on the surfaces. The numbers of cells on each sample were counted. To evaluate the ability of cells to adhere to and spread on the substrate, the average area of the focal adhesions was detected using ImageJ software**.** The area of the focal adhesion in each condition was analyzed by ROI manger.

3.2.5.6 STATISTICAL ANALYSIS

Statistical analysis was conducted using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were accepted as statistically significant at $p < 0.05$.

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3.3 RESULTS

3.3.1 SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDX) OF SOL-GEL HYDROTHERMAL COATINGS

Polished and SA Ti substrates, as well as substrates coated with bioactive glass under different hydrothermal conditions were examined by SEM (Figure 3.1). The coatings produced under 0.25 M NaOH conditions displayed nanowire structures. The coatings under 0.5 M NaOH showed square-shaped particles. Whereas, the coatings prepared under 1 and 2 M NaOH conditions exhibited non-uniform structures consisting of fine and coarse plates.

EDX analyses revealed the elemental compositions of the coated Ti substrates (Figure 3.2). The coarse SEM structures ("A" regions) had different chemical compositions than the fine structures ("B" regions). Region "A" showed higher amount of silicon content. In contrast higher amount of calcium was detected in region "B". The elemental compositions of sodium, oxygen, silicon and Ti in the "A" region of specimens hydrothermally coated at different NaOH concentrations were compared (Figure 3.3). The elemental compositions at 0.5 M and 1 M NaOH were not significantly different. Significant differences of the elemental compositions at 2 M NaOH were observed. Sodium and silicon contents were lower at 2M compared to 0.5M and 1M. No significant differences in elemental composition of the "B" regions were observed for all NaOH concentrations expect for sodium content at 0.25 M that was significantly similar to 1 M only, Figure 3.4. Of note, calcium was present in the "B" regions, but not in the "A" regions.

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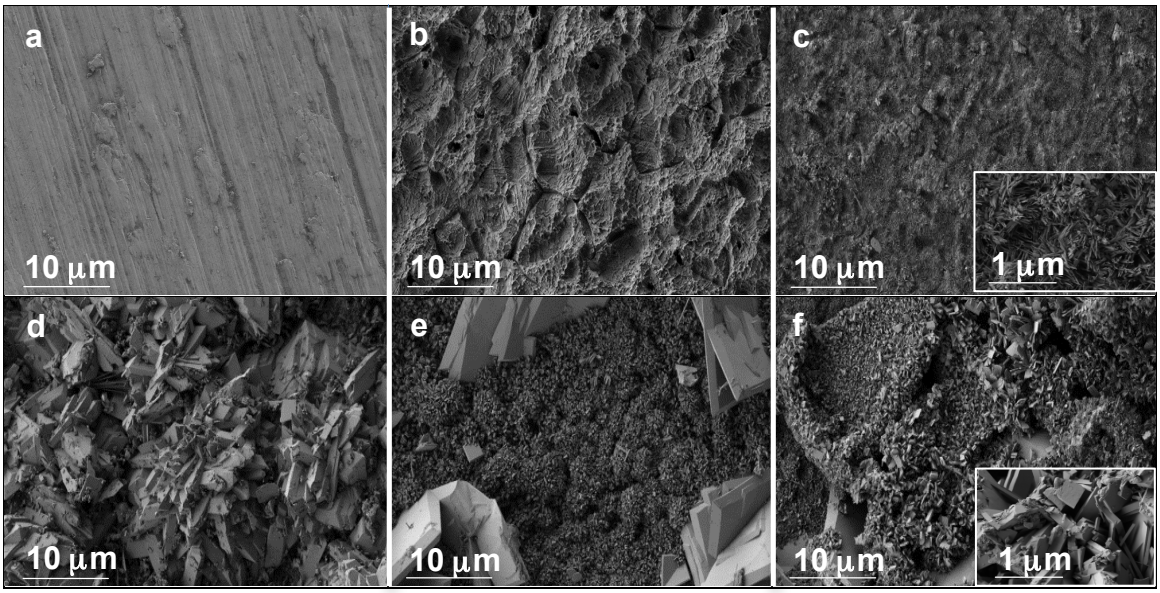

Figure 3-1 a) Polished Ti surface as control. b) Sand-blasted and acid-etched Ti surface. c) Titanium substrate coated with bioactive glass dispersed in 0.25 M NaOH under hydrothermal condition at 170ºC for 24 h. d) Titanium substrate coated with bioactive glass dispersed in 0.5 M NaOH under hydrothermal condition at 170ºC for 24 h. e) Titanium substrate coated with bioactive glass dispersed in 1 M NaOH under hydrothermal condition at 170ºC for 24 h. f) Titanium substrate coated with bioactive glass dispersed in 2 M NaOH under hydrothermal condition at 170ºC for 24 h. (n=3)

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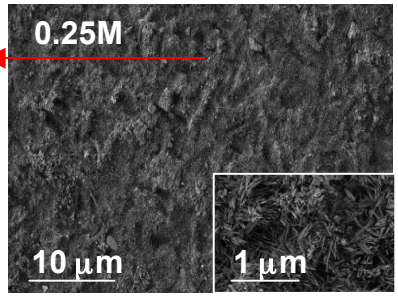
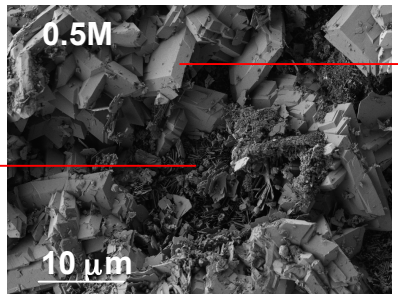
Oxygen	65.35 (0.06)
Na	4.11 (0.19)
Si	0.68 (0.01)
Ca	0.31 (0.02)
Ti	29.53 (0.15)

B



Oxygen	49.73 (7.68)
Na	7.05 (1.31)
Si	4.70 (0.04)
Ca	9.47 (2.21)
Ti	29.02 (6.84)

B

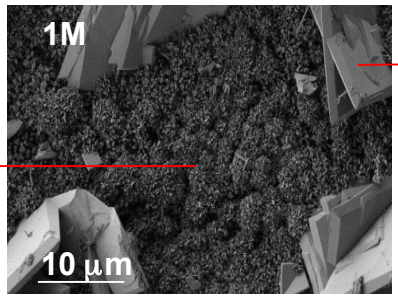


A

Oxygen	56.97 (2.10)
Na	20.80 (0.73)
Si	11.44 (0.88)
Ti	10.69 (0.70)

Oxygen	59.14 (2.11)
Na	6.05 (0.34)
Si	2.97 (1.00)
Ca	5.70 (2.39)
Ti	26.13 (1.35)

B

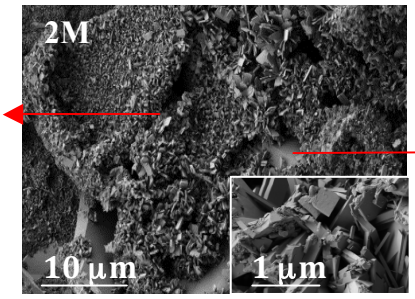


A

Oxygen	56.09 (2.45)
Na	19.39 (2.31)
Si	11.16 (0.63)
Ti	12.73 (3.94)

Oxygen	62.15 (1.78)
Na	7.31 (0.30)
Si	1.29 (0.15)
Ca	2.45 (0.55)
Ti	26.79 (1.37)

B



A

Oxygen	61.97 (0.70)
Na	7.18 (1.07)
Si	3.77 (0.42)
Ca	6.27 (0.68)
Ti	21.34 (0.89)

Figure 3-2 Elemental analysis of the hydrothermally coated titanium surfaces under various NaOH concentrations. The mean values are triplicate measurements of the atomic percent of each element from random fields of view. The numbers in brackets are SD.

