ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

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M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

TİTANYUM SERT DOKU İMPLANT YÜZEYLERİNİN MAGNEZYUM KATKILAMA İLE MODİFİKASYONU

YÜKSEK LİSANS TEZİ

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EYLÜL, 2014

Okan MAZMANOĞLU, a **M.Sc.** student of ITU **Department of Molecular Biology**, student ID 521121148, successfully defended the **thesis** entitled **"Surface Modification of Titanium Hard Tissue Implants By Magnesium Doping"**, which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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FOREWORD

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SEPTEMBER 2014 Okan Mazmanoğlu

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LIST OF ABBREVIATIONS

EDS:	Energy Dispurive X-Ray Diffractometer
XRD:	X-ray Diffractometer
GDOES:	Glow Discharge Optical Emission Spectroscopy
NADP:	Nicotinamide adenin diphosphate
NADPH:	Nicotinamide adenin diphosphate hydrogen
fhOB :	Fetal human osteoblast
MTS:	3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt

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SURFACE MODIFICATION OF TITANIUM HARD TISSUE IMPLANTS BY MAGNESIUM DOPING

SUMMARY

Hard tissue biomaterials have a great demand from all over the world according to increasing health care issues including hard tissue injuries, diseases or ageing. The most common materials for hard tissue biomaterial applications are metals due to their superior mechanical properties especially for load bearing applications.

Titanium (Ti) and its alloys are commonly used as hard tissue biomaterials, such as implants for total joint replacements or dental applications, since the discovery of their potential as biomaterials. However they encounter problems such as lack of corrosion and wear resistance in long term applications. The waste majority of these problems end up with implant failures and consequent revision surgeries, which are painful for the patients and also expensive.

In this thesis, magnesium (Mg) was used to address the potential issues of titanium based hard tissue biomaterials. Mg is a biocompatible and biodegradable metallic element so that it is considered as a good candidate for improving titanium surface properties. On the other hand, pure Mg cannot be used because of its rapid corrosion behavior in physiological media.

Cathodic arc physical vapor deposition technique which is the one of the main techniques for coating technology was used to modify Ti surfaces by Mg. The aim was to adjust Mg amounts on and within the layers of the new structured surfaces to be able to control corrosion behavior and the biocompatibility of the modified surface. Fine control of atomic Mg percentages below 10 at % was aimed to achieve this goal.

Second step was to determine the structural layer components of created modified surfaces and determine the depth of Mg deposited layers on the surface. The analysis of the surfaces was mainly made optical profilometer, GDOES and XRD. Both duration and current values were changed to determine their effect on doping and Mg amounts ranging from 27.1 ± 1.2 at. % in ~0.50 μm depth to 3.8 ± 0.7 at. % in ~0.75 μm depth were found using optical profilometer and GDOES analysis. XRD profiles also showed a metal oxide rich structure formation including titanium and titanium oxides. MTS assay of the coatings showed that lower Mg contents in the coatings were better for cell poliferation and viability. 3.8 ± 0.7 at. % Mg containing coating showed the best performance for cell proliferation even than pure Ti (0% at. Mg).

Results are promising for improving present Ti based hard tissue biomaterials by this relatively practical approach including Mg.

TİTANYUM SERT DOKU İMPLANT YÜZEYLERİNİN MAGNEZYUM KATKILAMA İLE MODİFİKASYONU

ÖZET

Geniş kapsamlı bir tanımlama ile biyomalzemeler çoğunlukla tıbbi veya tıbbi olmayan amaçlarla kullanılan, yaşama ve büyüme yeteneğinde olmayan ve biyolojik sistemler ile etkileşime girmeye yatkın tüm malzemeleri içermektedir. Biyomalzemeler genel olarak metalik, seramik, polimerik ve kompozit materyallerden oluşmaktadırlar. Bu materyal tiplerinin beraber kullanıldığı sistemler de literatürde mevcuttur.

Sert doku biyomalzemeleri, tüm dünyada ihtiyaç duyulan sert doku yaralanmaları, hastalıklar veya yaşlılık kaynaklı sağlık sorunlarının tedavisinde sert doku implantlarının fabrikasyonunda kullanılan malzemelerdir. Özellikle yük mukavemeti gibi üstün mekanik özelliklerinden dolayı metalik biyomalzemeler bu alanda yaygınlıkla uygulanmaktadırlar. Paslanmaz çelik, kobalt-krom ve titanyum alaşımları sert doku biyomalzemelerinin yapımında yaygın olarak kullanılmaktadırlar.

Metalik biyomalzemeler üzerine yapılan bilimsel çalışmalar genellikle toksisitenin düşürülmesi, korozyon direncinin arttırılması, aşınma direncinin arttırılması, biyouyumluluğun geliştirilmesi , elastik modül değerinin doğal kemik yapısıyla karşılaştırıldığında uygunluğunun arttırılması , biyoaktivitesinin arttırılması, yüksek dayanıklılık, düşük kalıntı oluşturma ve iyi osteoentegrasyonun sağlanması üzerinde durmuştur.

Titanyum (Ti) ve alaşımları tam eklem protezleri veya diş implantları gibi alanlarda en sık kullanılan metallerdendir. Ancak metalik biyomalzemelerin karşılaştığı temel sorunlar Ti için de geçerlidir. Bu sorunlar genellikle korozyon dirençliliğin düşük olması veya uygulanan biyomalzemenin uygulama bölgesindeki çeşitli aşınma ve sürtünme kaynaklı problemlerden etkilenmesi ile oluşur. Bu sorunların çoğu uygulanan biyomalzemenin, özellikle uzun dönem kullanım gerektiren uygulamalarda başarısızlığa uğramasına neden olur. İmplantın görevini yapamaz hale gelmesi, ikinci bir operasyonu gerekli kılar ve bu operasyonlar uygulama yapılan kişilere acı vermesinin yanında daha düşük başarı oranlarına ve yüksek maliyete sahiptirler.

Magnezyum (Mg) çeşitli medikal uygulamalarda uzun bir kullanım tarihi olan ve bahsi geçen Ti yüzey özellik yetersizliklerinin bir çaresi olarak son yıllarda popüleritesi artan biybozunur ve biyouyumlu bir metalik elementtir. Mg vücutta en fazla bulunan katyon olmasının yanında kemik dokusunun doğal yapısında da bulunmaktadır. Bu özelliği Mg tabanlı biyomalzemelerin aynı zamanda yüksek

biyouyumluluğa ve yüksek doku etkileşimi yeteneğine sahip olmasını da sağlamış olur. Mg doğal kemik dokusu ile karşılaştırıldığında diğer geleneksel metalik biyomalzemelerden yaklaşık 3 kat daha iyi elastik modül değerlerine sahiptir. Bu özellikleri bakımından avantajlı olan Mg, saf olarak kullanıldığı uygulamalarında yüksek korozyona ve bunun sonucu olarak belirli stokiyometrik denklemlere bağlı olarak fizyolojik ortamda hidrojen gazı oluşumuna neden olmaya eğilimli olduğundan, istenilen özellikleri sağlayabilmesi için diğer malzemelerle birlikte kullanılması daha uygun olabilmektedir.

Bu tezde yukarıda bahsedilen sorunlara bir çözüm olarak, Ti yüzeylerin Mg ile kontrol edilebilir biçimde modifiye edilerek in vivo performansı iyileştirilmiş sert doku implantları elde edilmesini amaçlanmıştır. Bu amaçla bilinen bir kaplama tekniği olan katodik ark fiziksel buhar biriktirme yöntemi (katodik ark FBB) ile Ti yüzeylerinin modifikasyonu ve aynı zamanda Mg'un Ti yüzeylerinde, yüzey altında veya alt-ara yüzeylerinde depolanarak korozyon miktarının fizyolojik ortamlarda kontrol edilmesi amaçlanmıştır.

Bu amaca yönelik olarak 1cm^2 Ti altlıklar kimyasal olarak temizlenmiş ve katodik ark FBB yöntemi için tasarlanan Mg ve Ti katodları arasında bulunan, bir perde sistemi üzerinde sabitlenmiştir. Kaplama denemeleri toplam kaplama süreleri sabit tutularak, farklı katod akım değerleri ve iyon bombardımanı zamanlarında gerçekleştirilmiştir. İlk olarak katod akım değerleri sabit tutulmuş ve iyon bombardımanı zamanları kademeli olarak değiştirilmiştir. Bu sayede iyon bombardıman zamanının kaplamaların ihtiva ettiği Mg miktarı üzerindeki etkisi araştırılmıştır. Daha sonra iyon bombardıman zamanı ve Ti katod akım değeri sabit tutularak, Mg katod akım değerleri düşürülerek seri denemeler ile üretilen yeni kaplamalardaki Mg oranlarının düşürülmesi sağlanmıştır.

İlk adım, kaplama sonrası yapılan elementel analizler ışığında atomik Mg oranlarının % 10 ve altına indirilmesinin başarılmasıyla tamamlanmıştır. Bu oranın üstündeki Mg miktarlarının korozyonu hızlandırdığı bilindiğinden % 10 ve altı hedefinin gerçekleştirilmesi önemlidir. Bu adımdan sonra Mg un Ti yüzey altında gerçekten depolanıp depolanmadığı ve depolandıysa kaplama kalınlığı ile karşılaştırıldığında ne kadar kalınlıkta Mg içeren ara yüzey oluşturulduğunun tayini yapılmıştır. Bu yapılan ölçümler ışığında Mg'un en fazla oranda bulunuduğu 27.1 ± 1.2 at. % Mg içeren kaplama da ~0,50 μm derinliğe kadar ve en düşük oranda bulunduğu 3.8 ± 0.7 at. % Mg içeren kaplamalarda ~0,75 μm derinliğine kadar tespit edilebildiği GDOES ve 3D optik profilometre analizlerinin sonuçları doğrultusunda ortaya çıkarılmıştır. Ayrıca yapılan XRD analizlerinden kaplama sonrası birçok titanyum ve titanyum-magnezyum oksit zengini bir yüzey yapısına kavuşulduğu düşünülmektedir.

Elde edilen kaplamaların hücre canlılığı ve hücre proliferasyonu açısından performanslarının ölçülmesi amacaıyla yapılan MTS deneyleri sonucunda, düşük Mg içerikli kaplamalarının hücre proliferasyonu açısından daha iyi olduğu ve en az Mg içeriğine sahip olan 3.8 ± 0.7 at. % Mg içeren kaplamada en yüksek hücre proliferasyonun olduğu görülmüştür.Ayrıca bu sonuç saf titanyumdan (0 at. % Mg) daha iyi performans göstermiştir. MTS deneyi sonucunda elde edilen veriler,

kaplama üzerine fikse edilen hücrelerin SEM vasıtasıyla görüntülenmesi ile de desteklenmiştir.

Elde edilen bu olumlu sonuçlar Ti yüzeyler içerisine ve üstüne ayarlanabilir oranlarda biyobozunur ve biyouyumlu özellikteki Mg'un yerleştirilebildiği ve bu yöntemin biyomalzeme özelliklerinin modifikasyonunda kullanılabileceği ortaya konmuştur.

1.INTRODUCTION

1.1 Purpose of Thesis

Titanium (Ti) and its alloys are extensively used as metallic biomaterials for hard tissue biomaterials. Despite their unique properties such as relatively low modulus, high strength and biocompatibility, there are several reported cases for failed long term applications due to low osteointegration performance and wear resistance. Magnesium (Mg) is considered as a good alternative tool for surface modifications of Ti based biomaterials to overcome these issues in last decades (Geetha et al, 2009; Staiger et al., 2006).

This thesis proposes a new approach for designing Mg modified Ti surfaces via cathodic arc physical vapor deposition (cathodic arc PVD), which is one of the important coating techniques that allows the modification of the surface in atomic level. The aim is to do fine tuning of Mg amount on Ti surfaces through a novel surface design to control corrosion rate and to obtain a higher degree of osteoconduction and osteointegration.

1.2 Biomaterials: General Aspects

A broad definition is that biomaterial is a nonviable material used in a medical device intended to interact with biological systems (Williams, 1987).

Biomaterials are generally used in different applications including medical and non-medical uses. While artery grafts, dental implants, intraocular lenses, hip,knee and shoulder joints, artificial heart valves, drug delivery systems, stents, catheters and wound dressing are being introduced in medical field, arrays for DNA and diagnostics, bioremediation systems, biosensors, bioseparation and chromatography systems, fuel cells and nanofabrications may be placed under biomaterials for non-medical purposes (Ratner and Bryant, 2004).

If we classify biomaterials under generations there would be three generations which has strong informational and methodic connections between them: bioinert materials as first generation, bioactive and biodegradable materials as second generation and materials having the ability to induce specific cellular responses at the molecular level as third generation. All generations include metallic, ceramic, polymeric and composite type materials and their combinations. Polymeric biomaterials could be easily manufactured and modified towards desired properties and used for especially in soft tissues and drug delivery systems. On the other hand, they have tendency to be degraded and could deform with time. Metallic biomaterials are generally used in the design of hard tissue implants and have powerful mechanical properties such as toughness and strength. However they may encounter corrosion problems. Ceramics are one of the most biocompatible class of inert biomaterials whereas they are brittle and not resilient. (Navarro et al, 2008; Banerjee, 2012).

First generation biomaterials have first been studied and used extensively in twentieth century and still are in use. They had been produced to fulfill the demand of replacements especially for total joint replacements, bone fractures, low back pain, osteoporosis etc. as other biomaterial generations. These biomaterials were designed on simple requirements such as matching or suitable mechanical, physical and chemical properties. First generation metallic materials can be simply grouped to stainless steel (i.e. 316L stainless steel), cobalt-chrome (Co-Cr) based alloys, Ti and its alloys, and NiTi alloys as shape memory alloys. The research on metallic materials are focused on improving toxicity, corrosion resistance, wear and shear resistance, biocompatibility, elastic modulus sufficiency compared to natural bone structure, bioactivity, high strength, low residual debris formation, good osteoconduction and osteointegration properties of selected and produced materials for instance orthopedics and different load-bearing applications. Ceramic and polymeric biomaterials both consists of first, second and third generation biomaterials. Alumina (Al_2O_3, zirconia, hydroxyapatite ($Ca_{10}(PO_{4)6}(OH)_2$), β-tricalcium phosphate (β-TCP, $Ca_3(PO_4)_2$) are the main materials in bioceramic class. Polymeric materials which are commonly preferred are silicon rubber, poly ethylene (PE) and ultra high molecular weight PE, acrylic resins, polyurethanes, polypropylene (PP), polymethyl-methacrylate (PMMA) and biodegradable polymers

such as polyglycolide (PGA), polylactide (PLA), polydioxanone (PDS), poly(ε-caprolactone) (PCL), polyhyrdoxybutyrate (PHB), polyortoester, chitosan, poly (2-hydroxy methacrylate (PHEMA), hyaluronic acid and hydrogels (Navarro et al, 2008).

1.3. Metallic Biomaterials

Metallic biomaterials are mostly used for substitution or repairing components hard tissues which are shown in Figure 1.1 . Stainless steel, cobalt chrome and titanium alloys can be considered as common materials which take place in implant production and their implantation surgeries. Commercially available and well identified metallic alloys with different elemental compositions are available. (Geetha et al, 2009).

