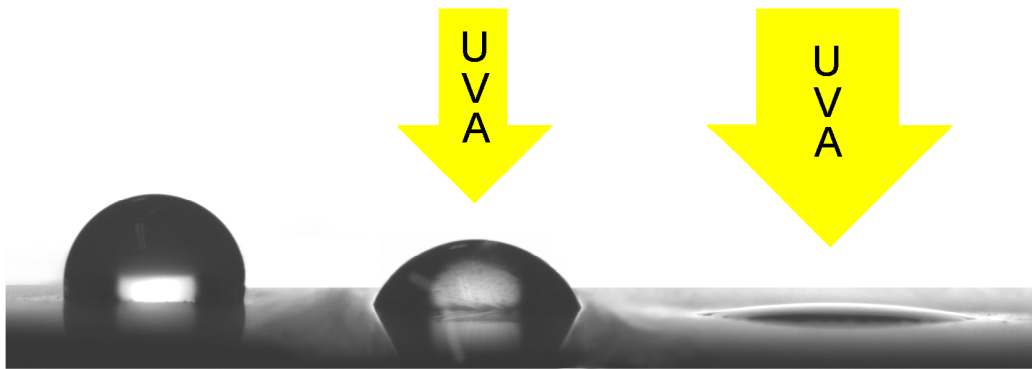


CHALMERS



Effects of UV-exposure of titanium-based dental implant materials

Master's Thesis

CAROLINE KLANG

Department of Applied Physics
Division of Biological physics
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2010
Master's Thesis

MASTER'S THESIS

Effects of UV-exposure of titanium-based dental
implant materials

Master's Thesis
CAROLINE KLANG

Department of Applied Physics
Division of Biological physics
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2010

Effects of UV-exposure of titanium-based dental implant materials
CAROLINE KLANG

©CAROLINE KLANG, 2010

Master's Thesis
Department of Applied Physics
Division of Biological physics
Chalmers University of Technology
SE-412 96 Göteborg
Sweden
Telephone: + 46 (0)31-772 1000

Cover:
UV exposure on a surface related to hydrophilicity.

Chalmers Reproservice
Göteborg, Sweden 2010

Effects of UV-exposure of titanium-based dental implant materials

Master's Thesis

CAROLINE KLANG

Department of Applied Physics

Division of Biological physics

Chalmers University of Technology

Keywords: Contact angle, Methylene blue, XPS, Hydrophilicity, Biocompatibility, Photocatalytic effect, UV, TiO₂

Sammanfattning

Det här är ett examensarbete som har genomförts som ett samarbete mellan Chalmers Tekniska Högskola och Nobel Biocare. Målet med examensarbetet var att utvärdera den fotokatalytiska aktiviteten och de hydrofila egenskaperna på olika titanbaserade ytor när de blivit exponerade av UV strålning. Det var även av intresse att undersöka hur lagring i olika miljöer luft, etanol och plastpåsar påverkar materialen. En UV lampa bestående av LED ljus har även jämförts med en kommersiell befintlig UV lampa. De olika materialen som är studerade i den här studien är maskintillverkade titandiskar, titandioxid diskar och TiUnite diskar. Teknikerna som använts för att studera den fotokatalytiska effekten var nedbrytning av metylen blå, kontaktvinkel mätningar och röntgen fotoelektron spektroskopi, XPS.

Slutsatsen kan dras att den fotokatalytiska effekten kunde aktiveras på både TiUnite och TiO_2 diskarna genom att belysa dessa med UV strålning. De maskintillverkade titandiskarna visade en mycket liten fotokatalytisk effekt som enbart kunde observeras i experimentet med nedbrytning av metylen blå. För de olika diskarna lagrade i luft och plastpåsar ökade kontaktvinkeln medan för diskar lagrade i etanol minskade kontaktvinkeln. Den största kontaktvinkeln observerades då diskarna lagrades i plastpåsar. Mängden kol ökade på TiUnite och TiO_2 diskarna under lagringstiden men kunde minskas igen genom att belysa diskarna med UV strålning.

UV strålning visade sig fungera som aktiveringsmekanism för den fotokatalytiska effekten på TiUnite och TiO_2 diskarna då både kontaktvinkeln minskade och metylen blå i vissa fall bröts ned efter diskarna blivit belysta. Eftersom effekter kan observeras då de olika materialen belyses med UV strålning skulle detta kunna leda till dentala material med förbättrade egenskaper som potentiellt skulle kunna öka antalet lyckadade dentala implantat.