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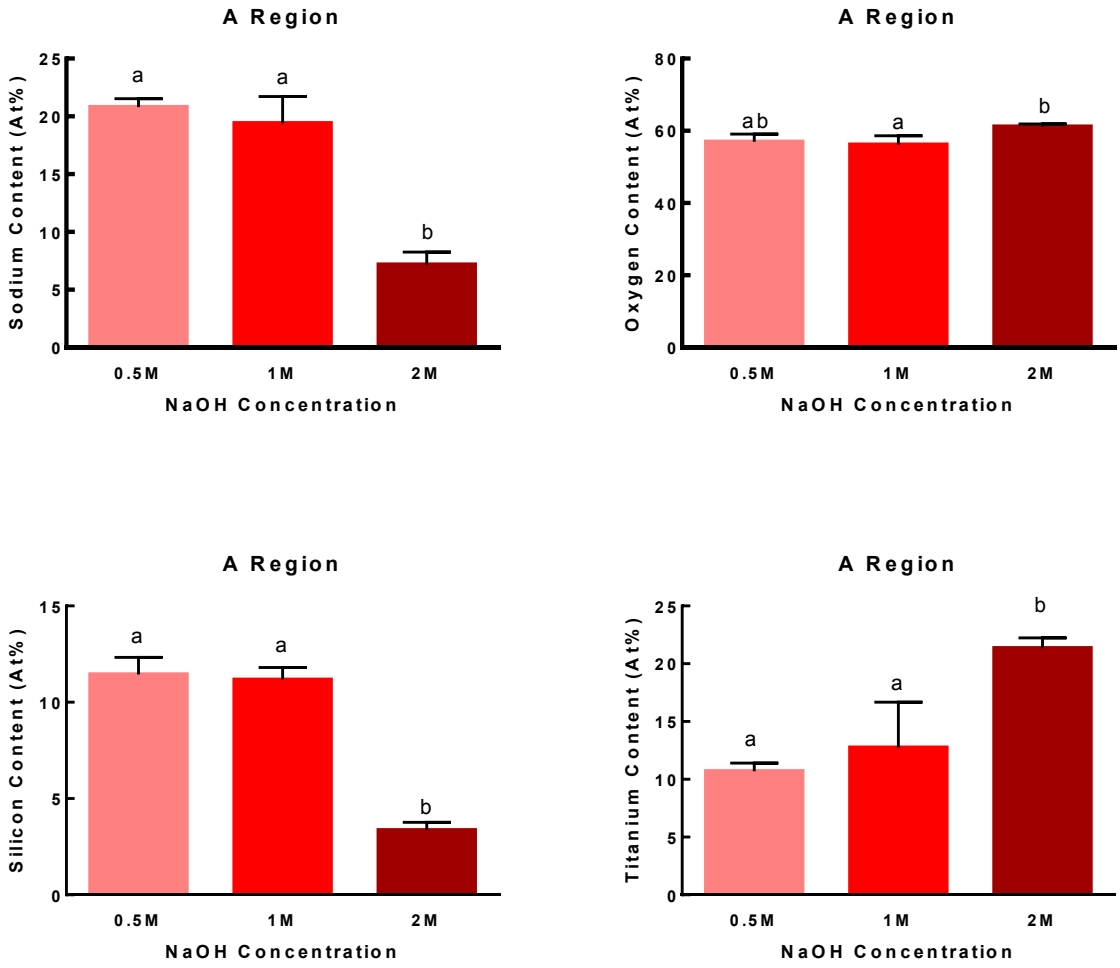

Figure 3-3 Histogram comparing the sodium, oxygen, silicon and titanium contents in the "A" region as a function of NaOH concentration. Data are means ± SD of triplicate measurements from random fields of view (n=3). Different lower case letters indicate significant differences at $p < 0.05$.

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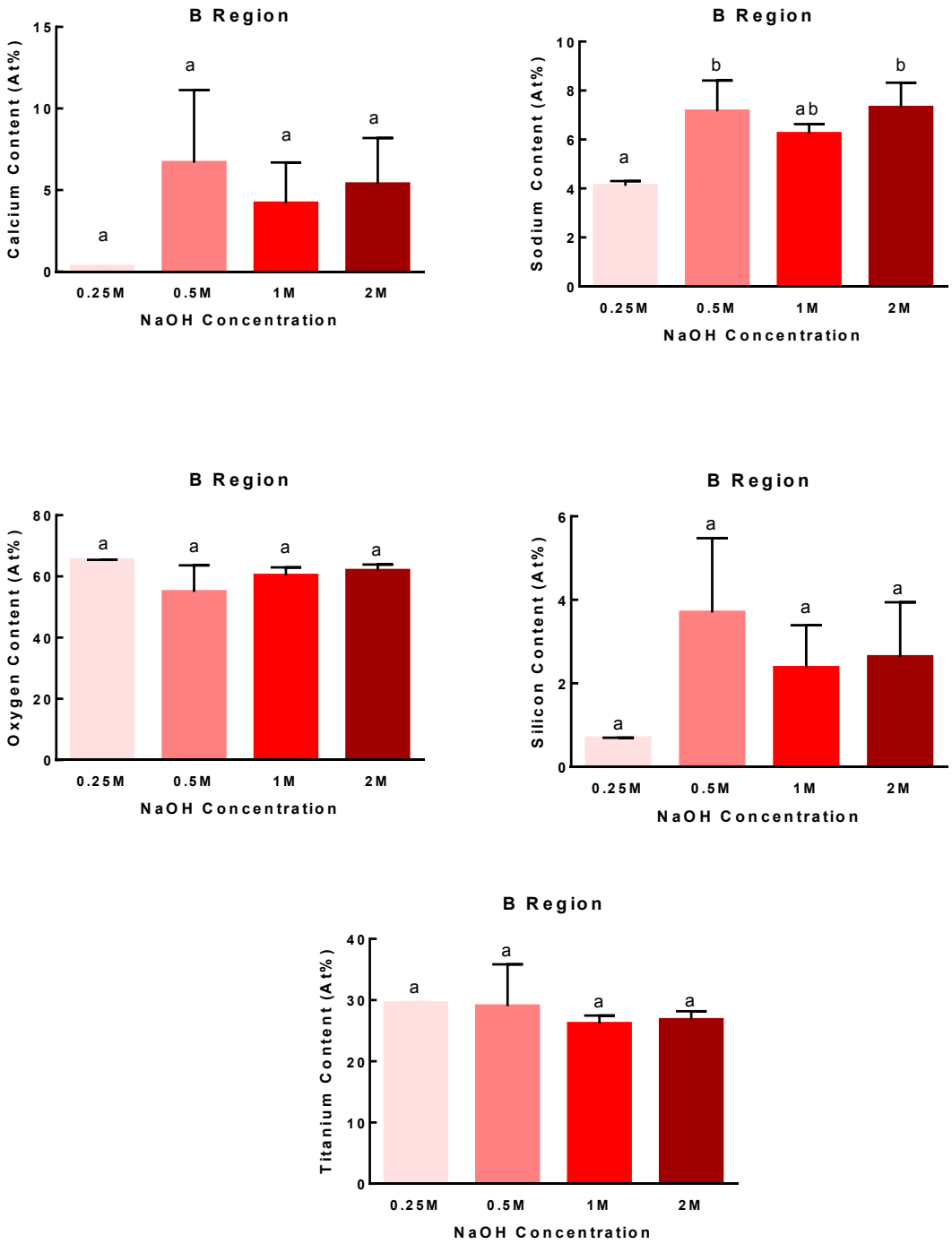

Figure 3-4 Histogram comparing the calcium, sodium, oxygen, silicon and titanium contents in the "B" region as a function of NaOH concentration. Data are means ± SD of triplicate measurements from random fields of view (n=3). Different lower case letters indicate significant differences at $p < 0.05$.