Stainless steel, especially 316L stainless steel, is the most common metallic biomaterial for implant manufacturing. It contains 0.003 wt% C, 2.00 wt % Mn 17-20 wt % Cr, 12-14 wt % Ni, 2-3 wt% Mo, 0,003 wt % P, 0.003 wt % S and other minor elements (Park and Bronzino, 1992). It is preferred because of its high corrosion resistance due to Cr content which forms an oxide layer. On the other hand, it is prone to wear stress which leads to debris release as a result of friction on application site and in turn to implant failure due to implant loosening. For that reason Co-Cr based alloys could be considered for a more enhanced implant performance. These materials exhibit a good corrosion resistance even in chloride solutions. However the lack of mechanical stimuli may cause implant failure or loosening. 316L stainless steel and Co-Cr based alloys have approximately same elastic modulus (ca. 200 GPa for stainless steel and 230-240 GPa for Co-Cr alloys) which is higher than natural bone (4-30 GPa) (Figure 1.2) (Bauer et al, 1999)

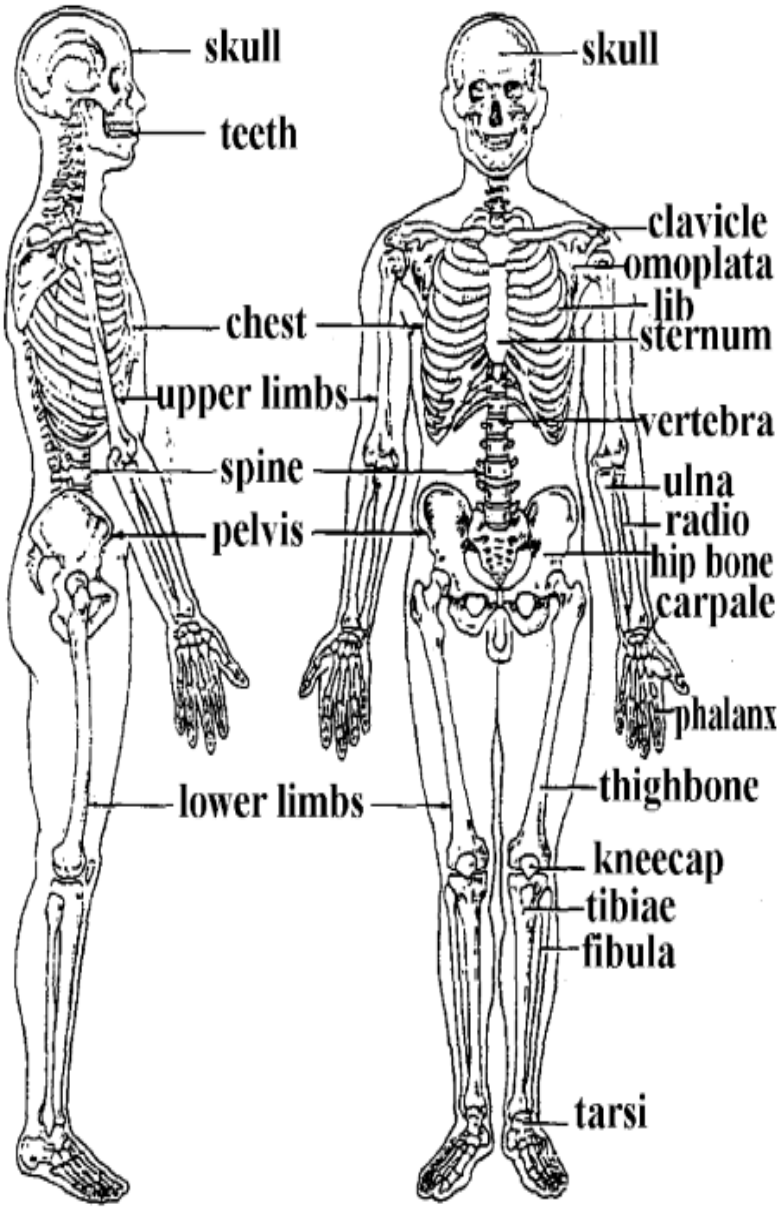

Figure 1.1: Hard tissues in the human body for metallic biomaterial applications (Liu et al, 2004) .

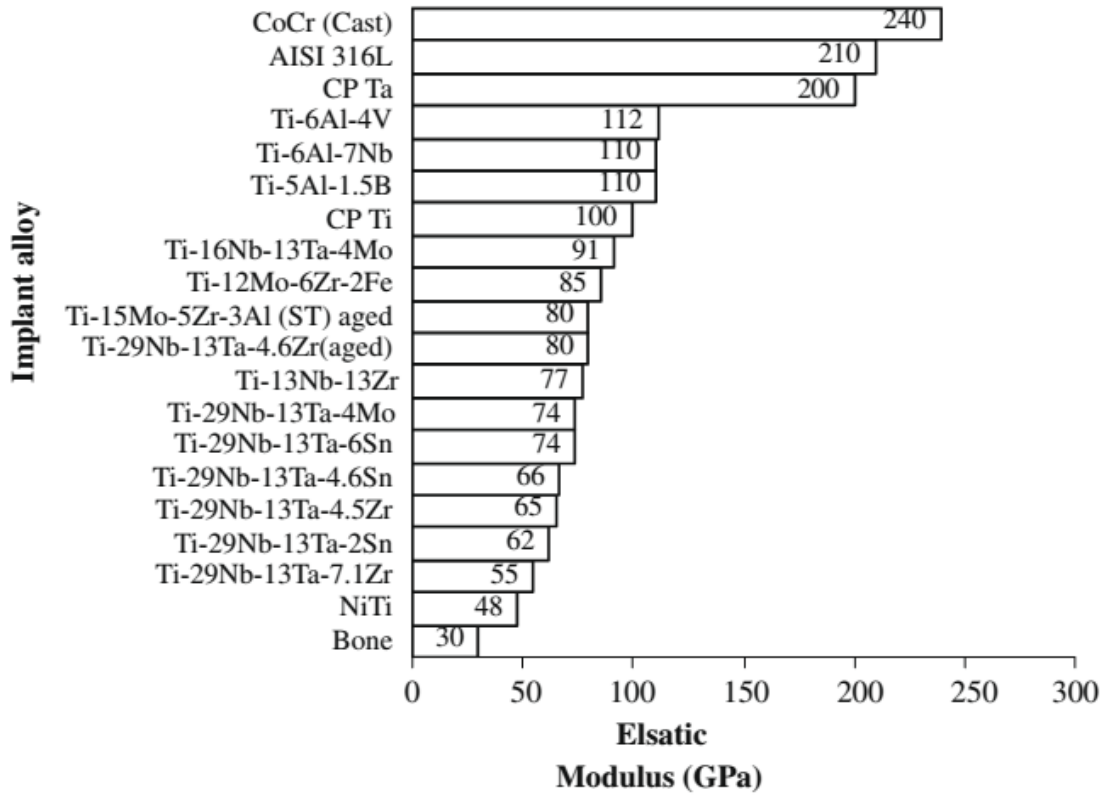

Figure 1.2: Comparison of elastic modulus of several metallic alloys with natural bone (Geetha et al, 2009).

1.4. Requirements for metallic biomaterials

A metallic biomaterial especially for load bearing applications should be non-cytotoxic, highly biocompatible without causing inflammatory or immune response in physiological environment, and should have high corrosion, wear and fatigue resistance, low modulus of elasticity and high strength (Figure 1.2) (Geetha et al, 2009; Long et al, 1998).

Apart from all these, metallic implants used in hard tissue replacement should encourage osteointegration. The failure of implants can occur with debris generation from wear and corrosion, fibrous encapsulation as a result of nonbonding with connected tissue, rejection, low fracture toughness and fatigue, and mismatch in elastic modulus resulting in stress shielding effect that restricts cell vitality around the implant (Geetha et al, 2009).

Figure 1.3: Requirements of implants (Long et al, 1998).

1.4.1 Mechanical properties

Hardness, strength, fatigue resistance, corrosion and wear resistance and elastic modulus properties defines a specific metallic material performance in implant applications. Long term usage of implants depends on these parameters and determines the performance of the implants for their specific purposes. Fractures, loosening problems and ion release according to well-known mechanisms and inadequate osteointegration between implant and bone tissue may otherwise appear and that leads to revision surgeries, which is an undesired procedure after implantations. Metallic alloys have different strength and modulus which can be seen in Table 1.1. (Geetha et al, 2009; Okazaki et al, 2005.)

Table 1.1: Different alloys and their mechanical properties (Navarro et al, 2008)

Material	**elastic modulus (GPa)**	**Yield strength(MPa)**	**maximum strength(MPa)**
Stainless Steel (316L)	205-210	170-750	465-950
CoCrMo F75	220-230	275-1585	600-1785
Ti grade 4	105	692	785
Ti4Al6V	110	850-900	960-970
Ti6Al7Nb	105	921	1024
Ti35Nb5Ta7Zr(TNZT)	55	530	590
NiTi	20-70(martensite) 70-110(austenite)	50-300(martensite) 100-800(austenite)	755-960
TiNb	60-85	-	-

1.4.2 Biocompatibility

Biocompatibility is the key parameter that designates the performance of biomaterials and determination of biocompatibility is a multi-level detailed protocol to evaluate newly designed or in use biomaterials. According to the consensus of the majority of researchers in the field, biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific situation. The presence of a biomaterial such as an implant in the body, should not cause any toxicity and immune response or,

in other words, host response. A few of generic host response characteristics are protein adsorption and desorption mechanisms on biomaterial surfaces, cytotoxic effects, neutrophil activation, macrophage activation, foreign body giant cell production, granulation tissue formation, fibroblast behavioral interferences and fibrosis, microvascular changes, tissue-organ specific cell responses (i.e. osteoclasts and osteoblasts for bone, endothelial proliferation, activation of clotting cascade, platelet adhesion, activation and aggregation, complement activation, antibody production, acute hypersensitivity/anaphylaxis, delayed hypersensitivity, mutagenic responses, genotoxicity reproductive toxicity and tumor formation (Franz et al, 2011). On the other hand materials that could influence the host response depend on major material variables like bulk material composition, micro or nano structure and morphology, crystallinity and crystallography, elastic constants, water content and hydrophilicity, porosity at macro or micro level, surface characteristics as chemical composition, gradients and molecular mobility; surface topography (i.e. roughness); surface energy and electrical/electronic properties, corrosion parameter, ion release profile, metal ion toxicity for metallic biomaterials; degradation profile, leachables, additives, catalysts, contaminants and toxicity for polymeric biomaterials; dissolution/degradation profile and degradation product toxicity for ceramic biomaterials; and finally wear debris release profile. (Williams, 1987, 2008; Helmus et al, 2008).

1.4.3 Osteointegration

Osteointegration is another important phenomenon that dijudicate the performance of inserted biomaterials in the body. Osteointegration is a type of bone-biomaterial(i.e. implant) interface interaction conducting by numerous molecular processes. Figure 1.4 shows the events at a bone-implant interface.

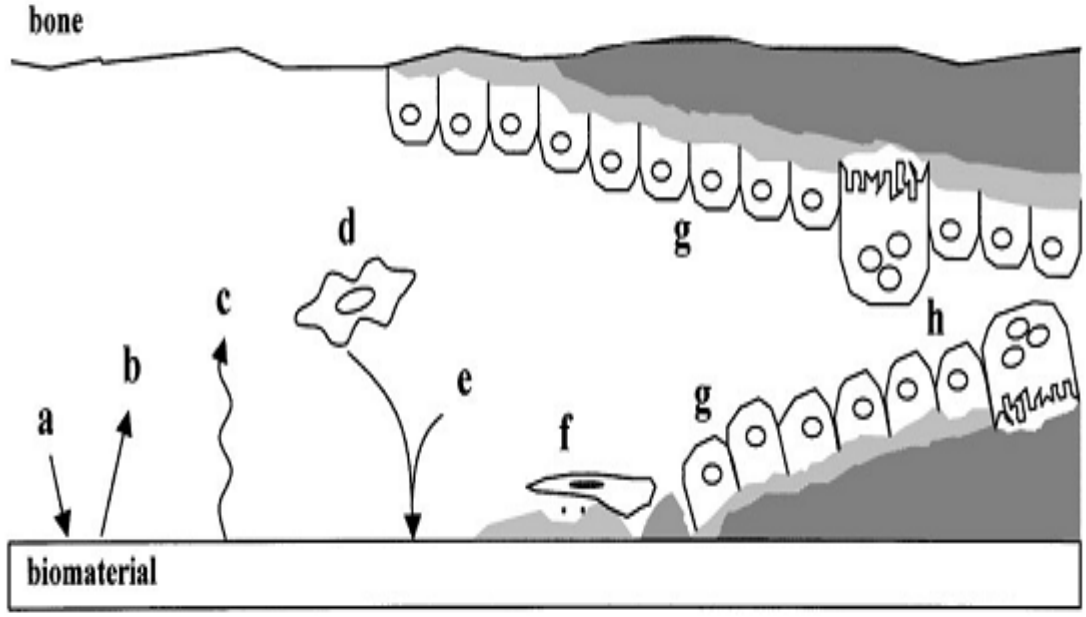

Figure 1.4: Representation of events at the bone-implant interface.(a) Protein adsorption from blood and tissue fluids, (b) protein desorption, (c) surface changes and material release, (d) inflammatory and connective tissue cells approach the implant, (e) possible targeted of matrix proteins and selected adsorption of proteins, (f)adhesion of osteogenic cells, (g) bone deposition on both the exposed bone and implant surfaces, (remodeling of newly formed bone (Puleo and Nanci, 1999).

1.4.4 Corrosion and wear resistance

Corrosion and wear mechanisms were described in literature according to several parameters and conditions (Figure 1.5). Prevention of metallic ion release is vital especially to solve numerous metabolic and allergic response issues. In the literature, there are several studies on the effect of debris and ion release on chromosomes of tissue culture cells and stimulation of allergic responses (Navarro et al, 2008; Daley et al, 2004). Implants should meet suitable corrosion and wear resistance properties to become a candidate for bio-applications (Antunes and de Olivia, 2012).

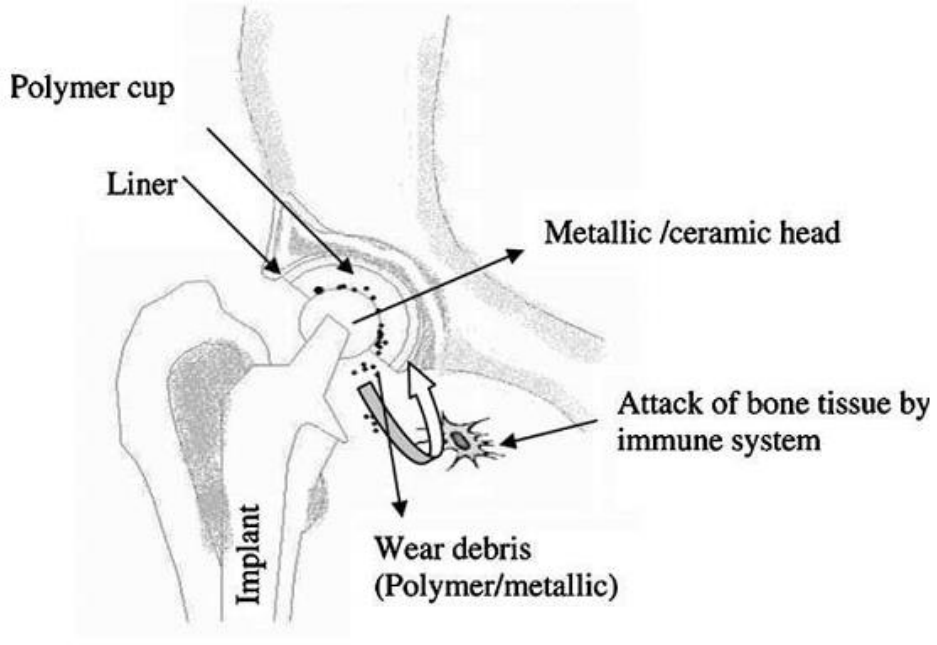

Figure 1.5: Wear mechanism of a total hip joint implant(Geetha et al, 2009).

1.5 Titanium and its Alloys

Titanium is one of transition metals with two crystallographic forms. At room temperature, elemental form of pure titanium has a hexagonal close-packed (hcp) crystal structure known as α-phase. At 883°C this formation differs into body centered cubic structure (bcc) and it is called β-phase. Controlling of these crystallographic properties allows researchers to create a broad range of alloys which have different properties. Titanium alloys can be classified as either α, near-α, α + β, metastable β or stable β according to their room temperature microstructures. Alloying elements have different effects of titanium. α alloys contains α-stabilizing elements such as Al, O, N, C; β alloys includes Mo, V, Nb, Ta, Fe, W, Cr, Si, Ni, Co, Mn β-stabilizers and α + β alloys have a composition of α and β alloys. Every alloy type has different properties which determines the whole material structure (Table 1.2) (Figure 1.6) (Long et al, 1998; Zhecheva et al, 2005; Liu et al, 2004; Rack et al, 2006).

Titanium and its alloys in medical field comprise huge diversity of applications i.e. dental implants, joint replacement parts for knee, hip, shoulder, spine, elbow and wrist, bone fixation units like nails, screws, nuts and plates and surgical components

Table 1.2: Common properties of titanium alloy types(Long et al, 1998; Zhecheva et al, 2005; Taddei et al, 2004).