Abstract

This work has been carried out as a collaboration between Chalmers University of Technology and Nobel Biocare. The aim of this master thesis was to evaluate the photocatalytic activity and hydrophilic properties of different titanium surfaces when they were exposed to UV radiation. It was also of interest to investigate the influence of storing the materials in different media; air, ethanol and plastic bags. Further it has been explored if a custombuilt UV activation device built with LED lamps would be comparable to a commercially available UV lamp. Materials included in this study are machined titanium discs, titanium dioxide discs and TiUnite discs. The techniques that were used to evaluate the photocatalytic effect were degradation of methylene blue, contact angle measurement, and x-ray photoelectron spectroscopy, XPS.

It can be concluded that the photocatalytic effect could be activated for both the TiUnite and TiO₂ discs by exposing them to UV radiation. The machined surface showed a small photocatalytic effect that could only be observed in the experiments of degradation of methylene blue. Storage of the different discs in air and plastic increase the contact angle, where as storage in EtOH decrease the contact angle instead. The largest contact angle was observed when the discs were stored in plastic bags. The amount of carbon increased with storage time, on the TiUnite discs and the TiO₂ discs, however these carbons could be removed by exposing the discs to UV light.

The use of UV radiation to activate the photocatalytic activity of TiUnite and TiO₂ surfaces was shown to be successful when the contact angle decreased and the methylene blue degraded in some cases after exposure. Exposing these surfaces to UV radiation could give rise to materials with improved properties, used in dental applications, that could potentially increase the success rate of dental implants.

I Preface

During my first three years at Chalmers University of Technology I studied a wide range of very interesting but relatively abstract subjects in physics, chemistry and biology. I chose the Applied Physics master program because it allowed me to further study and compare these fields while gaining an appreciation for the connection between the practical and the theoretical.

This thesis work was initiated by Nobel Biocare AB and was performed in collaboration with Chalmers University of Technology. This report, therefore, was written with consideration for the potential usage of some results by Nobel Biocare, and for this reason, not all experimental details may be explained in full.

II Abbreviations

LED	Light emitting diode
UV	Ultraviolet
CA	Contact angle
CA1	Contact angle experiment 1
CA2	Contact angle experiment 2
CA3	Contact angle experiment 3
MB	Methylene blue
XPS	X-ray photoelectron spectroscopy
OD	Optical density
TiM	Machined titanium disc
TiU	TiUnite disc
TiO	Titanium dioxide disc

III Keywords

Dental implant TiO₂
Anatase Photocatalytic effect
UV
Hydrophilicity
Methylene Blue
XPS
Contact angle

Contents

Abstract	I
I Preface	I
II Abbreviations	II
III Keywords	II
1 Introduction	1
1.1 Challenges today of dental implants	1
1.2 Aim	1
1.3 Earlier done studies similar to the project	2
1.3.1 Effects of degradation of methylene blue	3
1.3.2 Historical overview of photocatalytic effect on TiO_2	3
1.3.3 Changes in temperature give rise to changes in contact angle	3
1.3.4 Hydrophilic antibacterial properties	4
1.3.5 Photocatalytic activity as antibacterial treatment	4
1.4 Project outline	5
2 Background	6
2.1 Dental implant history	6
2.2 Nobel Biocare	6
2.3 Components of common dental implants used today, produced by Nobel Biocare	7
3 Theory	9
3.1 Biomaterials and biocompatibility	9
3.2 Materials used in dental implants	10
3.2.1 Titanium	10
3.2.2 Titanium dioxide	10
3.2.3 TiUnite	11
3.2.4 Zirconium dioxide	12
3.3 Photocatalytic effect	12
3.3.1 Ultraviolet radiation	12
3.3.2 Quantification of the photocatalytic effect (Methylene blue assay)	13
3.3.3 Photocatalytic activity, organic breakdown and other effects of UV treatment on surfaces	14
3.4 Characterization of biomaterial surface properties	15