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3.3.2 X-RAY DIFFRACTION

Specimens hydrothermally coated with bioactive glass under different NaOH concentrations were subjected to XRD analysis (Figure 3.5). The data showed many peaks reflecting the presence of different crystalline phases. The most evident phases included silicon oxide, sodium titanate, titanium oxide, beta-calcium phosphate and sodium silicate. Because the data are a partial match to several possible phases that only partially match phases in the ICDD, as such XRD does not give a unique solution. For any partially matching International Centre for Diffraction Data (ICDD) standard phase, only a subset of lattice planes in the standard were represented by peaks in the sample. This suggests that there was significant preferred orientation in the coated specimens. Notably, in the case of preferred orientation, the relative peak heights in the diffraction pattern do not quantitatively reflect the amount of the phases [22].

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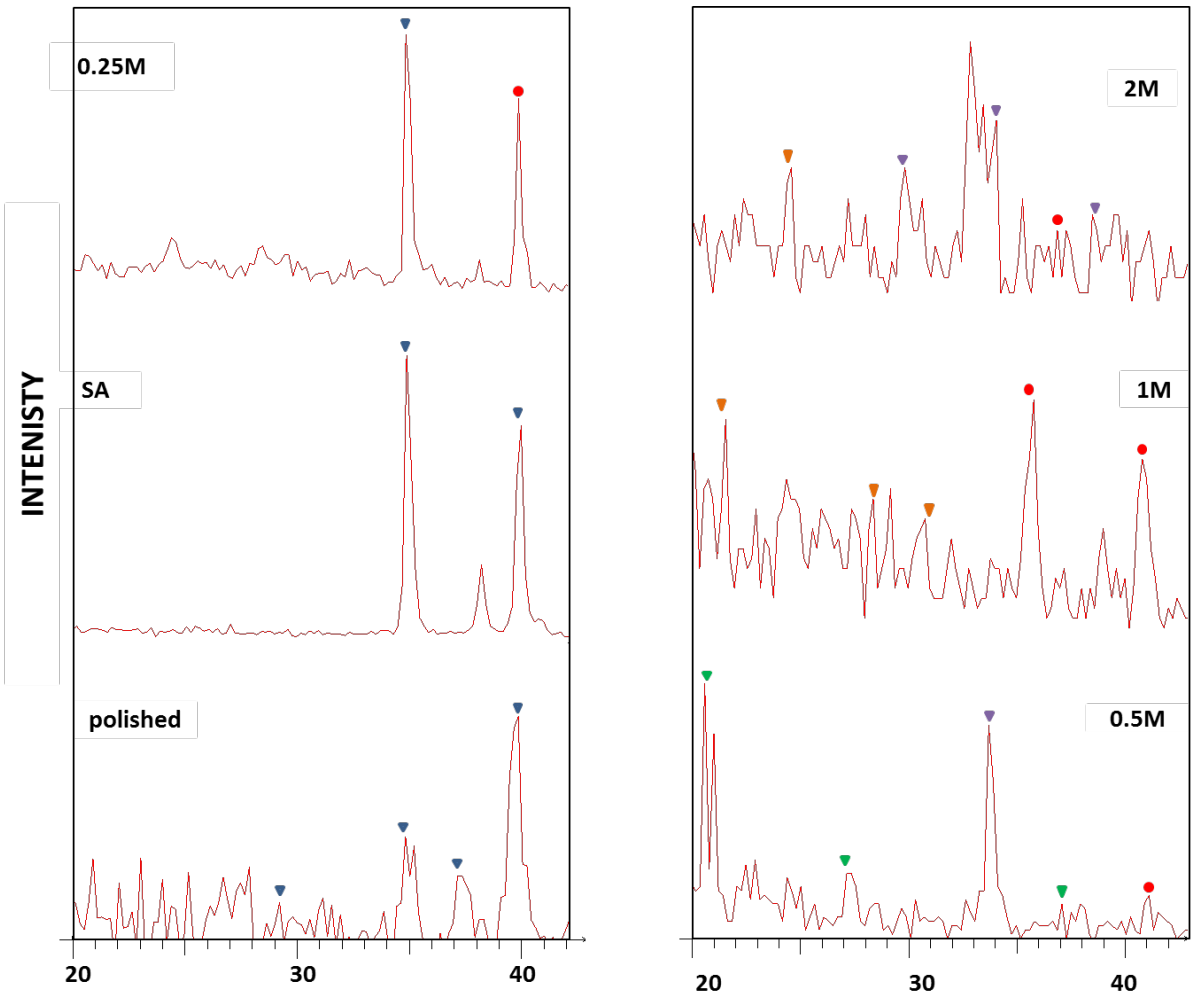

Figure 3-5 XRD analysis of Polished Ti and SA-Ti surfaces reveal 00-050-0787 (N)-Titanium oxide (▼) on its surface. Ti surface treated under 0.25 M NaOH showed -00-002-1359 (D)- Titanium Oxide (▼) and 01-089-0799 (C)- sodium titanate (●). Ti surfaces treated under 0.5 M NaOH showed 00-011-0039 (l)-beta calcium phosphate (▼) and 00-010-0179-(*) sodium phosphate (▼). Ti surfaces treated under 1M NaOH showed 01-089-0800 (C)-sodium titanate (●) and 00-051-1380 (*)- SiO_2 (▼). Ti surfaces treated under 2 M NaOH showed 00-018-1243 (D)- sodium silicate (▼), 00-031-1329 (*)-sodium titanate (●) and 00-046-0570 (C)- SiO_2 (▼). Data was analyzed using EVA software.

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3.3.3 SURFACE ROUGHNESS

The surface roughness of Ti substrates prepared under different conditions was quantified by profilometry (Figure 3.6). As expected, the polished surface was the smoothest. The roughness values of the ground glass (control) and polished surface were not significantly different with average Ra value of about 0.33 µm and 0.43 µm respectively. In contrast, the surfaces of SA, 0.25 M and 0.5 M were significantly more rough than polished. The surface roughness value of Ti substrate treated under 1 M NaOH was significantly lower than that of Ti substrate coated under 2 M NaOH, $P<0.05$. The hydrothermally coated Ti specimens under 2 M NaOH had significantly higher surface roughness value, $P < 0.05$ amongst the other Ti surfaces.

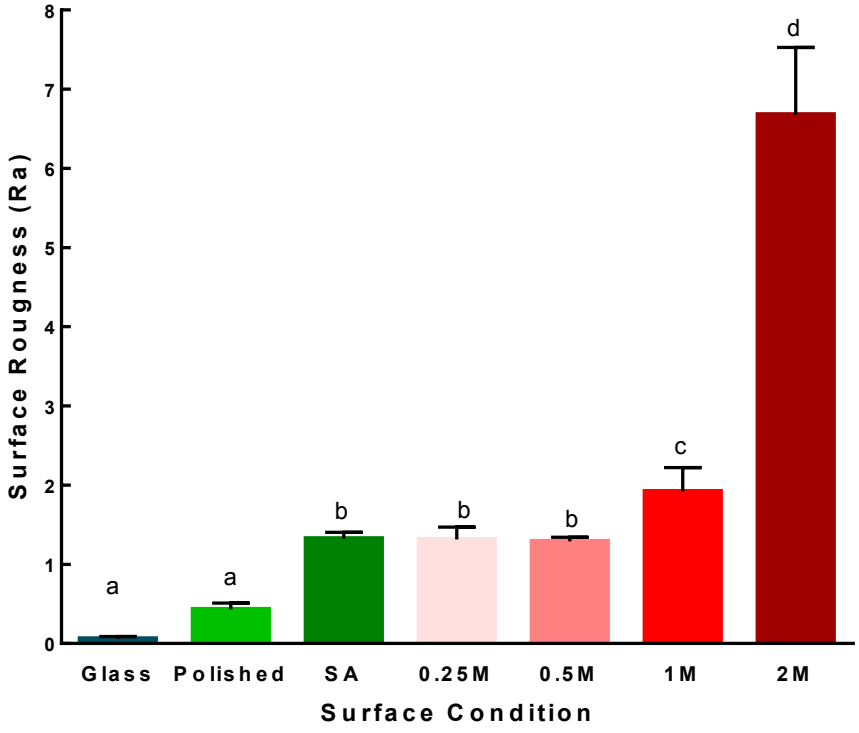

Figure 3-6 Surface roughness values of different Ti surfaces. Data are means ± SD of triplicate specimens. Different lower case letters indicate significance differences at $p < 0.05$.