Alloy type	Properties
α and near-α	Superior corrosion resistance Limited strength
B	High strength Good formability High hardenability High corrosion resistance Availability to reach low elastic modulus
α+β	Higher strentgth than α and near-α alloys (depends on α/β ratio)

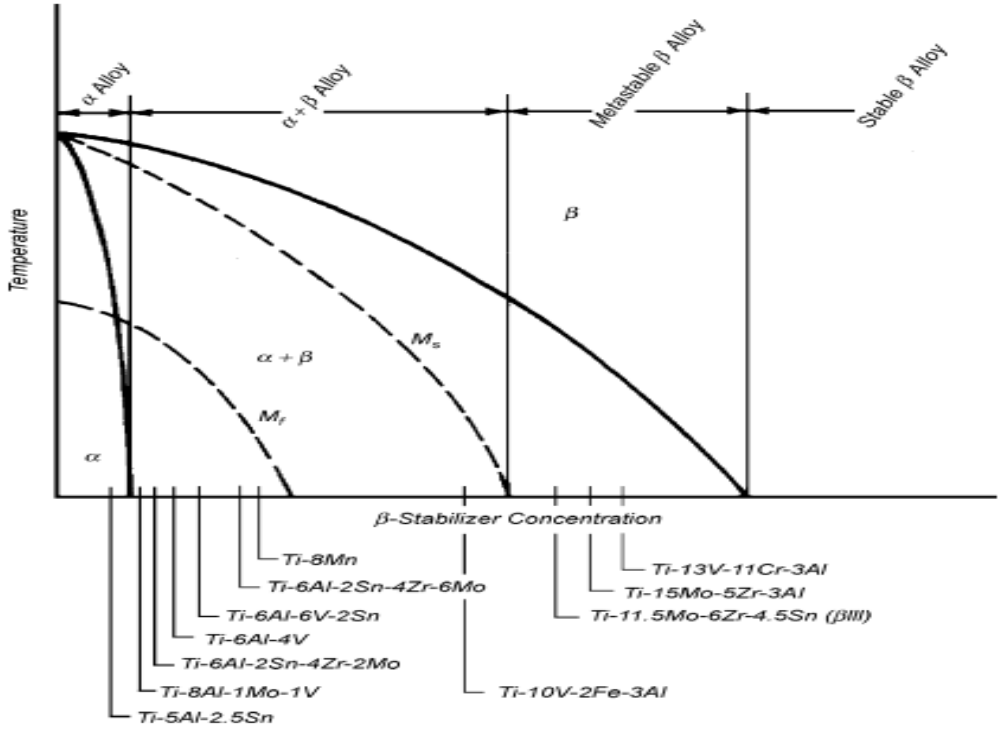

Figure 1.6: Composition of U.S technical titanium alloys β-isomorphous phase diagram (Zhecheva et al, 2005).

1.5.1 Limitations of titanium and its alloys

Although titanium and its alloys are known as highly biocompatible and corrosion resistant, there are numerous concerns about wear and ion release from the implants in

long term load bearing applications. For instance Ti64 (Ti-6Al-4V) alloys are reported for their Al and V release and formation of V_2O_5 oxides at the surface of alloys that causes long term health problems like Alzheimer disease, neuropathy and ostemomalacia (Geetha et al, 2009).

Furthermore, titanium has insufficient shear strength and high friction coefficient which lead to wear debris formation and subsequent failure of the implant. As a result of these problems, a revision surgery is necessary for replacing damaged components in 10-15 years. These surgeries have limited success compared with the first ones and recovery ratio is less (Geetha et al, 2009).As an example, femoral heads and accetubular cups made of ultra-high-molecular weight polyethylene (UHMWPE) components of a common hip joint implant may address to specific issues regarding wear of titanium implants. Failure of implants concludes with loosening and high concentration of metal ions surrounding of bio-application sites (McGee et al, 2000; Geetha et al, 2009).

1.6 Magnesium As an Alternative to Improve Metallic Biomaterial Performance

Non-degradable conventional metallic materials are widely used in biomaterial sciences and medical applications in load bearing applications for their mechanical properties. However third generation biomaterials which includes functional tissue engineering constructs and bio-absorbable materials may not be produced with non-degradable metallic materials. Compared to these materials, magnesium (Mg) has some unique properties such as biodegradability, biocompatibility and superior mechanical specifications so could offer an alternative (Yang et al, 2010; Farraro et al, 2013).

Mg implants were studied from the beginning of twentieth century and several examples of implants were tried to be involved in implant technology (Witte, 2010).Mg based biomaterials, pure Mg and its alloys, are gaining popularity to be integrated into both hard and soft tissue applications. Mg also offers a better elastic modulus and tissue interaction than metallic biomaterials (Table 1.3).

Mg has several remarkable advantages including being the most abundant cation in human body and existing in natural bone structure. These properties make Mg based implant materials highly biocompatible when introduced into human body and prevent

revision surgeries which lead to additional cost and complications. Additionally, issues explained for conventional metallic biomaterials such as corrosion and wear problems can be reduced with biodegradable Mg biomaterials (Witte et al, 2008).

Table 1.3: Summary of physical and mechanical properties of several materials compared to natural bone (Staiger et al, 2006).

Properties	**Natural bone**	**Magnesium**	**Ti alloy**	**Co-Cr**	**Stainless stees**	**Synthetic hydroxyapatite**
Density (g/cm^3)	1,8-2,1	1,74-2,0	4,4-4,5	8,3-9,2	7,9-8,1	3,1
Elastic modulus(Gpa)	3-20	41-45	110-117	230	189-205	73-117
Compressive yield strength(MPa)	130-180	65-100	758-1117	450-1000	170-310	600
Fracture toughness ($MPam^{1/2}$)	3-6	15-40	55-115	-	50-200	0,7

Pure Mg usage in biomedical applications has some limitations due to its corrosion behavior in aqueous solutions or physiological media. Mg crystals has hexagonal closed-packed and formed by agglomeration of random distribution of small crystals. Corrosion happens from boundary regions rather than bulk crystals because of lattice irregularities. Impurities causing galvanic corrosion tend to place these regions. This phenomena is used to characterize direct corrosion or stress corrosion cracks from grain boundaries in the literature (Salunke et al, 2011). Also Mg corrosion in aqueous solutions leads to hydrogen evolution based on stoichiometric reversible equations below (Witte et al, 2008, 2005, 2006; Wu et al, 2013):

Overall equation: $Mg_{(s)} + 2H_2O_{(aq)} \rightarrow Mg(OH)_{2(s)} + H_{2(gas)}$ **(1.1)**

Anodic reaction: $Mg_{(s)} \rightarrow Mg^{2+}_{(aq)} + 2e^-$ **(1.2)**

Cathodic reaction: $2H_2O_{(aq)} + 2e^- \rightarrow H_{2(gas)} + 2OH^-(aq)$ **(1.3)**

Product formation: $Mg^{2+}_{(aq)} + 2OH^-(aq) \rightarrow Mg(OH)_{2(s)}$ **(1.4)**

Hydrogen evolution is a common problem in replacement surgeries because of subcutenous gas bubbles after implantation within a short period of time. Mg biocorrosion should be controlled in every situation to sustain a complete recovery after surgeries. There are so many methods to measure corrosion rate, for instance mass loss measurement, hydrogen evolution measurement, pH monitoring and polarized experiments (Kirkland et al, 2012) (Figure 1.7).

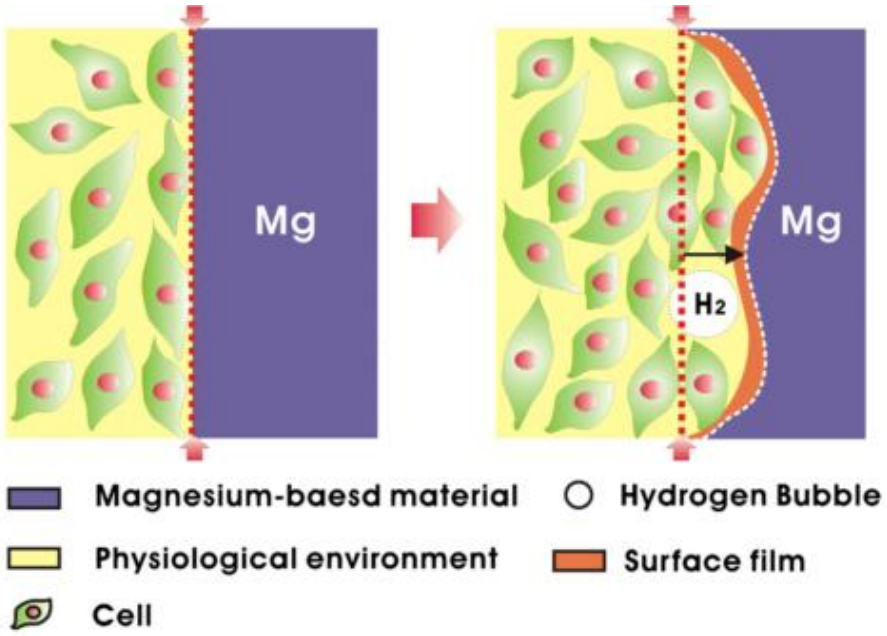

Figure 1.7: Dynamic interface between Mg-based biomaterials and bio-environment during biocorrosion (Wu et al,2013).

1.7 Titanium - Magnesium Systems

It is reported that there is very low solubility of magnesium and titanium at temperatures above 400 °C and there is not any intermetallic compounds reported in the literature (Figure 1.8).

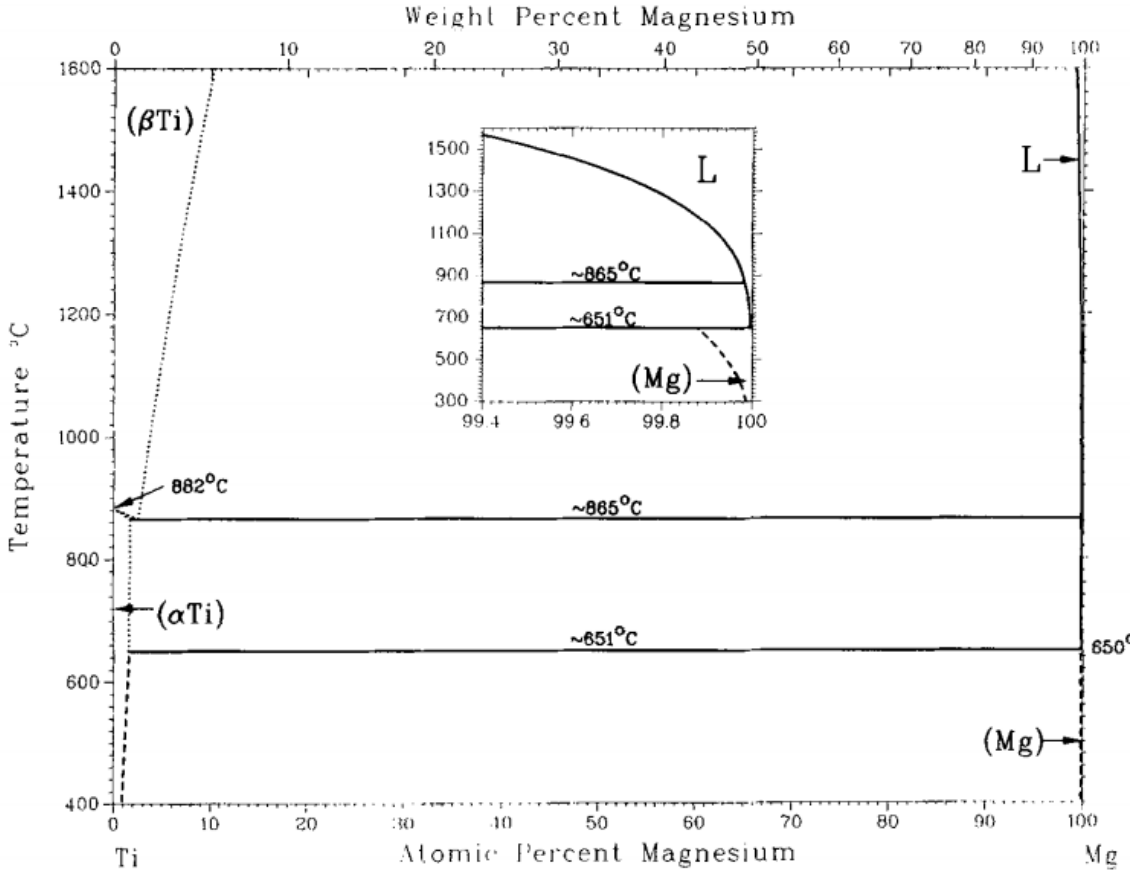

Figure 1.8: Ti-Mg Binary Phase Diagram (Murray, 1986).

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Titanium plates and cathodes

Titanium plates and cathodes with their suppliers are listed in Appendix A.

2.1.2 Laboratory equipment and chemicals

Laboratory equipment and chemicals with their suppliers are listed in Appendix B.

2.2 Methods

2.2.1 Preparation of titanium substrates

Titanium grade II plates with 99.9 % purity were cut as ca. 1cm^2 squares using guillotine cutter and cleaned chemically about 4-5 minutes with acid mixture etchant including hydrofluoric acid, nitric acid and distilled water (60 ml nitric acid 25 ml hydrofluoric acid 45ml distilled water).

2.2.2. Cathodic arc PVD ion treatment procedure

In this study, the samples were coated and bombarded with titanium ions using cathodic arc PVD technique so as to alter the magnesium content diffused into the titanium substrate surfaces. The magnesium contents were adjusted by changing the titanium and magnesium cathode currents. Because the evaporation temperature of magnesium is around 600 °C, the coated magnesium atoms are re-sputtered during the bombardment with titanium ions. In order to prevent from Mg re-sputtering, the magnesium and titanium were coated as very thin layers by rotating the sample holder with a shutter

system. The shutter system was designed as shown in Figure 2.1 to separate the magnesium and titanium plasmas so that the magnesium and titanium were coated as separate layers and the magnesium was entrapped between subsequent titanium layers.

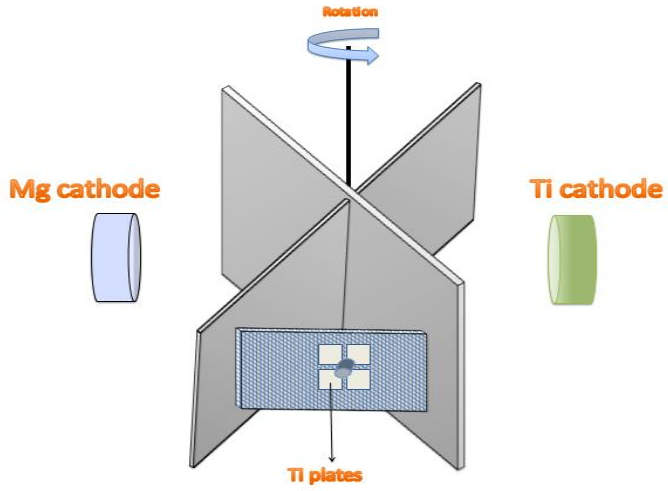

Figure 2.1:Schematic of the system for cathodic arc PVD.

After placing the substrates on the substrate holder, the chamber was evacuated to the ultimate pressure of ca. 6.00×10^{-3} Pa. The coating and bombardment treatment were carried out at 1×10^{-3} Pa. The chamber pressure was adjusted using Ar gas. Before the coating and bombardment procedures, the titanium substrates were etched in Ar glow-discharge plasma using -800 V bias voltages. During the coating and titanium ion bombardment for all samples, the bias voltages were -50 V and -600 V, respectively. The other experimental parameters for ion treatment procedures were given in Table 2.1.

Table 2.1: Parameters for cathodic arc PVD coatings.

Experiments	**Current(A)**		**Coating**	**Ion bombardment**
	Ti	Mg	**Time(min)**	**Time(min)**
1	85	55	10	2
2	85	55	10	5
3	85	55	10	8
4	85	40	10	8
5	85	25	10	8

2.2.3. Characterization of coatings

Atomic Mg and Ti percentages and micrographs of coating surfaces were determined by scanning electron microscopy (SEM) including energy dispersive X-ray spectroscopy (EDS: 10kV, take-off angle 25.0°, line: Kα.). Crystallinity and phase structures of the coatings were analyzed using X-ray diffraction (XRD) with Cu-Kα radiation and thin film attachment. In order to analyze the concentration profiles of the samples, glow discharge optical emission spectrometry (GDOES) technique was used. The depth of the sputtered area was measured for all samples using a 3D optical profilometer. Visualization of the coated and uncoated samples were carried out via SEM again.

2.2.4. Cell proliferation studies by MTS assay

Cell proliferation studies were conducted in all of the coatings in order to determine the effects of Mg contents and surface properties of the coatings on cell proliferation.

Samples were first placed in 12-well plates and sterilized in 70% ethanol for 1 hour. After removing the ethanol, all samples were washed with 1X PBS (phosphate buffer saline, pH 7.4). Human fetal osteoblastic cells (hFOB) were seeded onto samples as 1000cells/well and incubated in CO_2 incubator with 5% CO_2 and 37°C.. The medium (DMEM low glucose without phenol red with glutamine) was changed every 3 days. MTS ([3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) assay was used for the determination of the cell number on the coatings. This formazan compound is reduced by viable cells due to NADP and NADPH produced by dehydrogenases and gives optical density differences. These differences can be measured as absorbances and related to viable cell amount.

MTS assay was performed for 1, 2 and 7 days of incubation. All samples were transferred to another 12-well plate by forceps after the incubation period. MTS solution (Promega Cell Titer 96$^{®}$AQ$_{ueous}$One Solution) was prepared by mixing the medium and MTS with a ratio of 5:1. This mixture was added onto each well containing the samples (750µl per well) and incubated in CO_2 incubator with 5% CO_2 and 37°C for 2.5 hours .All materials and solutions were prepared in the dark.

After incubation, 200 µl of the solution on each well were transferred to a 96-well plate and the absorbance values were read at 490nm wavelength using Elisa Plate Reader. Calibration curve with R^2=0,9913 were used to calculate cell numbers on the coating samples (Figure 2.2). Also Student's two tailed t-test were applied to do statistical analysis using Microsoft Excel. Significant difference was considered at the level of $p \leq 0,05$ (n=3).

For SEM analysis, cells were fixed with cocadylic acid buffer (16 g/1L) and glutaraldehyde solution (25% stock solution diluted to 2.5% with cocadylic acid buffer) for 1.5 hours and washed with again cocadylic acid buffer. Cocadylic acid buffer were prepare

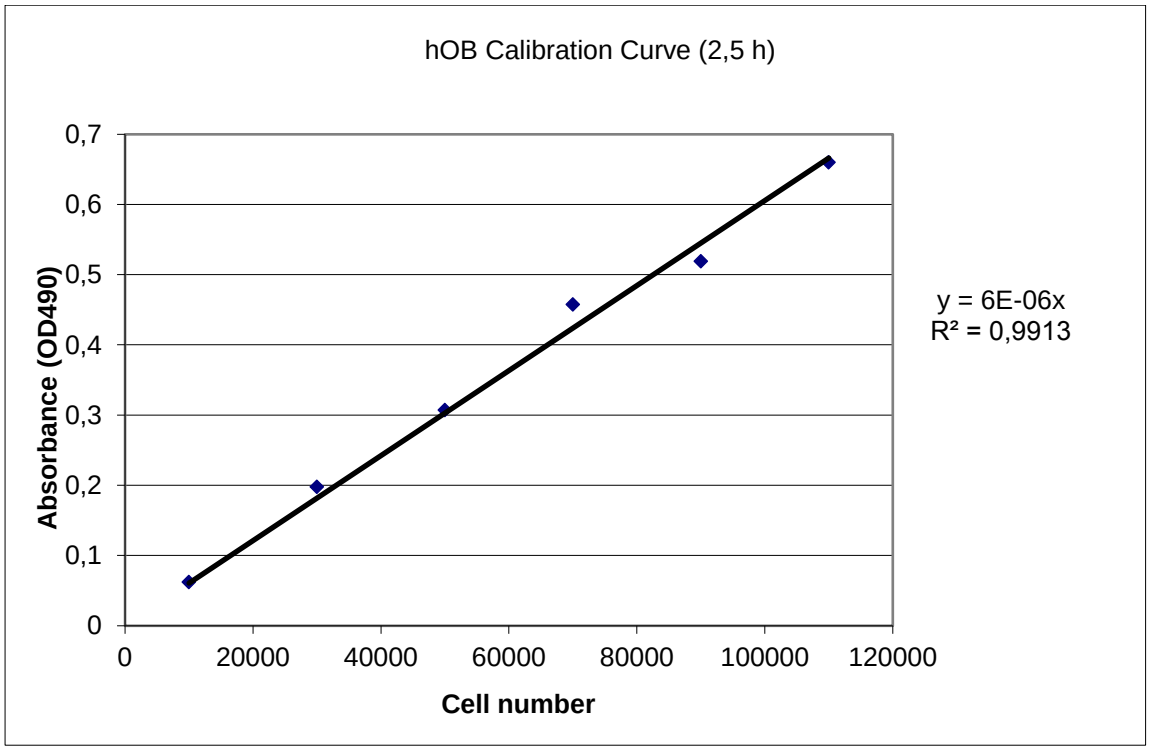

Figure 2.2 : MTS calibration curve (R^2=0,9913).

3. RESULTS AND DISCUSSION

3.1 EDS Analysis and Visualization of The Surfaces

EDS is a tool to gather information about elemental composition of a surface. An incident electron causes x-ray emissions by the atoms at surface of a material. Elemental composition can be determined by measuring intensities and energy of these x-ray emissions. The penetration of the electrons (e.g. the information depth) depends on the electron accelerating voltage and the atomic weight of the surface atoms. In this study, the accelerating voltage was 10kV for all samples. The analyses were carried out using kα x-ray lines for both Ti and Mg. EDS spectra obtained from the sample surfaces were shown in Figure 3.1. The measured Mg contents of the samples were given in Table 3.1.

Table 3.1: Atomic Mg amounts of the coatings.

Experiments	**Current(A)**		**Coating Time(min)**	**Ion Bombadment Time(min)**	**Atomic Mg (%)**
	Ti	Mg			
1	85	55	10	2	27.1 ± 1.2
2	85	55	10	5	12.3 ± 0.2
3	85	55	10	8	11 ± 2.0
4	85	40	10	8	10 ± 1.1
5	85	25	10	8	3.8 ± 0.7

In order to determine the bombardment time effect on Mg concentrations, Ti and Mg cathode currents were kept constant at 85A and 55A, respectively and the bombardment time was changed. For this purpose, three different bombardment time 2, 5 and 8 min were selected. Depending on the bombardment time, the magnesium content was changed from 27.1 ± 1.2 to 11 ± 2.0 (Table 3.1). To be able to obtain lower Mg content, Mg cathode currents were lowered down to 40 A and 25A by keeping the bombardment time constant at 8 minutes. The result of these experiments showed that Mg amounts below 10 at% could be achieved with these parameters.

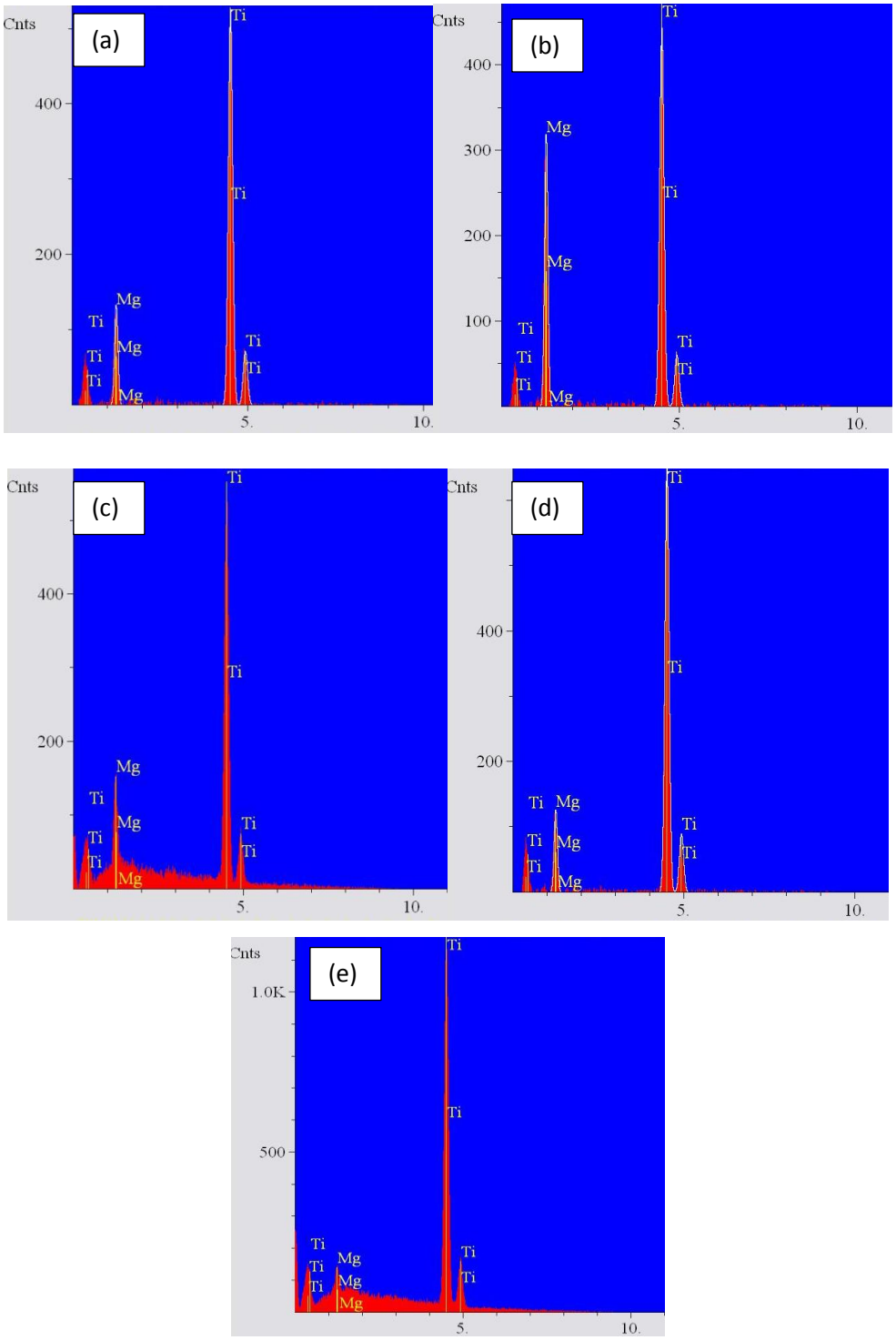

Figure 3.1: EDS results of the coatings (a) 27.1 ± 1.2 at % Mg, (b) 12.3 ± 0.2 at % Mg, (c) 11 ± 2.0 at % Mg, (d) 10 ± 1.1 at % Mg, (e) 3.8 ±0.7 at Mg % (kV=10kV (*x* axis), take-off angle=25.0° line = Kα).

According to SEM micrographs in Figure 3.2, all coatings with different Mg concentrations showed same characteristics. Ti droplets caused by titanium ion bombardments were observed for the all coatings.

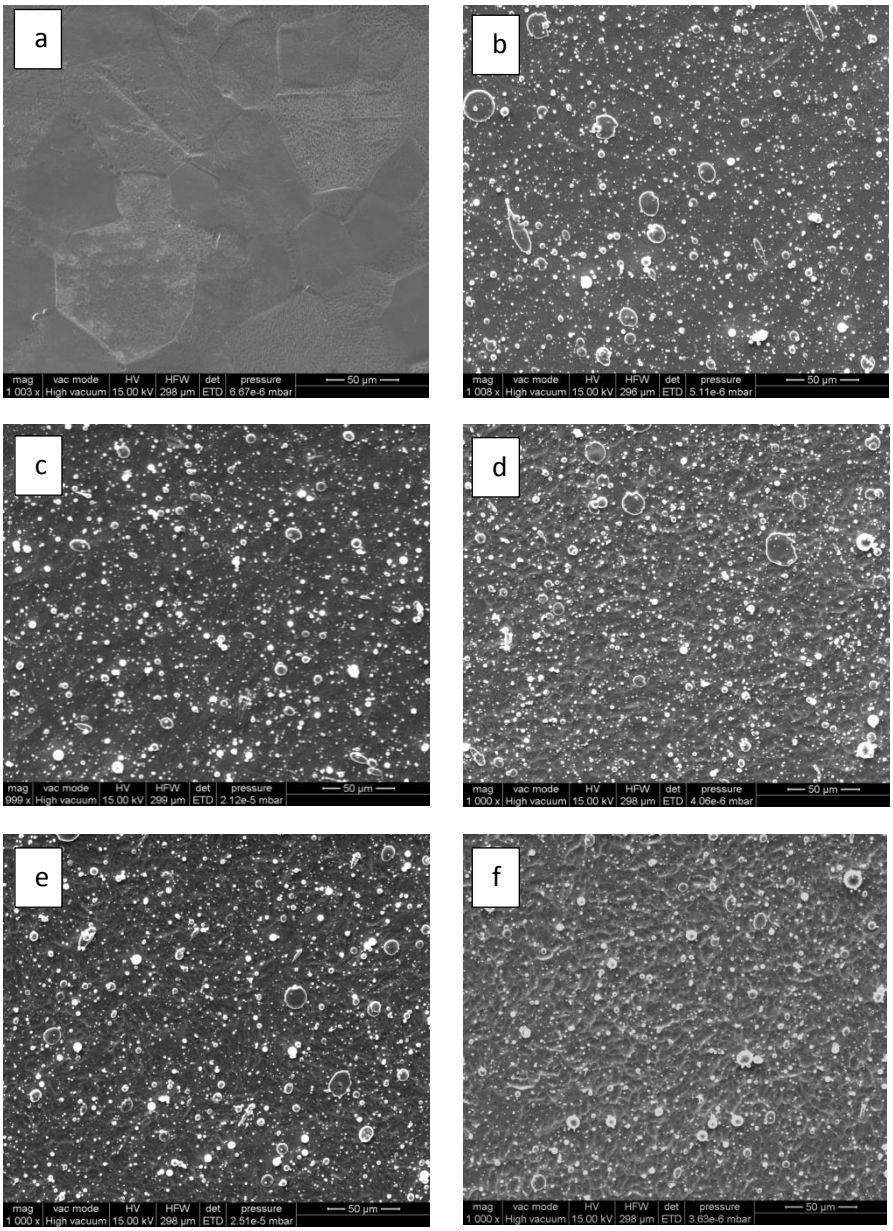

Figure 3.2: SEM micrographs of the uncoated and coated samples. (a)Uncoated (b) 3.8 ±0.7 (c) 10 ± 1.1 (d) 11 ± 2.0 (e) 12.3 ± 0.2 (f) 27.1 ± 1.2 at % Mg.

3.2 XRD Analysis

XRD is an important technique to obtain information about crystal structures of materials. A XRD pattern gives also information about crystallite size, lattice formation and chemical composition (Suryanarayana, 1998).

In order to define the diffraction peaks, raw data from XRD analysis were processed using "Philips X'Pert High Score" software with PDF 2-2003 database. According to database patterns all diffraction peaks were identified.

XRD analysis of the Mg coated and as received Ti substrates were carried out to determine Mg and Ti peaks (Figure 3.2 and Figure 3.3). These data was then used as reference in analysis of the other coatings (Figure 3.4).

XRD pattern of the coatings showed that titanium-ion-treated samples were composed of Ti (ref code: 00-044-1294), Ti_2O (ref. code:011-0218) and Ti_2O_3 (ref. code: 00-044-0086) phases according to PDF 2-2003 database (Figure 3.4). Although EDS spectra showed that the samples contained magnesium up to 27 at.%, there was not any magnesium related peaks. Magnesium is immiscible in titanium and there is not any intermetallic between these metals. In magnesium containing titanium structures, the magnesium is expected to be at grain boundaries of the titanium. In such cases, the crystals formed at grain boundaries having very small crystal sizes might not be determined using XRD patterns.