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3.3.4 BIOACTIVITY

The surface morphology of Ti substrates soaked in SBF for 7 days was examined by SEM (Figure 3.7). Polished Ti and SA substrates did not exhibit any crystal deposition on their surfaces. In contrast, Ti substrates hydrothermally coated under different alkaline conditions did exhibit deposits resembling hydroxyapatite after soaking in SBF for 7 days. A dense particles of HA were fully deposited on the surface of coated substrates. The presence of hydroxyapatite was confirmed by XRD (Figure 3.8), indicating that the glass-coated surfaces were bioactive.

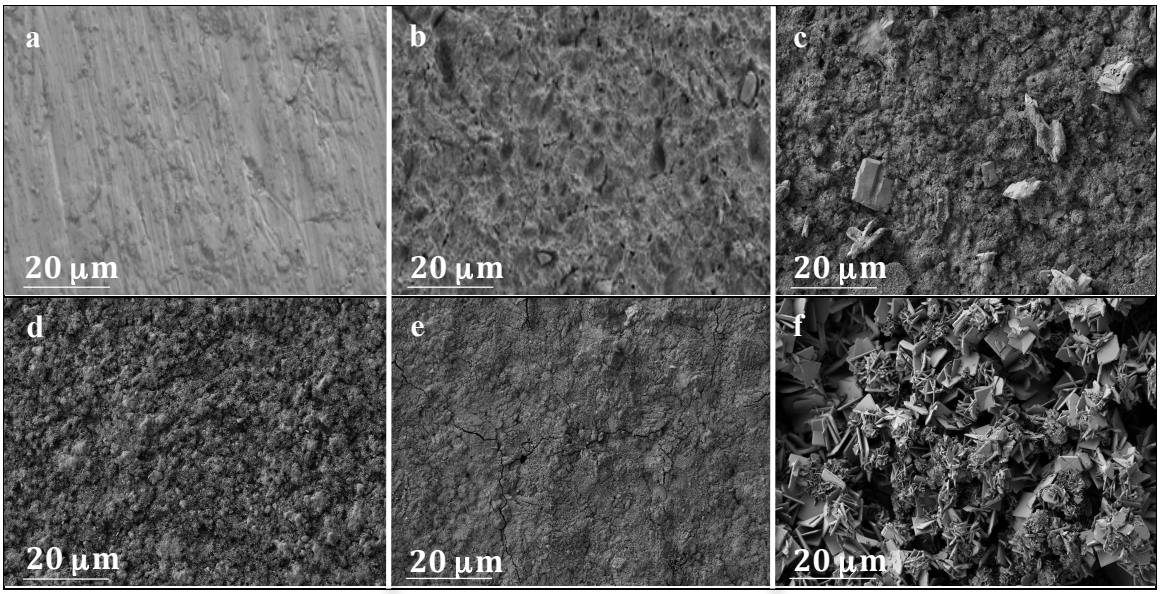

Figure 3-7a) Polished Ti (control) and b) SA Ti did not exhibit hydroxyapatite deposition on their surfaces after 7 days immersion in SBF at 37 ºC. c, d, e, f) Ti surfaces coated with BG under different alkaline conditions (0.25, 0.5, 1 and 2 M NaOH) showed hydroxyapatite-like crystals on their surfaces after soaking in SBF for 1 week. (n=3)

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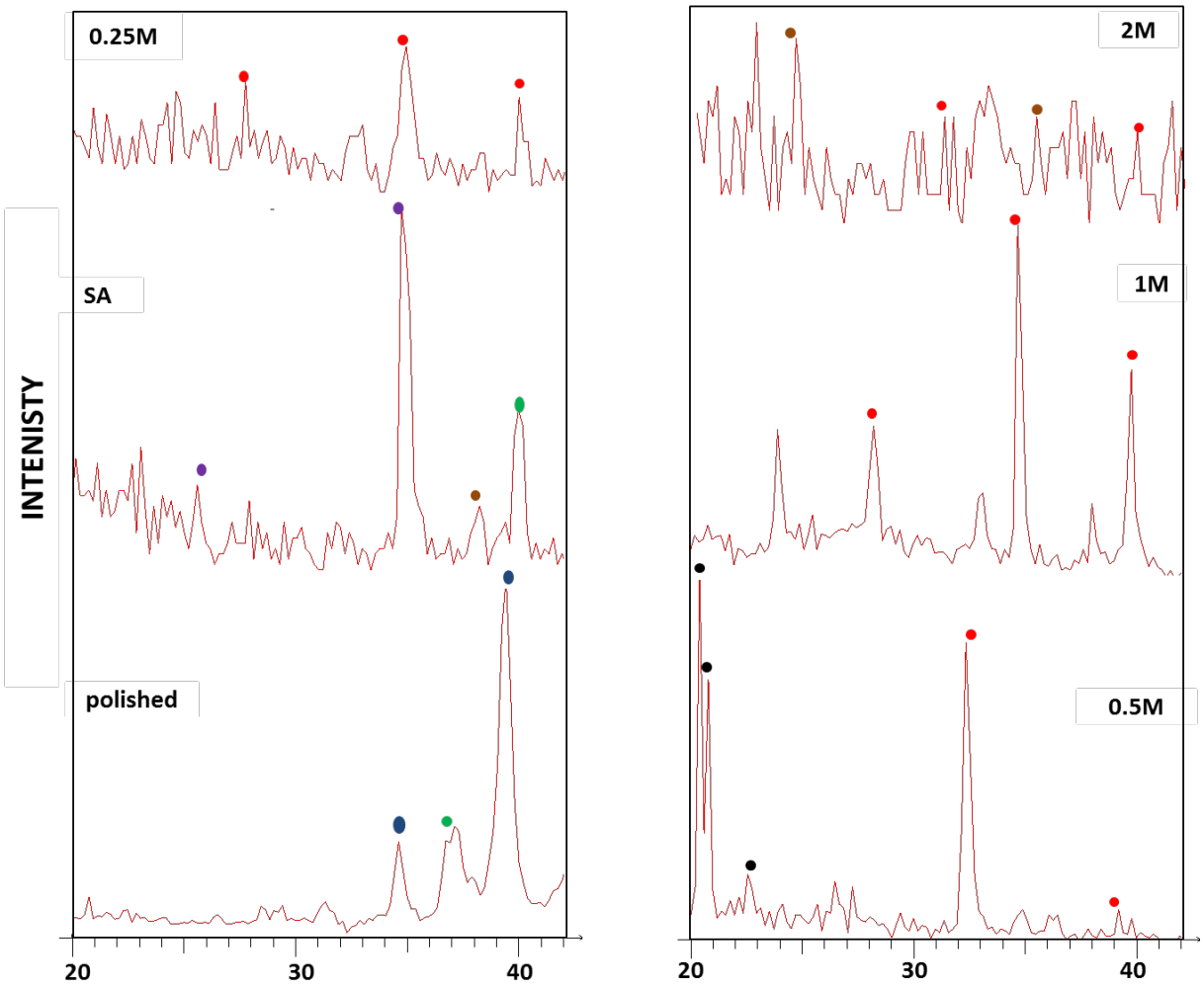

Figure 3-8 XRD analysis after immersion of substrate in SBF for 7 days. Polished Ti and SA surfaces showed 00-011-0029 (N)- calcium titanate (●) and 00-011-0177 (D)-beta calcium phosphate (●). Ti surfaces treated under 0.25 M NaOH showed 01-086-0740 (C)-hydroxyapatite (●). Ti surfaces treated under 0.5 M NaOH showed 00-044-0763 (D)-calcium phosphate hydrate (●) and 01-086-0740 (C) -hydroxyapatite (●). Ti surfaces treated under 1 M and 2 M NaOH showed 01-086-0740 (C)-hydroxyapatite (●) and 00-032-1369 (N)- titanium phosphate (●). Data was analyzed using EVA software.

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3.3.5 CELL INTERACTION TO SUBSTRATE

MC3T3-E1 osteoblast-like cells were cultured on Ti substrates coated with BG under 0.25, 0.5, 1 and 2 M NaOH. Glass and polished Ti discs were used as controls. SA was used as a positive control owing to its biocompatibility in clinical applications. Specimens were seeded with equal numbers of cells and then incubated for 24 h.

Fluorescence images showed that cells were uniformly distributed over the sample surfaces (Figure 3.9). On glass and polished Ti substrates, the cells were well spread with more flattened nuclei compared to other conditions. Higher magnification images revealed the formation of focal adhesions. In contrast, the cells on Ti substrates coated with BG under 0.25, 0.5, 1 and 2 M NaOH were a mixture of polygonal and spindle-shaped with more focal adhesions. Cells were mainly detected at the peripheries of the substrates.

Cell attachment was quantified by counting the number of cells on each sample. Statistical differences were assessed using ANOVA (Figure 3.10). No significant difference was observed regarding the number of cells between glass and polished Ti substrates. There was significantly greater cell attachment to Ti hydrothermally coated under 0.25 M NaOH than to glass, polished, SA or Ti hydrothermally coated under 2 M NaOH. There was no significant difference in cell number on Ti substrates coated under 0.25, 0.5 and 1 M NaOH.

The extent of focal adhesion formation was quantified (Figure 3.11). The surface area of focal adhesion of cells on smooth surfaces including glass and polished Ti as well as Ti hydrothermally coated under 2 M NaOH were significantly greater than on surfaces

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coated under 0.25 M, 0.5M and 1 M NaOH, which exhibited the smallest areas of focal adhesion.

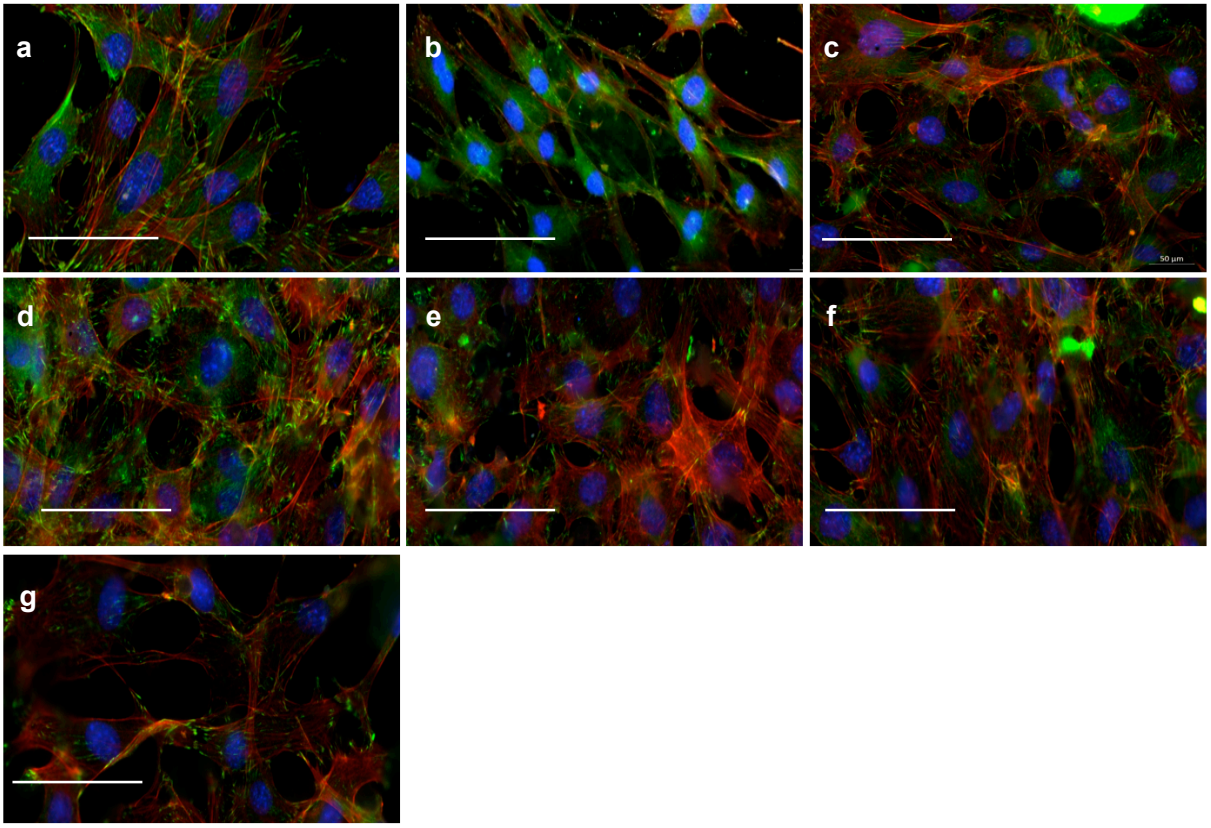

Figure 3-9 MC3T3-E1 osteoblastic cells were cultured on the substrate for 24 hours. Cells were then fixed and labeled for nuclei (blue), filamentous actin (red) and vinculin (green). Cells were imaged by fluorescence microscopy. a) Fluorescence image of MC3T3-E1 cells on control glass. b) Fluorescence image of MC3T3-E1 cells on Polished Ti. c) Fluorescence image of MC3T3-E1 cells on sand-blasted and acid-etched Ti. d) Fluorescence image of MC3T3-E1 cells on glass-ceramic coating prepared with 0.25 M NaOH. e) Fluorescence image of MC3T3-E1 cells on glass-ceramic coating prepared with 0.5 M NaOH. f) Fluorescence image of MC3T3-E1 cells on glass-ceramic coating prepared with 1 M NaOH. g) Fluorescence image of MC3T3-E1 cells on glass-ceramic coating prepared with 2 M NaOH. Scale bars represent 50µm. Images are representative of triplicate independent experiments.

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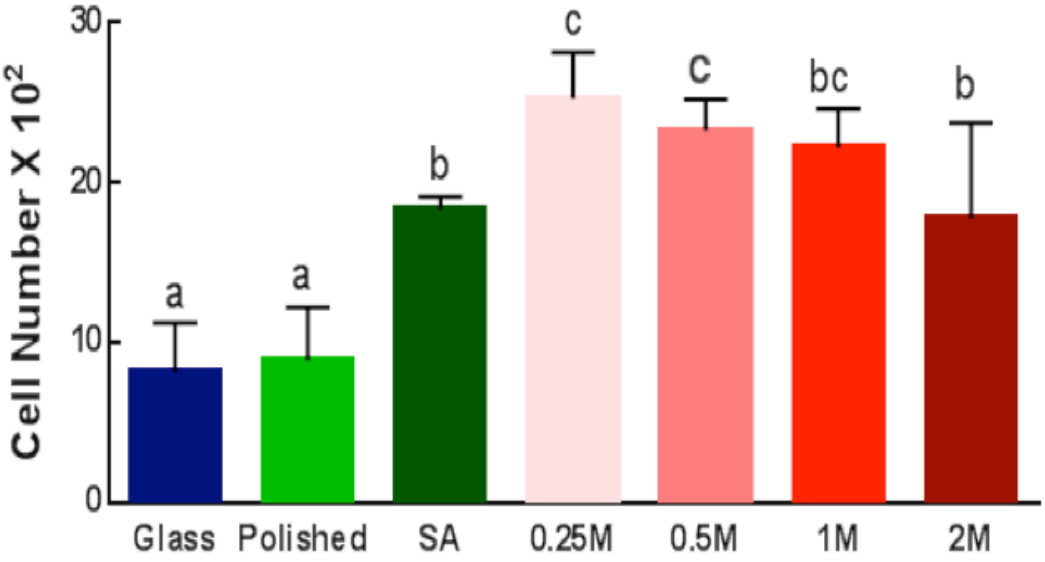

Figure 3-10 Histogram comparing the number of cells on glass and different Ti substrates. Data are means ± SD of triplicate independent experiments (n=3). Different lower case letters indicate significant differences at $p < 0.05$.