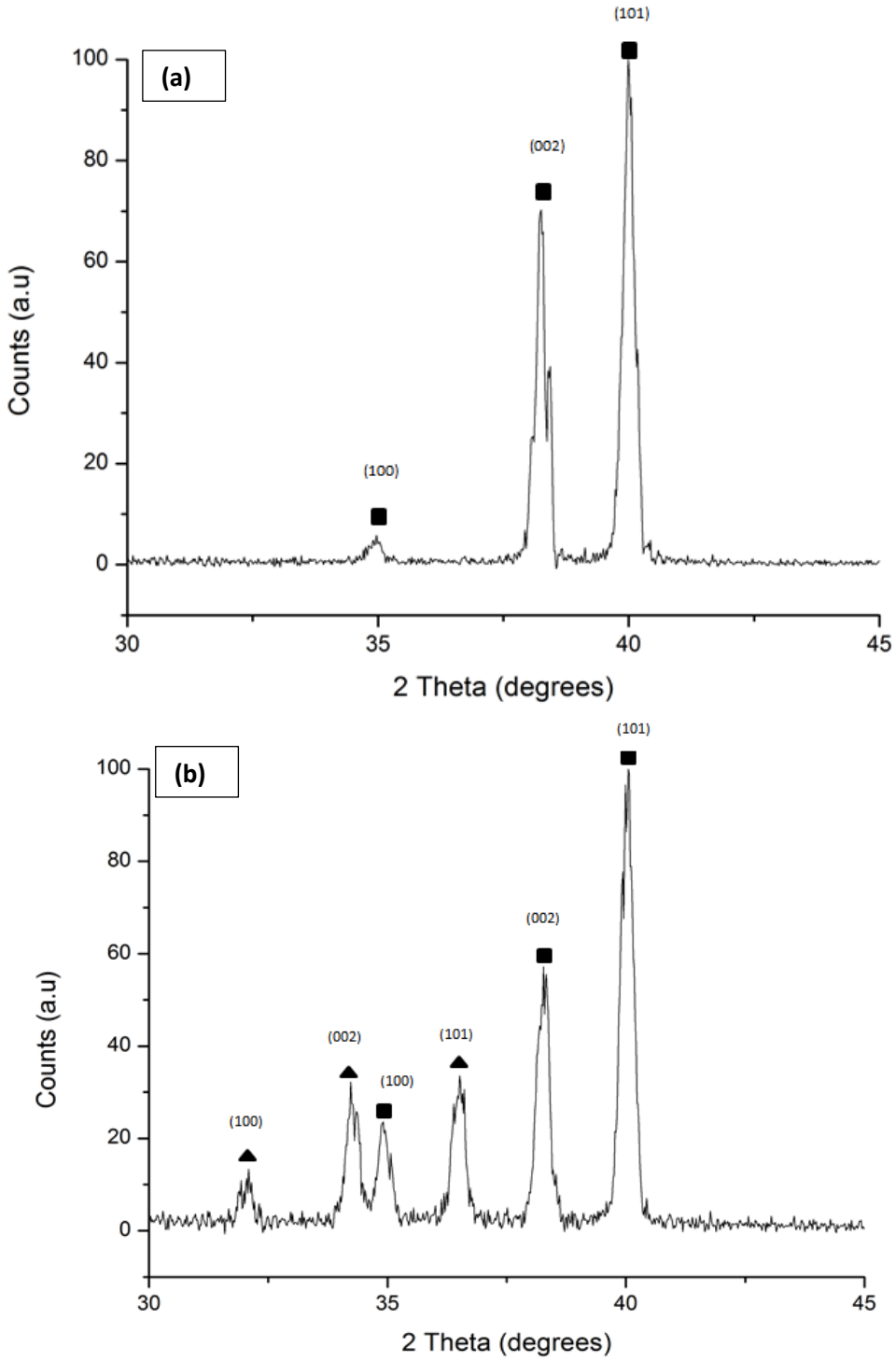

Figure 3.3: XRD pattern (30°-45° 2θ) of uncoated Ti substrates (a) and Mg coatings* on Ti substrates (b) for reference. (*10 minutes coating, Mg cathode current value: 55A without rotation) (▲ : Mg peaks ■ : Ti peaks).

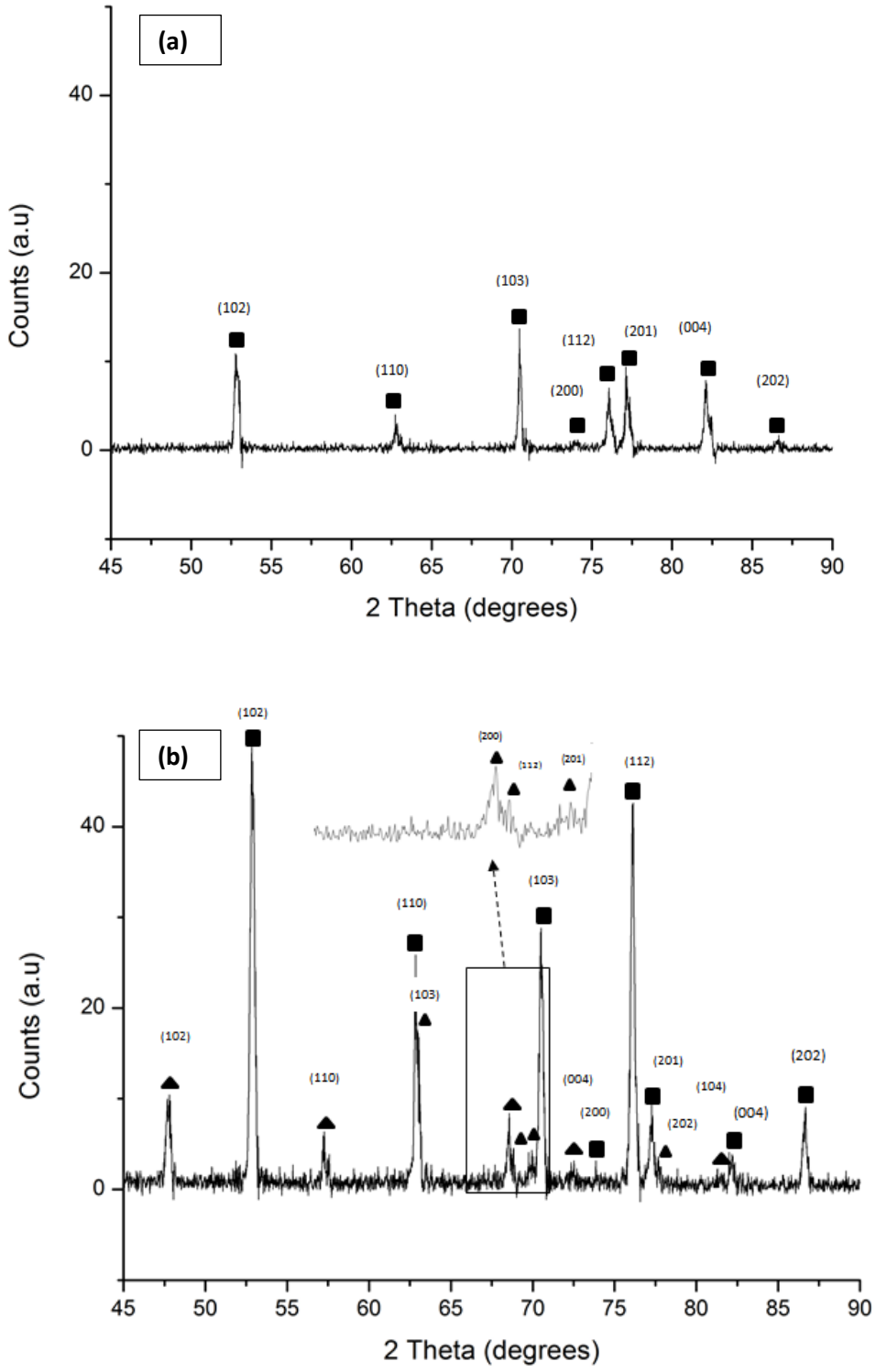

Figure 3.4: XRD pattern (45°-90° 2θ) of uncoated Ti substrates (a) and Mg coatings* on Ti substrates (b) for reference. (*10 minutes coating, Mg cathode current value: 55A without rotation) (▲ : Mg peaks ■ : Ti peaks).

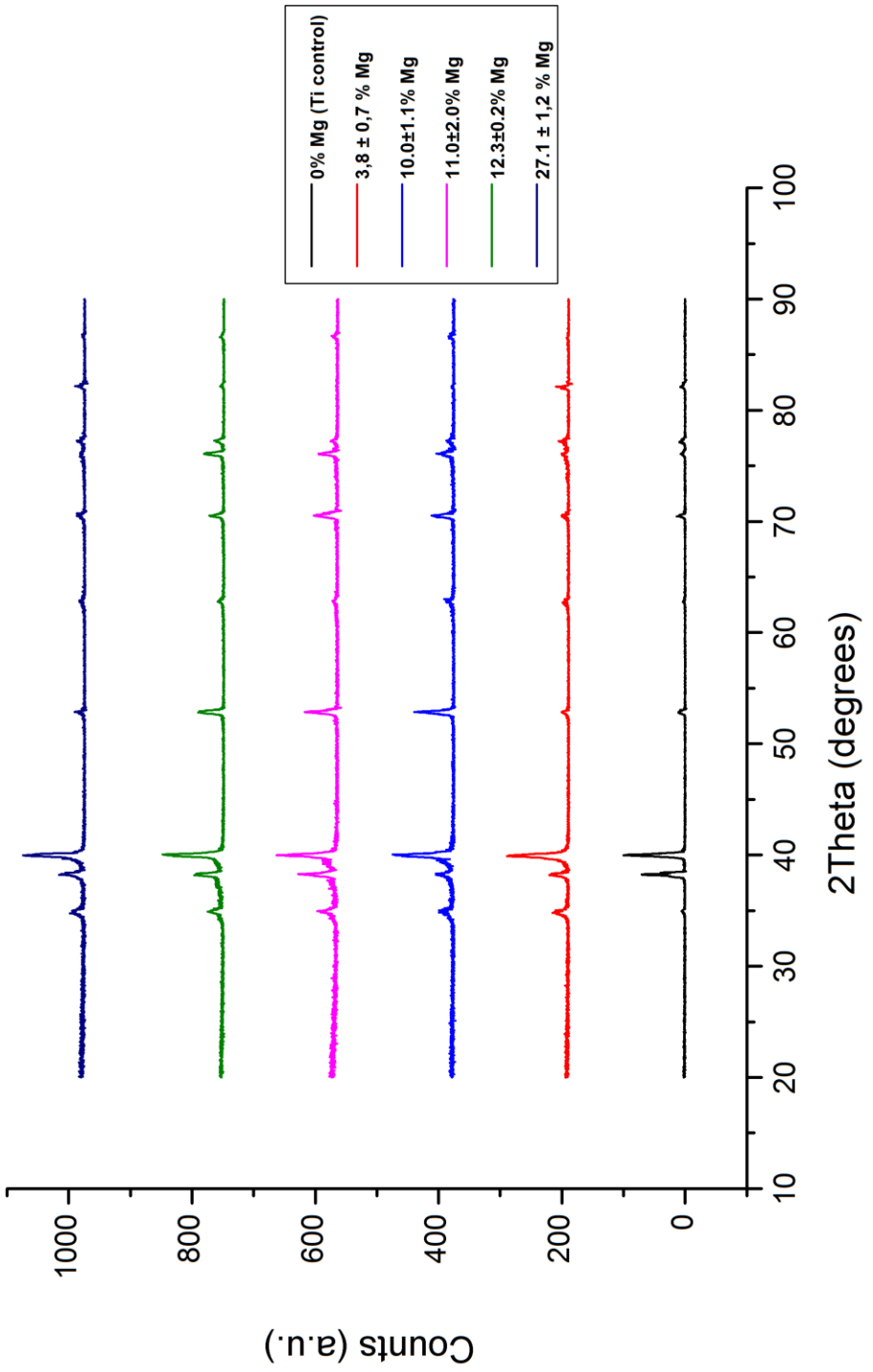

Figure 3.5: XRD patterns of the coatings.

3.3 GDOES analysis and 3D optical profilometer measurements

The glow discharge optical emission spectroscopy (GDOES) results and related 3D optical profilometer profiles of the samples were given in Figures 3.5, 3.6, 3.7, 3.8, 3.9, 3.10, 3.11and 3.12.

GDOES and 3D optical profilometer analysis of the coatings were performed to reveal the elemental depth profiles. After GDOES analysis, average depths of sputtered areas on the coatings were measured as seen in Figure 3.4, 3.5, 3.6 and 3.7. Total sputtered depths were 5.0 ± 0.6 μm for the sample with 3.8 ± 0.7 at. % Mg, 4.3 ± 0.1 μm for the sample with 10 ± 1.1 at. % Mg, 4.00 μm for the sample with 11± 2.0 at. % Mg, 4.35 ± 0.05 μm for the sample with 12.3 ± 0.2 at. % Mg and 2.0 ± 0.1μm for the sample with 27.1 ± 1.2 at. % Mg . This time differences were scaled according to 3D optical profilemeter data. According to the data from 3D optical profilometer and GDOES together, measured coating thicknesses were calculated.

GDOES depth profiles showed that Mg diffused into the depth of ~0.75μm for the sample with 3.8 ± 0.7 at. % Mg, ~0.70μm for the sample with 10 ± 1.1 at. % Mg, ~1 μm for the sample with 11± 2.0 at. % Mg, ~0.50 μm for the sample with 12.3 ± 0.2 at. % Mg and ~0.50 μm for the sample with 27.1 ± 1.2 at. % Mg.

Additionally, thin oxide rich layers on the surface were detected for all the coatings. Mg lines started to rise after the oxide layers. It is seen that Mg contents were re-sputtered from the surface and migrated towards the Ti substrate due to ion bombardment as seen in Figure 3.11(a) and 3.12. Also, the oxide layer depths are higher for higher bombardment time. This result also justifies XRD patterns of the coatings, which indicates titanium oxide formations given in section 3.2.

All results also showed that Mg distribution profile in Ti-Mg coatings on Ti substrates can be adjusted with arc current values and bombardment times. and Mg contents were able to be entrapped into Ti-Mg coatings between outer surface and the Ti substrate.

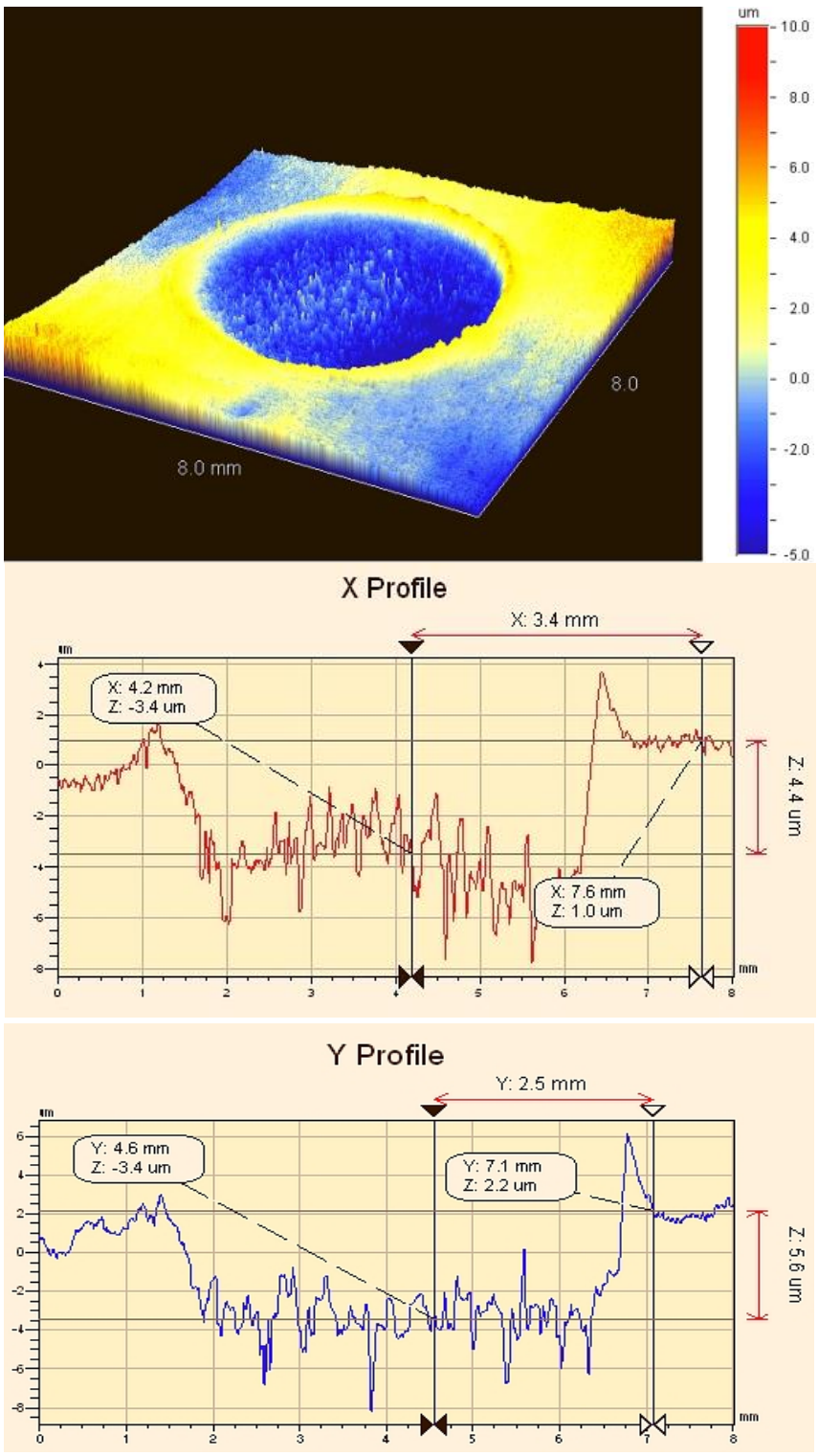

Figure 3.6: Optical profilometer analysis done after GDOES analysis. Total sputtered depth via GDOES analysis were measured to scale coating thickness. Profile of 3.8 ± 0.7 at. %Mg.