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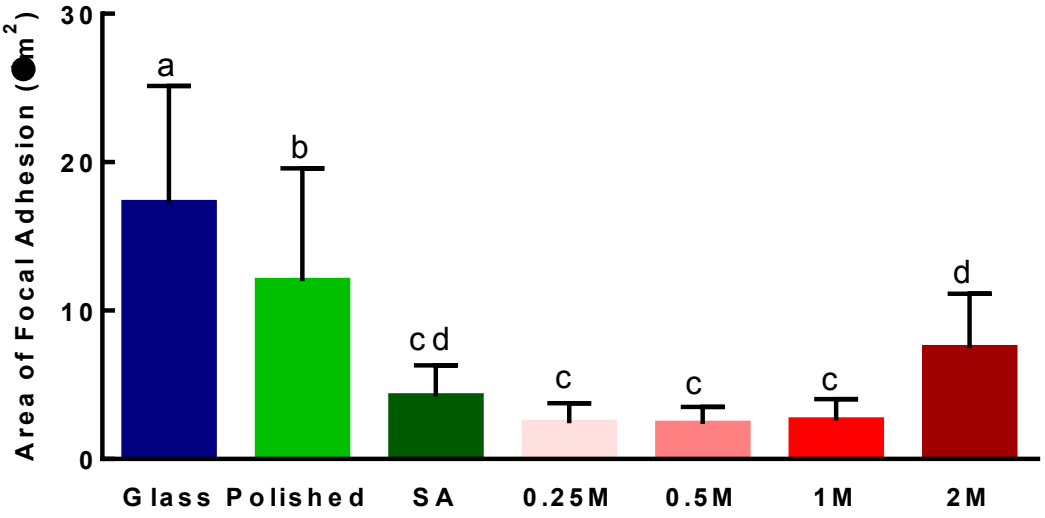

Figure3-11. Histogram comparing the area of focal adhesion on glass and different Ti substrates. Data are means ± SD of triplicate independent experiments (n=3). Different lower case letters indicate significant differences at $p < 0.05$.

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3.4 DISCUSSION

In this study, we developed a novel hydrothermal method for coating titanium surfaces with BG. This method involves a sol-gel synthesis of tertiary system of BG followed by hydrothermal treatment at 170ºC under different NaOH concentrations for 24 hours. In the late 1960s, Hench developed bioglass®, since then, different formulations of BGs have been developed as bone graft and bone contact materials [23]. BGs are ideal candidate coating materials due to the osteostimulative (osteoinductive and osteoconductive) characteristics of their dissolution products that enable them to bond directly to bone. In contrast, hydroxyapatite coating is reported to be an osteoconductive coating only [24, 25]. It is essential that implant materials be biocompatible, thus any modification to implant surfaces needs to be investigated for possible cytotoxicity [26]. Surface characteristics of the implant has a critical role in the process of osseointegration since the cascade of biological reactions that occur is closely related to the surface that is first exposed in the body [27, 28].

Different studies reported that treatment of commercially pure Ti and Ti alloy surfaces with NaOH followed by heat treatment produced bioactive Ti. Sodium titanate was detected after treating titanium under various NaOH concentrations (4-10 M) at 60◦C for 24 h followed by heat treatment at 400–800◦C for 1–24 h [25, 29, 30]. The produced sodium titanate layer enhanced the deposition of bone-like apatite crystals when immersed in SBF solution. On the submicron scale, this layer is highly porous and irregular [31]. In another study, commercially pure Ti and Ti alloys were soaked in SBF for 7 and 10 days at RT after being treated for 24 h at 60◦C with 5 M NaOH. HA was detected on the alkaline-treated surfaces only [32].

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In our study, commercially pure Ti discs were hydrothermally coated with BG under 0.25, 0.5, 1 and 2M NaOH for 24 h at 170ºC. The SEM and EDX analyses revealed that polished Ti substrates (control) were relatively smooth, whereas SA surfaces showed some irregularities. The coated samples exhibited different topographies when treated under 0.25, 0.5, 1 and 2 M NaOH conditions. Taken together with the EDX analyses, these results indicate that chemical reactions are taking place between the Ti surfaces, BG and sodium hydroxide during the hydrothermal process. The untreated Ti substrates displayed a planar micro-topography with smooth surface. In contrast, after being hydrothermally treated under different NaOH concentrations, the surface became more irregular with larger particles on its surfaces. EDX results showed differences in the atomic composition between region "a" and "b". This result may be due to a short reaction time. Hydrothermal coating for longer periods of time might result in more homogenous coating on the Ti surfaces.

As shown in figure (3.5), our XRD data revealed very weak and poorly defined peaks of sodium titanate as well as SiO_2. Considering the previous reported studies, it was shown that a porous film of amorphous sodium titanium oxide is produced in the case of alkaline heat treatment [25], yet ours appear poorly crystalline. Presence of lattice defects and alteration in the size of the crystals can result in poorly defined XRD peaks as well [25, 33].

XRD analysis of samples before and after being hydrothermally coated with BG revealed different results. In the case of polished and SA Ti substrates, no characteristic diffraction peaks of hydroxyapatite were detected, whereas in the case of Ti surfaces hydrothermally

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coated under different NaOH concentrations hydroxyapatite was detected after 7 days of soaking in SBF.

Bioactivity of Ti substrates coated with BG under different alkaline hydrothermal conditions was evaluated by soaking them in SBF to study their ability to produce HA on their surfaces. An apatite layer was deposited on the coated surfaces after 7 days of immersion, which was observed by SEM and confirmed by XRD. It should be noted that the uncoated Ti surfaces including polished and SA Ti did not exhibit any HA deposition, which indicates that surface chemistry plays an important role in HA deposition rather than surface roughness. The mechanism of HA deposition is likely similar to what has been described in the literature regarding bioactive glasses. The process involves the following steps. First, Na^+ and K^+ are exchanged with H^+ or H^3O^- from the solution. SiOH4 is released to the solution. Silicon-to-oxygen bonds break down forming silanol on the surface of the material. Thereafter, condensation and repolymerization of the SiO_2-rich layer on the surface occur. Ca as well as P ions deposit on the surface forming a layer of amorphous calcium phosphate. The amorphous calcium phosphate then crystallizes to form hydroxycarbonate apatite (HCA) [34, 35].

Another possible explanation of the bioactivity of the coated substrates was observed by Kokubo *et al.* In Kokubo's study, a bone-like apatite layer was deposited on titanium orthopaedic implants following sodium hydroxide and heat treatment, and immersion in SBF. In the proposed mechanism, H_3O^+ ions from SBF are exchanged for Na^+ ions from the titanate layer. TiOH groups are formed, that react with Ca^{2+} ions to produce amorphous calcium titanate. Thereafter PO_4^{3-} ions interact with it to produce amorphous calcium phosphate that is transformed into HCA. The mechanism of bioactivity is very

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important, especially the last two steps. Knowing that bioactivity test is time-dependent, the longer the time needed for a material to form HCA indicates poorer bioactivity [34].

Previous studies reported that micro-topography and roughness of Ti surfaces has a huge influence on the attachment rate of proteins and other biological macromolecules. Adsorption of these molecules affects cell attachment as well as subsequent proliferation that eventually enhance osseointegration. In our work, the average the Ra value of uncoated polished Ti and glass was about 0.33 µm and 0.43 µm respectively, whereas it increased significantly for coated Ti surfaces, indicating that the coating layer can significantly increase the surface roughness. It was detected that roughness of Ti surfaces enhances osteoblast-like cells proliferation [36]. In our work, Ti substrates hydrothermally coated with BG exhibited higher number of cells compared with glass and polished Ti surfaces.

The SLA titanium surfaces developed by Straumann reported excellent osseointegration, yet the studies explain it as an effect of the surface topography knowing that the role of surface chemistry is not clear [37]. Studies reported that Ti substrate with moderate surface roughness of (1-2 µm) have greater bone production levels compared to rougher and smoother ones [36]. In addition, acid-etched Ti showed superior levels of osseointegration compared to Ti plasma sprayed (TPS) as well as machined titanium, due to the micropore roughness produced [38-40]. The surface chemistry of SLA Ti as in the hydride Ti is the main reason for its bioactivity due to the release of hydride ions that break down the TiO_2 and form Ti-OH that acts as nucleation site for the hydroxyapatite [25, 28]. The formation of hydroxyapatite on the surface will eventually absorb serum proteins that are essential for osteoblast attachment, differentiation and proliferation.

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In our research, the glass and polished Ti surfaces exhibited lower roughness values compared to SA Ti and hydrothermally coated Ti. The Ra value for polished and glass surfaces was 0.4 µm. SA, 0.25 M and 0.5 M coated surfaces had average Ra value of 1.3 µm. Surfaces treated under 1 M exhibited higher surface roughness of 2 µm. Surfaces treated under 2 M NaOH had the largest Ra value of 6.67 µm. In our study, it was shown that the osteoblast-like cells attached best to the moderately rough surfaces (Ti substrates treated under 0.25, 0.5 and 1 M NaOH) compared to the smoother and roughest surfaces.

Knowing that the surface topography has a major influence on the osteoblast behavior, different methods of assessing osteoblast response have been used in previous studies including attachment, differentiation and biomineralization, all of which were shown to be enhanced on rougher surfaces [41]. In contrast, spreading and proliferation favor smooth surfaces [41]. Attachment as well as spreading represent the initial stages of cell-biomaterial interaction, which will determine whether cells will be able to form bone or not. Initial attachment alters morphology and subsequently cellular processes such as differentiation and proliferation [42, 43]. In the present study, we report that the BG coating, especially in the case of 0.25 M NaOH coated surfaces, enhanced cell attachment. Other studies indicate that increasing the surface roughness of HA coating on titanium enhanced cell attachment [44]. In our study, surface roughness increased cell attachment but to a certain limit. That is, moderate surface roughness of Ra value 1-2 µm was the most supportive for cell attachment. Our data suggests that surface roughness is not the only factor that controls cell responses. The surface chemistry of the coating also has a direct effect on the bone response.

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Focal adhesions connect the cytoskeleton of the cells to the extracellular matrix or biomaterial surface[46]. They are characterized by the localization of certain proteins including vinculin that support interactions between F-actin and integrin. Development of stable focal adhesions is strongly influenced by the topographical and chemical features of the biomaterial. The interaction of cells with biomaterials depends mainly on their ability to attach to the surfaces through specific proteins attached to the implant surface. Cell attachment, as well as cell response to absorbed proteins depends on integrin engagement [36, 47]. Variation of surface energy results in different cell attachment responses due to topographical differences [42]. The attachment of osteoblasts on the surface of an implant is reported to be essential for long-term survival of dental implants. For example, early stabilization is enhanced by rapid cell attachment decreasing the chance of fibrous capsule formation. It was reported that the TPS has higher levels of cell attachment compared to the SLA titanium [48]. Moreover, both SLA and TPS showed higher cell attachment levels than polished and machined Ti [49]. In our research, we showed that the cell attachment levels were greatest in case of the coated Ti under 0.25 to 1 M NaOH.

The size of focal adhesions formed by cells on the different surfaces was significantly different. Cells attached to the polished Ti as well as the glass control exhibited focal adhesions with the largest surface areas. In contrast, cells attached to surfaces treated under 0.25, 0.5 and 1 M NaOH exhibited focal adhesions with the smallest areas. The mean size of focal adhesion has a critical influence on cell migration [46]. Adhesion is also related to activation of cell signaling cascades that regulate many processes including differentiation [50, 51]. Moreover, changes in surface roughness influence

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expression of genes associated with matrix remodeling, specifically surfaces that prevent formation of stable adhesions. The exact role of focal adhesion size in the regulation of cell behavior is controversial, with several studies suggesting that the actual size may not be as important as which intracellular signaling cascades are activated.

It is not only the micro-scale features such as mechanical interlocking, but also the nano-scale structures that can affect the adhesion and differentiation of osteoblasts. Costa et al. described rat calvarial osteoblasts grown on HA surfaces with different surface roughness Ra values of 1, 1.3 and 2 μm for 6 hours [44]. In their study, the hydroxyapatite surfaces with Ra value of 2 μm showed the highest levels of cell attachment. However, surface roughness is not the only factor influencing cell attachment.

We found that osteoblasts cultured on BG exhibited more dorsal ruffles and filopodia. In contrast, osteoblasts on HA, Ti alloy, and stainless steel have flattened morphology with almost no filopodia or dorsal ruffles [52, 53]. Osteoblast attachment and spreading on BG may be superior compared to HA due to the high free surface energy of the BG that results in characteristic corrosion process at the surface of the BG [54, 55].

Although other investigators have developed BG coatings on the surface of Ti, this is the first study that achieved BG coating by hydrothermal coating under alkaline conditions. The main advantages of this method include the bioactivity of BG and the alkaline conditions of the hydrothermal reaction that provide multiple nucleation sites for HCA deposition. The ability of this novel method to develop bioactive surfaces with optimal surface roughness provides a useful alternative method for the coating of titanium. This

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would help to assure primary fixation of titanium dental implants that eventually support the long-term success of implants.

3.5 CONCLUSIONS

The dual effect of surface chemistry and surface topography of the bioactive glass coated titanium surfaces under alkaline conditions offers a promising approach for titanium surface modification. These modifications have the potential to directly influence the local response of the surrounding tissues and the process of new bone formation, resulting in better osseointegration and improving the long-term success of dental implants.

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CHAPTER 4: CONCLUSIONS

4.1 SUMMARY AND CONCLUSIONS

This study resulted in the successful synthesis of bioactive and osteoconductive coating onto Ti substrates through a two stages sol-gel-hydrothermal process. I also created a novel sol-gel hydrothermal coating method for Ti implants. This method involves sol-gel processing of a tertiary BG system followed by alkaline hydrothermal treatment at 170 ºC that produced a reaction between the BG and Ti substrates to produce a bioactive surface. A bioactive coating was successfully applied on Ti surfaces with clear differences among different hydrothermal conditions. EDX elemental analysis as well as SEM revealed that the BG coating was not uniform on the surface of Ti. Coatings prepared under 0.25M and 0.5M NaOH had a nano rectangular plates appearance while surfaces coated under 1M and 2M NaOH exhibited larger, coarser and non-uniform structures. This could be a limiting factor to this study. Our data suggests that longer reaction time is required in order to obtain a homogeneous coating on the surface of Ti.

The effect of reaction pH under hydrothermal conditions on the surface topography and chemistry of the coatings was investigated. Osteoblast-like cells were used to assess attachment to the BG coated Ti surfaces. The substrates with average surface roughness exhibited the highest cell attachment including Ti surfaces coated under 0.25M, 0.5M and 1M NaOH. However smoother and rougher surfaces resulted in lower levels of cell attachment including glass, polished-Ti and Ti surfaces coated under 2M NaOH. This data points to the importance of the surface roughness for cell attachment.