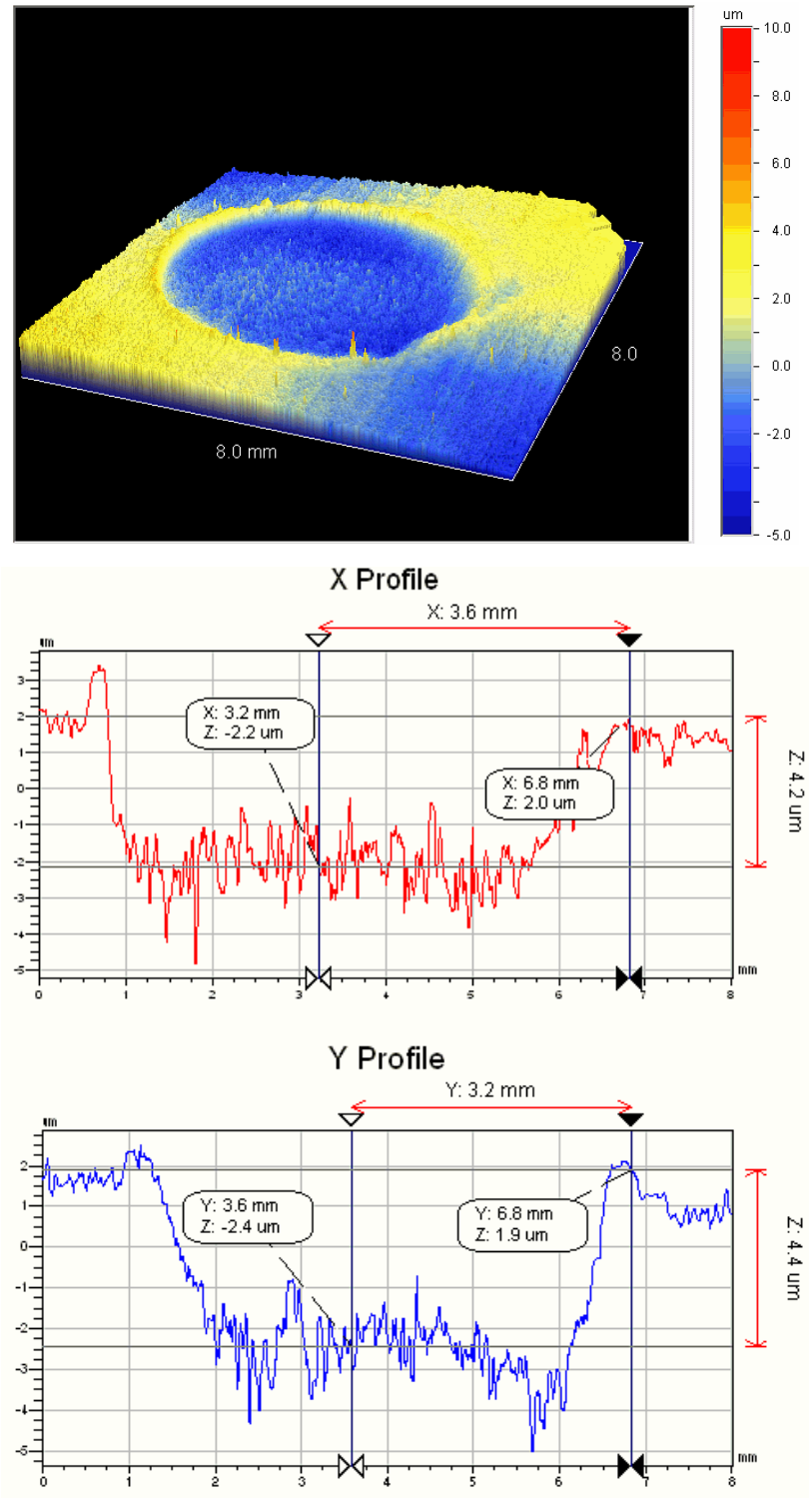

Figure 3.7: Optical profilometer analysis done after GDOES analysis. Total sputtered depth via GDOES analysis were measured to scale coating thickness. Profile of 10 ± 1.1 at % Mg.

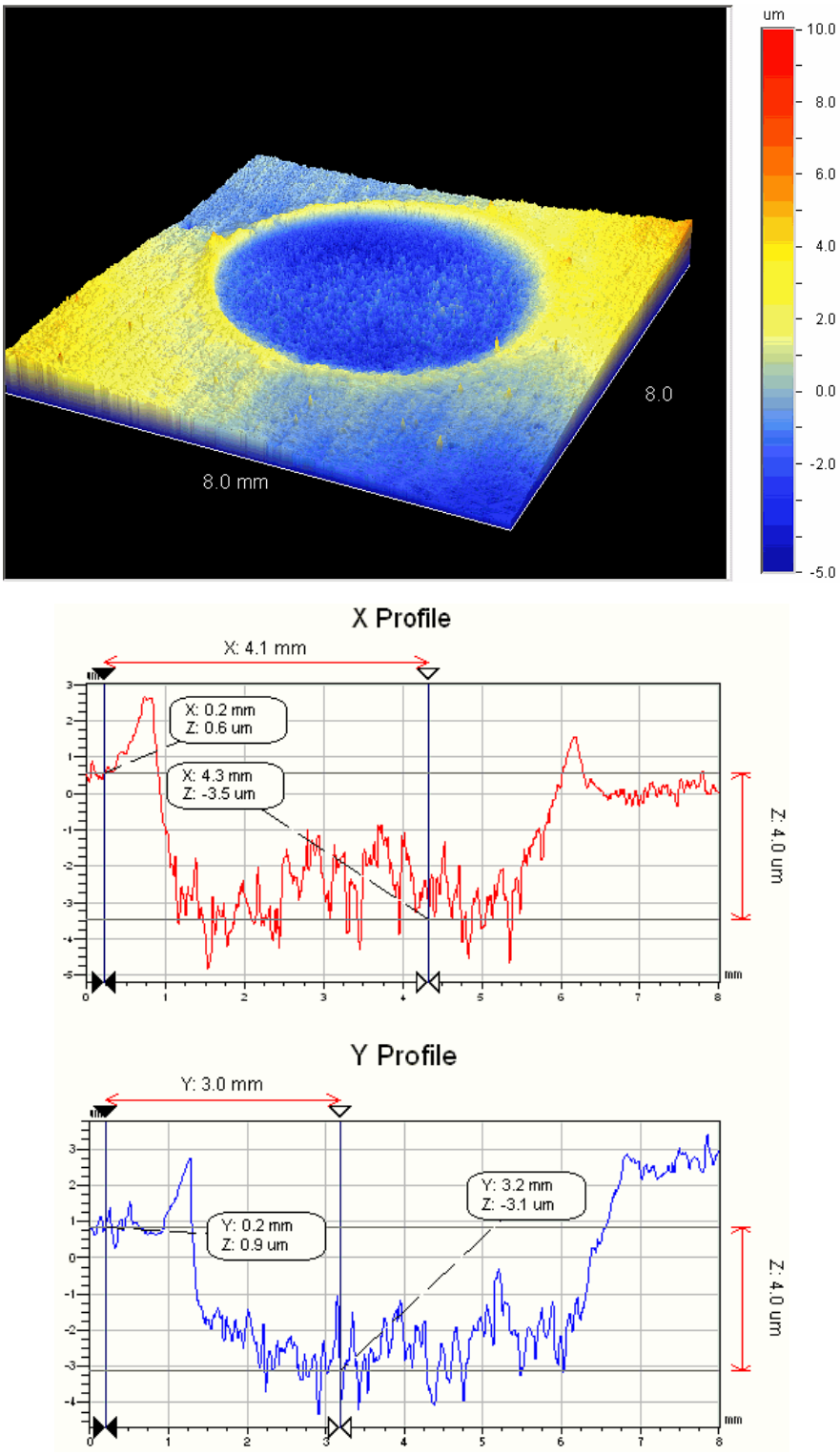

Figure 3.8: Optical profilometer analysis done after GDOES analysis. Total sputtered depth via GDOES analysis were measured to scale coating thickness. Profile of 11 ± 2.0 at % Mg.

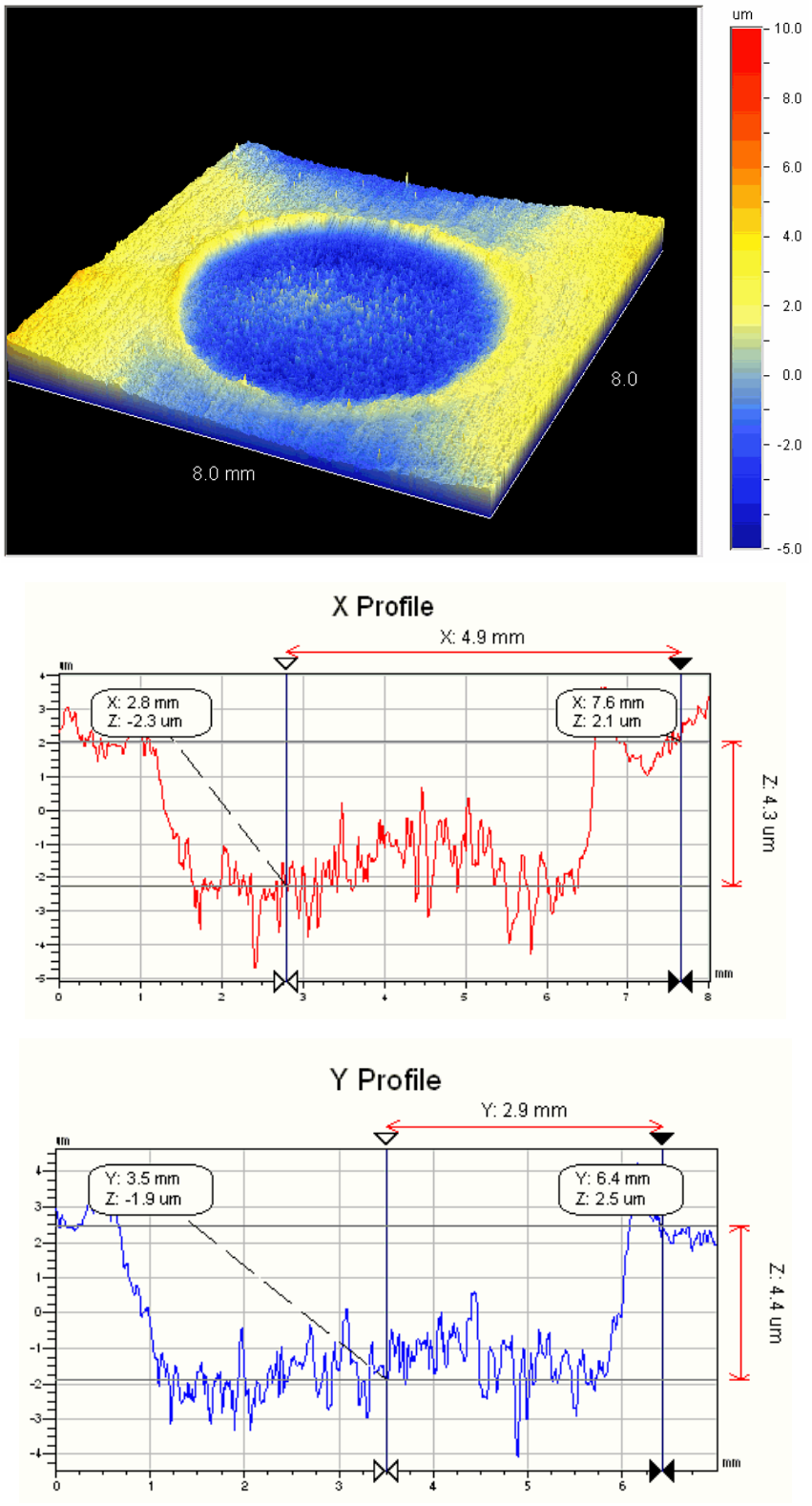

Figure 3.9: Optical profilometer analysis done after GDOES analysis. Total sputtered depth via GDOES analysis were measured to scale coating thickness. Profile of 12.3 ± 0.2 at % Mg.

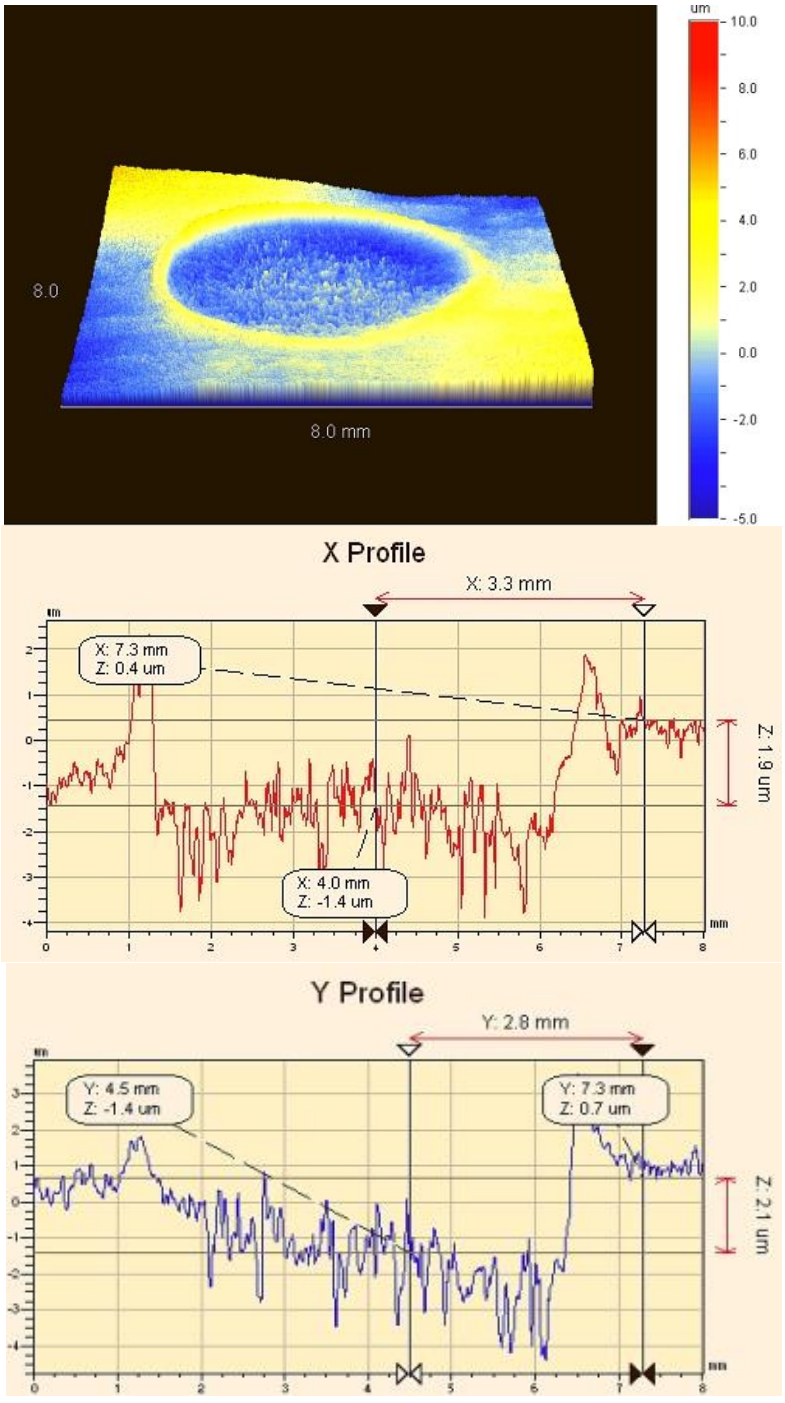

Figure 3.10: Optical profilometer analysis done after GDOES analysis. Total sputtered depth via GDOES analysis were measured to scale coating thickness. Profile of 27.1 ± 1.2 at. % Mg.