The early integration of implant in patients' bone is a critical challenge. The failure of early integration results in failure of implantation. Knowing that Ti is a bioinert material

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that does not form a biological bond with bone, different studies investigated the possibility of surface modification of Ti to enhance the process of bone formation and accordingly integration [1]. The surface modifications reported include surface coatings of HA, TiO_2 and bioactive glass using different methods of application [2, 3]. Various methods of applications reported some limitations that affected the abilities of the coatings applied. Our study reported an alternative method for BG coating application on Ti surface using low temperature hydrothermal process. Therefore, we decided to use BG synthesized by sol-gel chemistry as an alternative method over the melt-derived process due to the generation of porous BG of higher surface area which greatly enhances the bioactivity as well as the degradability [4]. Thereafter, BG is replaced by bone tissue. It was also reported that the silica layer or the silica gel formed on the surface of the BG as a result of leaching of ions plays a major role in the nucleation of HA [5, 6]. The production of HA on the coated surfaces in our study indicates the ability of these surfaces to enhance osseointegration. The *in vitro* cell work in our study demonstrated that all of the Ti coated surfaces are biocompatible. Based on the cellular response to the Ti coated surfaces, including BG hydrothermally coated under different NaOH concentrations. We can conclude that these surfaces provide favorable conditions for cell attachment and growth. In summary, the biological response of the Ti implants can be enhanced by modifying the non-physiological surfaces of Ti through the application of a bioactive coating that enhances the biological as well as the chemical bonding between the Ti surfaces and bone tissue. In that respect, our data suggests that the combination of the chemical properties of the BG coating as well as the modified surface roughness on the Ti substrates may result in an effective bone-resembling coating and as such to a new

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method of surface-modified titanium implants with improved functionality and biological efficacy. Our results show that favorable cell attachment can be reproduced in order to accelerate bone formation as a result of surface topography and chemistry.

Our major concern is the longer time for primary implant fixation. We claim that our study would offer an excellent method of bioactive coating for Ti implants which has the potential to accelerate bone formation and accordingly fixation.

4.2 CONTRIBUTIONS TO THE CURRENT STATE OF KNOWLEDGE

4.2.1 GENERAL SIGNIFICANCE

Considering the bioinert property of Ti that cannot form any chemical bond with bone tissue and knowing that early fixation of dental implants is of ultimate importance for long term success, the development of bioactive coating on the surface of Ti was conducted previously by different researchers [7]. Different limitations were encountered for these methods that alter the performance of the bioactive coating. We examined, the effect of surface roughness as well as surface chemistry of Ti implants on osteoblast attachment. It was also reported that the silica layer or the silica gel formed on the surface of the bioactive glass as a result of leaching of ions play a major role in the nucleation of hydroxyapatite. So the combination of bioactive glass synthesized by sol-gel and alkaline hydrothermal coating of Ti substrates at 170 ºC is quite novel and has not been published elsewhere. The low cost of the developed method in this study as well as its ability to support early fixation and bone formation greatly support its introduction to the market.

4.3 LIMITATIONS

The major limitation of this study is that uncoated control substrates including polished and SA surfaces did not undergo hydrothermal process under alkaline conditions. This

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would have given a better understanding of the evaluation of the effect of each of the surface topography and/or BG coating on Osteoblast interaction with the Ti surface.

Another limitation was the performance of polishing, sand-blasting and acid-etching in our laboratory that might not have been reproducible as in the case of the SLA process developed by Straumann. In order to overcome this issue, prepared samples from Straumann can be used.

In addition, our study did not evaluate the effect of reaction time under different alkaline hydrothermal conditions on surface topography, chemistry and osteoblast cell Ti interaction.

Moreover, for cell attachment evaluation, the cells were counted in this study following 24 h of incubation that might reflect a combination of cell attachment and proliferation. In other words, cell attachment should have been evaluated after 6 hours of incubation.

4.4 FUTURE DIRECTIONS

Different characterization methods should be conducted in order to fully understand and judge the performance of sol-gel hydrothermally coating of the BG coating on Ti surfaces. For example, X-ray photoelectron spectroscopy (XPS) can be used to analyze the chemistry of the modified titanium surfaces. XPS would provide further understanding of the influence of different crystalline phases produced as a result of the hydrothermal reaction. Atomic force microscopy (AFM) can also be conducted to study the effect of nanotopography on osteoblast cell-Ti interactions. The effect of reaction time under different alkaline hydrothermal conditions on surface topography, chemistry and osteoblast cell Ti interaction should be evaluated. Cell work can be extended to

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include the study of the effect of hydrothermal bioactive coating of Ti surfaces on cell proliferation and differentiation. This would allow better prediction of the performance of the coated surface in bone tissue. The investigation of the mechanical properties as well as the bond strength of the coating to the Ti surfaces should be conducted in order to evaluate the ability of the coating to withstand the physiological stresses.

Thereafter, *in vivo* biocompatibility of the hydrothermally coated Ti surfaces with BG should be conducted to investigate the effect of the coating on a living system such as rats or rabbits. This would provide more information about bone-to-implant response. Long-term research should include clinical studies prior to the application of this technology to the market place.

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APPENDIX AND CURRICULUM VITAE

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APPENDIX A: COPYRIGHT PERMISSIONS

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A01_First_Name: Mohamed
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A13_Requestor_Name:
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A16_Product_Title: Anatomy and Physiology: From Science to Life
A17_ISBN: 0471770671
A18_Author_Name: Jenkins, Gail W; Kemnitz, Christopher P; Tortora, Gerard J
A19_Page_Number:
A20_Number_of_Copies:
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CURRICULUM VITAE

Mohamed Gebril

Education

MESc, Biomedical Engineering (Biomaterials), University of Western Ontario, London, Ontario 2013-2015.
• Bioactive Glass Coating of Titanium Implants, Dr. Amin Rizkalla (supervisor)

Bachelor of Oral and Maxillofacial Surgery, Alexandria University, Alexandria, Egypt 2006-2011 with Honors.

Awards, Distinctions and fellowships

• Student of the Year 2010.
• Best Research Award 2009, 2011.

Presentations

• "Bioactive Glass Ceramic Coating for Titanium Implants"- 2013, Biomedical Engineering, Program seminar, University of Western Ontario.

• "The Relation between Chronic Periodontitis and Heart Diseases"- 2010, Department of oral medicine, Alexandria University.

• "Smoking and Prevalence of Oral Cancer"- 2009, Department of oral pathology, Alexandria University.

Work Experience

• Dentist (full time), Vahe Dental Clinic, Alexandria, Egypt 2012-2013
• Dentist (part time), Taj Dental Center, Alexandria, Egypt 2011-2012
• Internship Dentist, Alexandria Research Center, JAN- MARCH 2012
• Internship Dentist, Alexandria University, 2012

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Research Experience

• Graduate student, Biomedical Engineering, University of Western Ontario 2013-2014, "Bioactive Glass Ceramic Coating of Titanium Implants"

• Research assistant, Department of orthodontics, Alexandria University 2011, "Orthopedic Repair of Craniofacial Abnormalities"

• Research assistant, Department of Oral Medicine, Alexandria University 2010, "Smoking and Increased Risk of Gingivitis and Periodontitis"

• Research assistant, Department of Oral Pathology, Alexandria University 2009, "Clinical and Histological Study of Different Oral Cancers"

• Research assistant, Department Biomaterials, Alexandria University 2008, "Comparison between Amalgam and Composite Considering Advantages and Dis-advantages of each"

Teaching Experience

Teaching Assistant:

• Restorative Dentistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Winter 2015

Raw instructor

• Removable Prosthodontics, Dentistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Winter 2015

Raw instructor

• Engineering Science, Electrical Engineering, University of Western Ontario, London, Ontario. Fall 2014

Grading assignments and exams and instructing in tutorials

• MATLAB, Chemical Engineering, University of Western Ontario, London, Ontario. Winter 2014

Grading assignments and exams and instructing in tutorials

• Biomaterials, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario. Fall 2013

Grading assignments and exams and instructing in tutorials

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