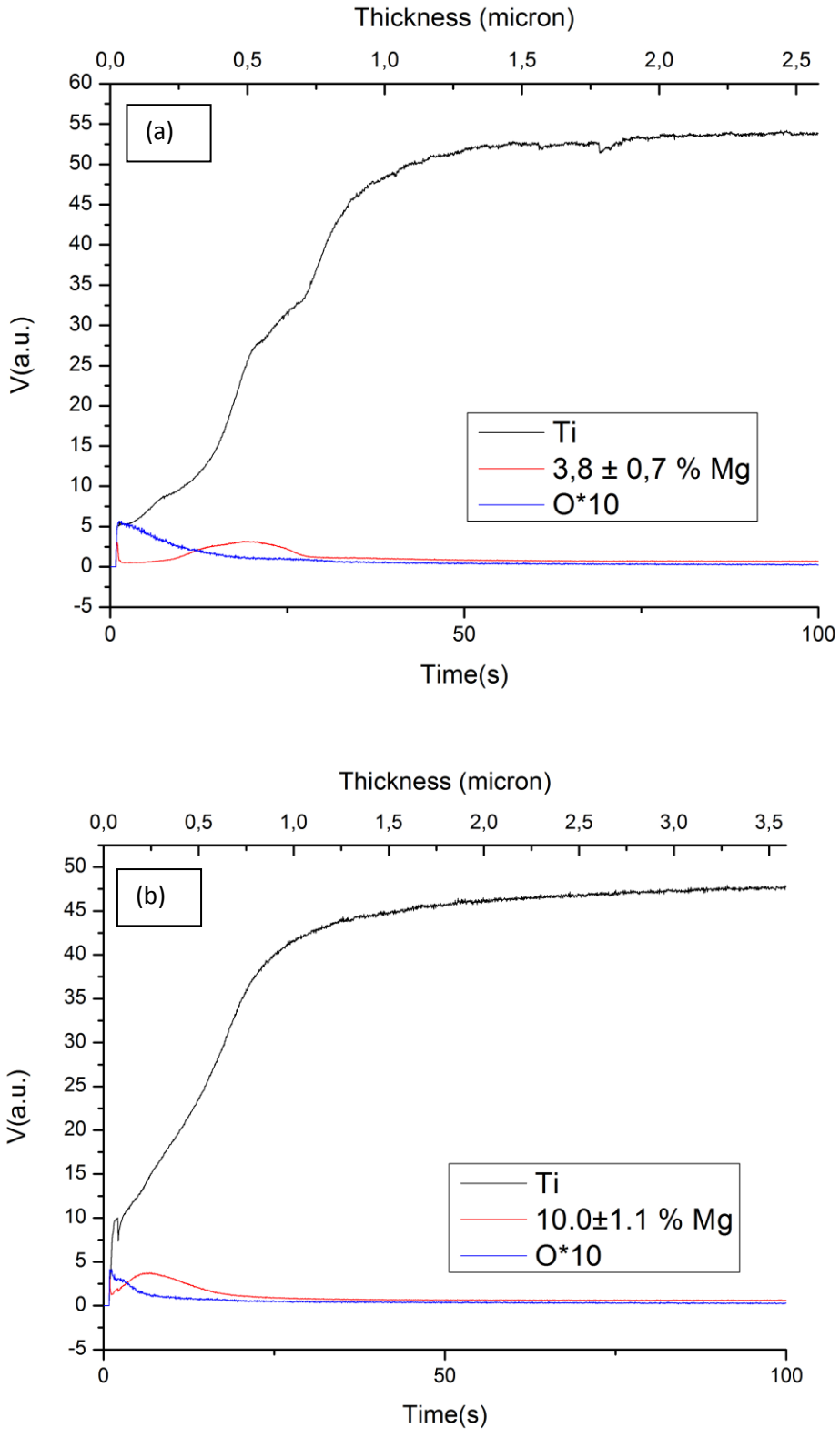

Figure 3.11: GDOES analyses to measure thickness of coatings and Mg phase in inner surface of Ti substrates. (a): 3.8 ± 0.7 at. %Mg, (b): 10 ± 1.1 at % Mg.

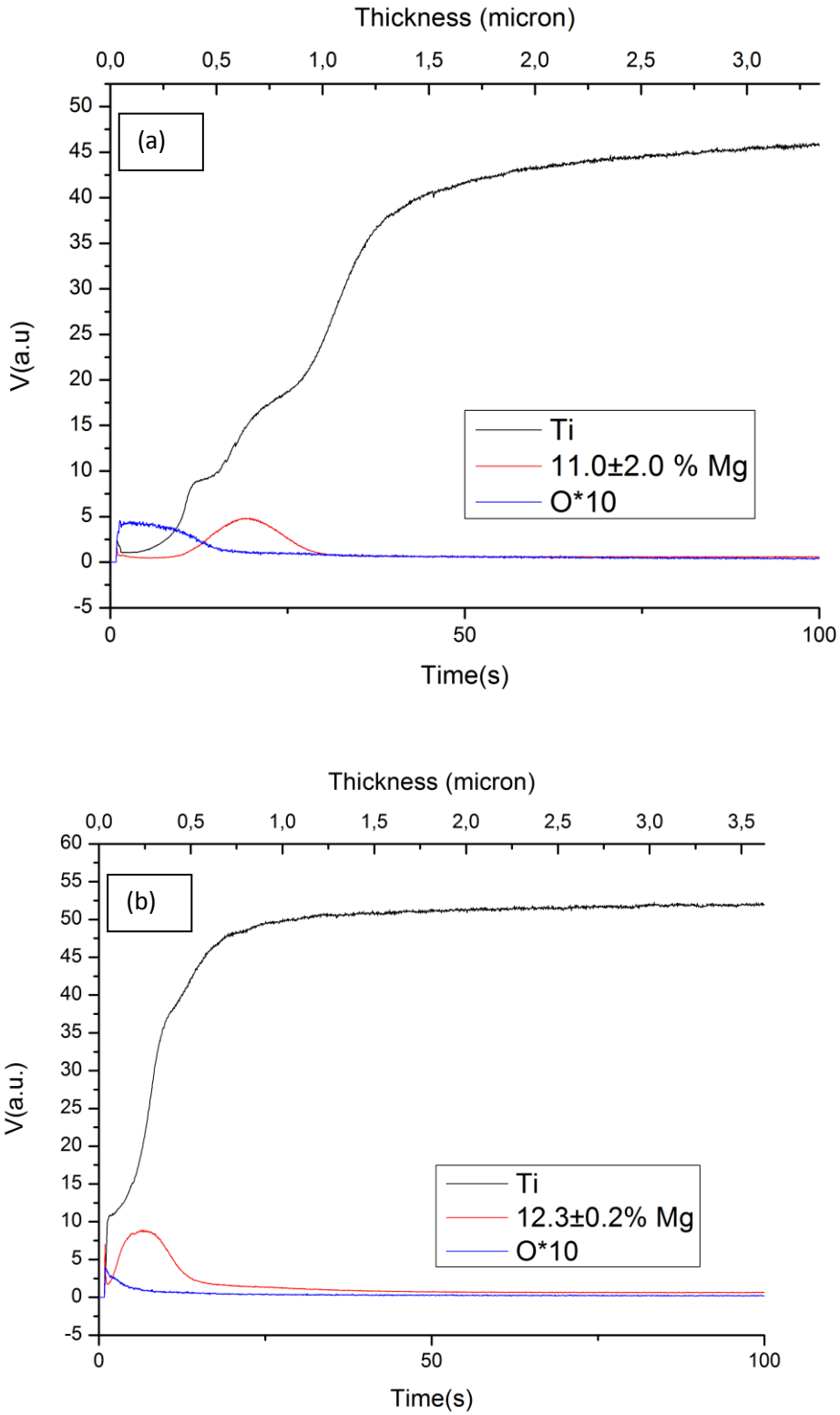

Figure 3.12: GDOES analyses to measure thickness of coatings and Mg phase in inner surface of Ti substrates. (a): 11 ± 2.0 at % Mg, (b): 12.3 ± 0.2 at % Mg.

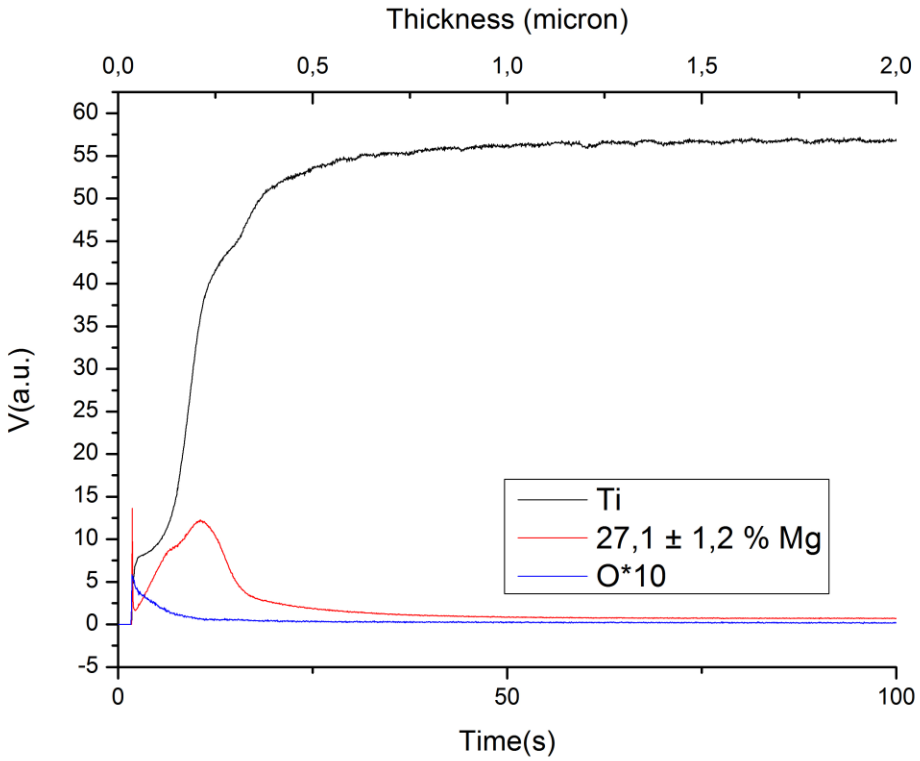

Figure 3.13: GDOES analyses to measure thickness of coatings and Mg phase in inner surface of Ti substrates. (27.1 ± 1.2 at. % Mg).

3.4 Cell proliferation studies by MTS Assay

MTS assay results were shown in Figure 3.13. Statistical significant differences with $p \leq 0.0005$ were shown in the graph. The rest ($0.0005 \leq p \leq 0.05$, n=3) were listed in Table 3.1.

The results showed that cell proliferation and attachment on the sample surfaces were affected directly from Mg content of the coatings. There was no cell proliferation on the 27.1 ± 1.2 at. % Mg containing coating which has the maximum Mg content. This issue was also observed in the previous studies of our group on arc-PVD coated (Ti,Mg)N coatings. Mg affects the cell proliferation positively for a limited range of concentrations. After certain concentration, however, cell proliferation and attachment are restricted and sometimes completely prevented because of rapid hydrogen gas evolution on the surfaces and cytotoxicity to the cells. For instance, it is reported that at the range of 10 – 1000 ppm, Mg accelerated the cell proliferation and viable cell

coverage in a study working with human embryonic stem cells. But at the higher concentrations, cell viability decreased and rapid cell death occurred (Nguyen et al, 2012, Önder, 2013).

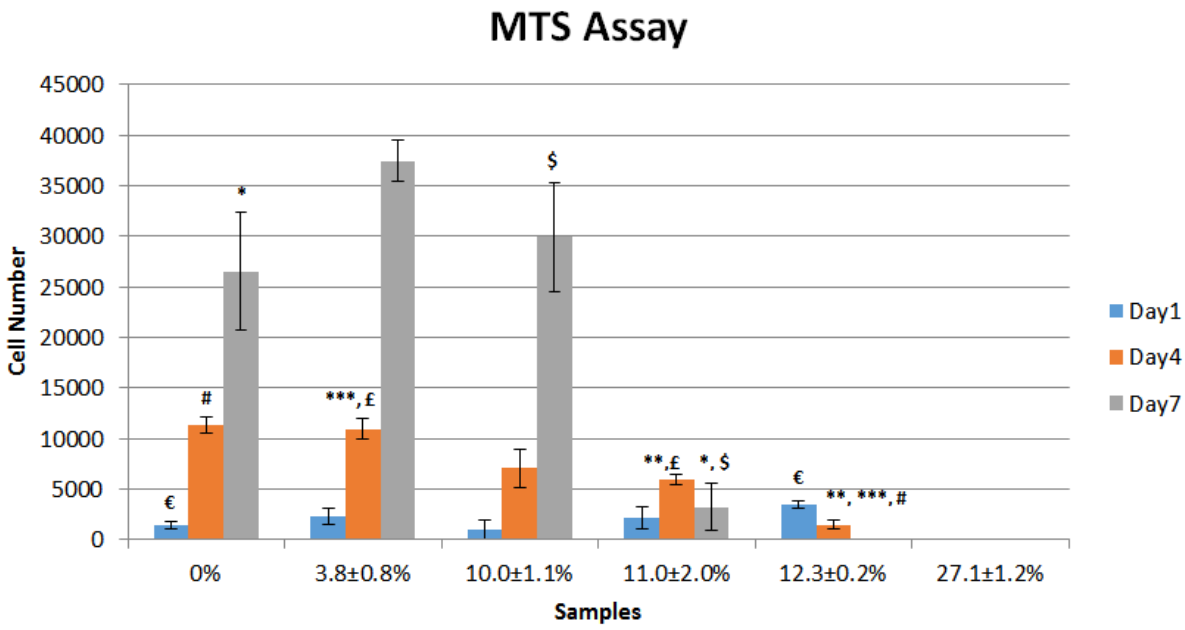

Figure 3.14: Cell proliferation on the coatings containing different amounts of magnesium using MTS assay (n=3, statistical significant differences with $p \leq 0.0005$ are indicated).

Table 3.2: Statistical significant differences between the coatings by Student's t-test ($0.0005 \leq p \leq 0.05$, n=3).

	Day 1	**Day 4**	**Day 7**
Samples (atomic % Mg)	3.8 ± 0.7 vs 0	12.3 ± 0.2 vs 10 ± 1.1	12.3 ± 0.2 vs 10 ± 1.1
	3.8 ± 0.7 - 10 vs 1.1		12.3 ± 0.2 vs 3.8 ± 0.7
	3.8 ± 0.7 vs 11± 2.0		

Increase of cell attachment and cell proliferation on the coatings were observed when the Mg contents were lowered to <10 at %. The best cell numbers were obtained on 3.8 ± 0.7 at. % Mg containing coatings at the end of seventh day. These coatings had similar proliferation profiles and better cell attachment performance than the pure titanium (0 at

%Mg). This result indicates the positive effect of low Mg concentrations for cell attachment and proliferation.

3.5. Visualization of Cells by SEM

Figure 3.15 shows the SEM micrographs of hFOB cells on the coatings containing 27.1 ± 1.2 and 3.8 ± 0.7 at. % Mg after 1 day, 4 days and 7 days incubation. It was seen that cell proliferation and attachment to the coating surfaces increased from first day to seventh day for lower Mg amounts. The micrographs with higher Mg containing coatings showed no cell proliferation or attachment. On seventh day it was clearly seen that maximum cell proliferation and attachment were achieved.

In Figure 3.16 SEM micrographs of all coatings could be seen at higher magnification after 7 days of incubation. Magnesium free and high Mg containing (> 10 at%) coatings seems to support lower cell proliferation compared to coatings with low Mg content (< 10 at%). SEM micrographs supported the MTS results and showed that the coating which supports the cell attachment and proliferation better was 3.8 at% Mg containing sample. Figure 3.17 shows the 1 and 7 day incubation results for this sample at two different magnifications. Cells were spread on the surfaces for both days and there was higher cell numbers at day 7, as also found by the previous MTS assay results.

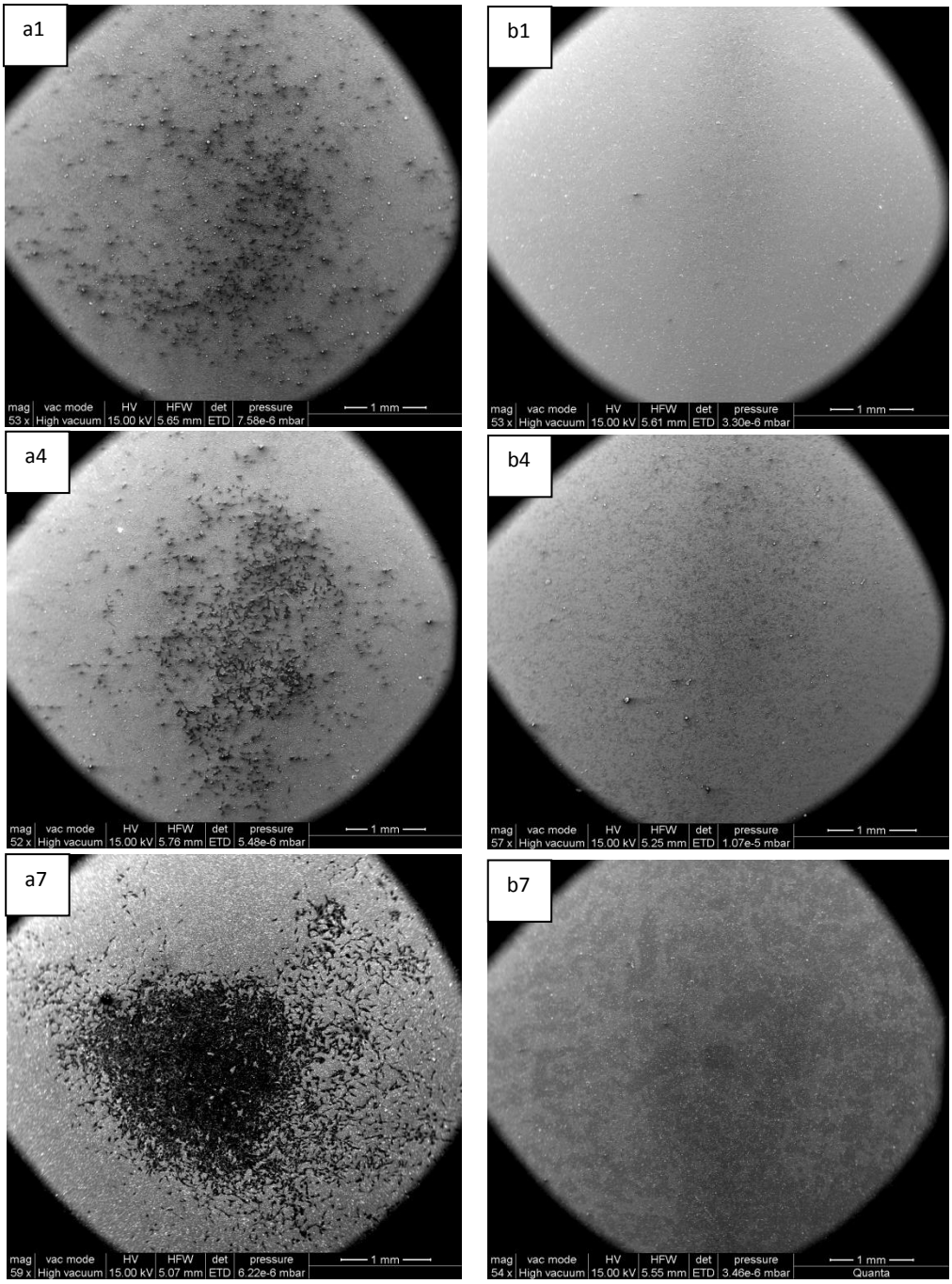

Figure 3.15: SEM micrographs of 27.1 ± 1.2 and 3.8 ± 0.7 at. % Mg containing coatings incubated with hFOB cells for general view. **a**: 3.8 ± 0.7 at. % Mg, **b**: 27.1 ± 1.2 at.%Mg **1,4,7**:days.

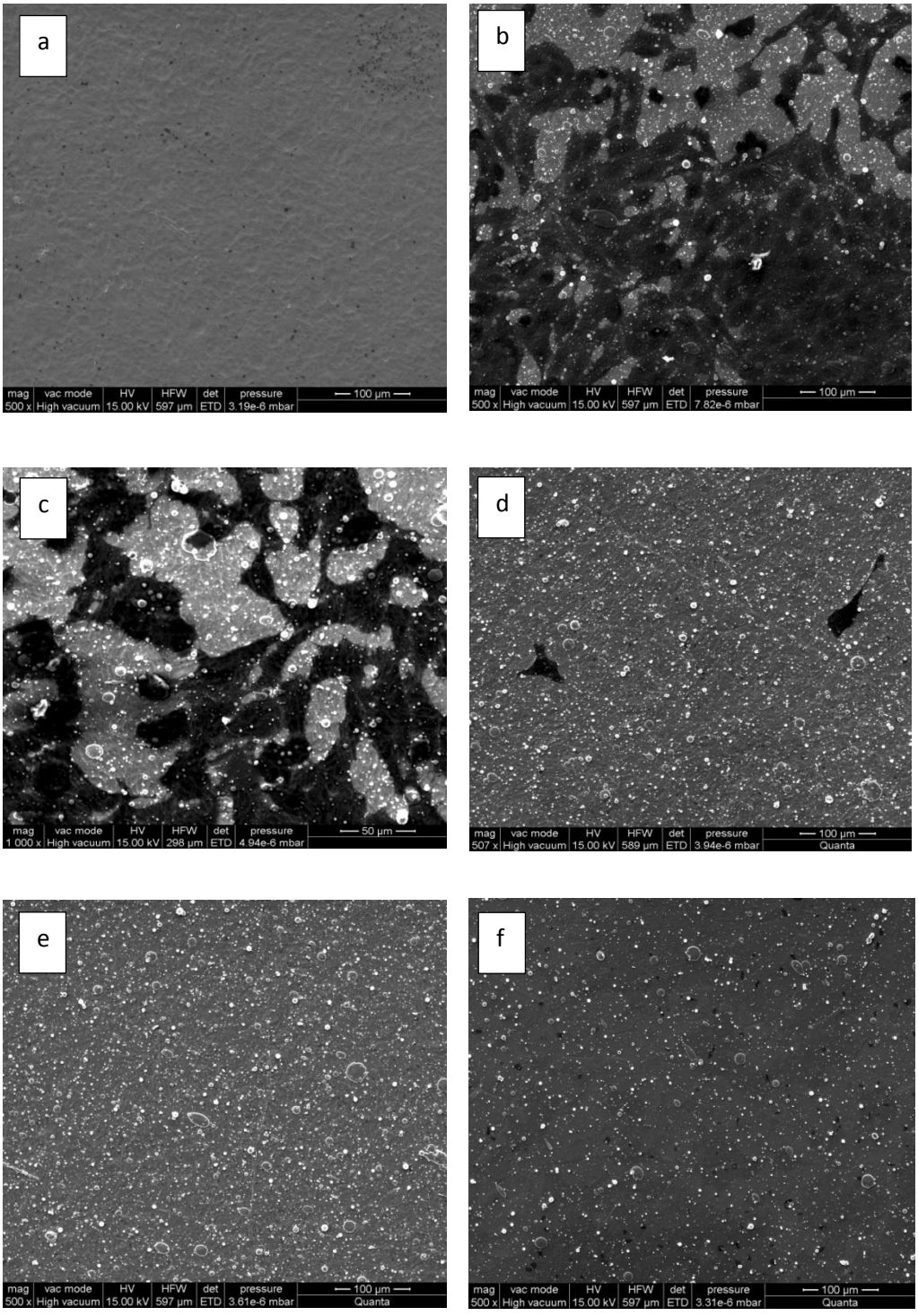

Figure 3.16:SEM micrographs of the coatings after 7 days incubation. (a)Uncoated (b) 3.8 ±0.7 (c) 10 ± 1.1 (d) 11 ± 2.0 (e) 12.3 ± 0.2 (f) 27.1 ± 1.2 at % Mg.

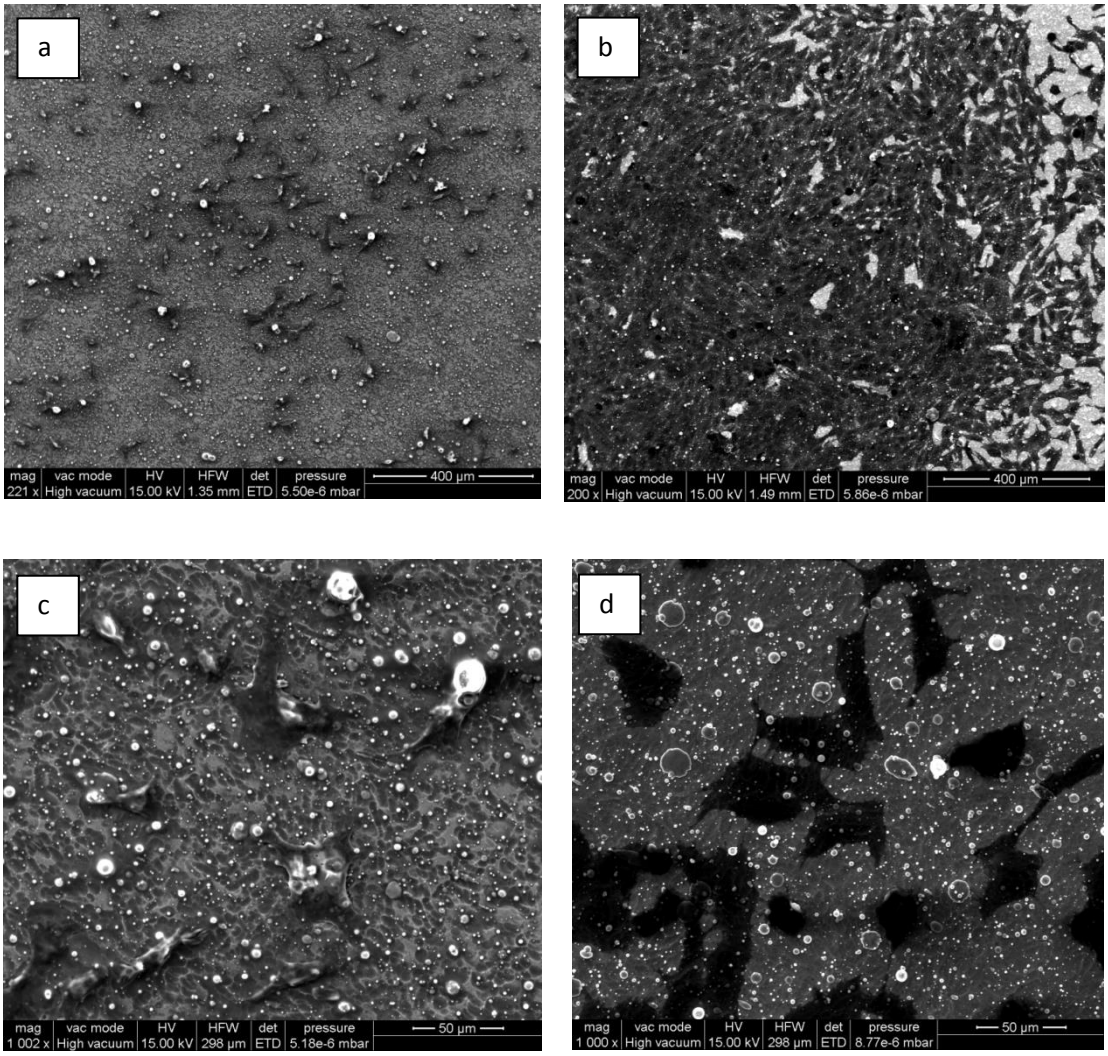

Figure 3.17: SEM micrographs of cell incubated coatings with 3.8 ± 0.7 at % Mg. (a)Day 1 lower magnification (b) Day 7 lower magnification (c) Day 1 higher magnification (d) Day 7 higher magnification.

4. CONCLUSIONS

Considering well-known issues regarding metallic biomaterials and Ti based hard tissue biomaterials, the aim was to modify Ti surfaces by Mg via cathodic arc physical vapor depositon technique to ameliorate the *in vivo* performance of these materials. This well-known coating process was used to create a novel Mg deposited Ti surface with tuned structures for hard tissue biomaterials that allow controlled Mg corrosion. Mg was selected for its biodegradable and biocompatible nature which provides an alternative to solve wear and corrosion issues related with Ti and its alloys. First goal was to adjust Mg proportions on Ti surfaces in order to decrease Mg biocorrosion by using the flexibility of the technique to produce desired structures.

Coating procedures were performed by optimizing different parameters such as current value and duration. Since the atomic Mg percentages above 10 % Mg were known to increase the corrosion rate, production conditions which would allow to produce coatings below that value was examined. Besides Mg amount, the depth of the Mg modified layer on Ti surfaces was determined. By changing the current value and the duration, Mg containing coatings were produced successfully with ~0.75μm depth for 3.8 ± 0.7 at. % Mg, ~0.70μm depth for 10 ± 1.1 at. % Mg, ~1 μm depth for 11± 2.0 at. % Mg, ~0.50 μm depth for 12.3 ± 0.2 at. % Mg and ~0.50 μm depth for 27.1 ± 1.2 at. % Mg containing coatings and structure properties were determined by using 3D optical profilometer in conjuction with GDOES analysis.

XRD analysis of the coatings showed that the nature of coating process might give rise to metal oxide rich structure formation including titanium oxides. This information was proved by GDOES profiles of the coatings in which all of the coatings has an thin oxide layer at sites before Mg contents.

MTS assay of the coatings showed that Mg contents lower than 10 at% in the coatings were better for cell proliferation and viability. The best performance was obtained with

the coatings having 3.8 ± 0.7 at. % Mg SEM results confirm that cell spreading and proliferation was better on these concentrations.

To sum up, the results showed that Mg amount in the Ti-Mg coating layer could be adjusted by controlling arc current values and ion bombardment time by using cathodic arc PVD technique. The proposed modified Ti surfaces can offer hard tissue biomaterials with better surface and biocompatibility properties.

4.1. Future Prospects

As a next study a complete micrographic examination of both cross section of the coatings and cell incubated coatings could be done for a better understanding on the newly designed coatings. Additionaly, the performance of the new method can be studied with the new parameters, for instance, number of rounds of the holder during rotation and count of layers related to this rotation until coating process ends.

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APPENDICES

APPENDIX A: Titanium plates and cathodes

APPENDIX B: Laboratory equipment

APPENDIX A: Titanium plates and cathodes

Titanium plate

Titanium plate (99.9%) (Grade II)

Cathodes

Titanium (Öncel Yüzey Teknolojileri)

Magnesium (Öncel Yüzey Teknolojileri)

APPENDIX B : Laboratory equipment and chemicals

Laboratory equipment

PVD :	Öncel Yüzey TEknolojileri
SEM with EDS :	JEOL JSM 700F
XRD:	Philips Model PW3710
3D Optical Profilemeter:	Wyko Nt1100
CO_2 Incubator:	Binder C-150
Centrifuge:	Beckman Coulter, Microfuge 18
Elisa Plate Reader:	Bio-Tek, Elx800

Chemcials

DMEM (1g/L glucose without phenol red)	Sigma-Aldrich
MTS(Cell Titer 96$^{®}$AQ$_{ueous}$One Solution)	Promega
FBS heat inactivated	Sigma-Aldrich

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PRESENTATIONS ON THE THESIS

- **Mazmanoğlu O., Onder, S., Kok, F. N., Kazmanli, K., & Urgen, M.,** Modification of magnesium coated titanium surfaces to control its corrosion rate, European Society of Biomaterials 26th Annual Conference, Poster Presentation, Liverpool, England , 29 Aug-04 Sep, 2014

- **Mazmanoğlu O., Onder, S., Kok, F. N., Kazmanli, K., & Urgen, M.,** Nano-modification of titanium surfaces to enhance surface properties, NANOTR'10, Poster Presentation, 17-21 Jun, 2014