3.4.1	Hydrophilicity	15
3.4.2	Catalytic activity	16
3.4.3	Chemical composition	17
4	Experimental techniques	18
4.1	UV-radiation device	18
4.2	Contact angle to measure hydrophilicity	20
4.3	Methylene blue assay to measure photocatalytic effect	22
4.4	X-ray photoelectron spectroscopy to measure surface chemical composition	23
5	Materials and methods	26
5.1	Surface materials	26
5.1.1	Machined Ti discs	26
5.1.2	TiO ₂ discs	26
5.1.3	TiUnite discs	26
5.1.4	Zirconia discs	27
5.1.5	Polished machined Ti discs	27
5.2	Cleaning of surfaces	28
5.3	Storage	28
5.4	Contact angle	29
5.4.1	Contact angle - storage, CA1	29
5.4.2	Contact angle - UV exposure on stored discs, CA2	30
5.4.3	Contact angle - UV exposure before storing discs, CA3	31
5.5	Methylene blue - photocatalytic effect	32
5.6	X-ray photoelectron spectroscopy - Hydrocarbons vs storage and UV exposure	33
6	Results	35
6.1	Hydrophilicity (measured by contact angle)	35
6.1.1	Cleaned and stored discs (CA1)	35
6.1.2	Cleaned, stored and UV exposed discs (CA2)	40
6.1.3	Cleaned, UV exposed and stored discs (CA3)	40
6.1.4	Comparison	41
6.2	Photocatalytic effect (measured by methylene blue assay)	43
6.2.1	Methylene blue assay, using UV - Black Ray 365 nm	44
6.2.2	Methylene blue assay, using UV - LED 365 nm	44
6.2.3	Methylene blue assay, using UV - LED 380 nm	45
6.2.4	Methylene blue assay, using UV - LED 385 nm	46
6.2.5	Comparison	46
6.3	Chemical composition (measured by XPS)	47

6.3.1	Survey spectra of chemical composition on different disc surfaces	47
6.3.2	Atomic-% of elements on different disc surfaces	50
6.3.3	Comparison	53
7	Discussion	54
7.1	Hydrophilicity (measured by contact angle)	54
7.1.1	Cleaned and stored discs (CA1)	55
7.1.2	Cleaned, stored and UV exposed discs (CA2)	56
7.1.3	Cleaned, UV exposed and stored discs (CA3)	57
7.1.4	Comparison	57
7.2	Photocatalytic effect (measured by methylene blue)	58
7.2.1	Comparison	58
7.3	Chemical composition (measured by XPS)	58
7.3.1	Comparison	59
8	Conclusion	60
9	Future perspectives	62
10	Acknowledgements	63
11	Text/Appendix A	68
11.1	Results of XPS measurement	68

1 Introduction

1.1 Challenges today of dental implants

Dental implants have more and more become a routine treatment replacing missing teeth. From the article of Norowski Jr. P.A. et al [1] it is known that the survival rate for an implant today is around 89% over a period of 10-15 years though the dental infection risk may be as high as 14%. The risk of peri-implantitis is higher the first months after implantation and also if the patient smoke or have poor oral health. A bacterial colonization on an implant could lead to inflammatory reactions and loss of osseointegration. One limitation of clinical success is the peri-implantitis, an inflammatory process that affects the soft and hard tissue around the implant. Development of surfaces that become antibacterial in response to infections is a part of the future strategies but research of how to prevent bacterial attachment and how to enhance normal cell attachment to the implant is still needed.

The group of Norowski Jr. P.A. writes that one of the most important challenges today is to further improve the success rate. It is important that the integration between biocompatible materials and soft and bone tissue is fast. To reach faster integration new materials, coatings and topographies need to be explored. The risk of bacterial infections needs to be reduced, during both the healing process/osseointegration and through the rest of the patient's life. Different methods for reducing the risks are under research. Examples of what can be done are antibiotic coatings, antiadherent surfaces, silver coatings, and antimicrobial peptides.

1.2 Aim

The aim of this master thesis is to evaluate the photocatalytic activity and hydrophilic properties of different titanium surfaces when they are exposed to UV radiation, see example in Figure 1.1. Questions that will be answered are what time, wavelength and intensity that are needed to activate the photocatalytic effect of the surfaces. It is also of interest to investigate the influence of storing the materials in different media; air, ethanol and plastic bags. Further it has been explored if a custombuilt UV activation device built with LED lamps would be comparable to a commercially available UV lamp. The purpose of this project is to improve the success rate of dental implants by treating the surfaces of commonly used, as well as novel materials, for dental applications to give rise to products with new enhanced properties.

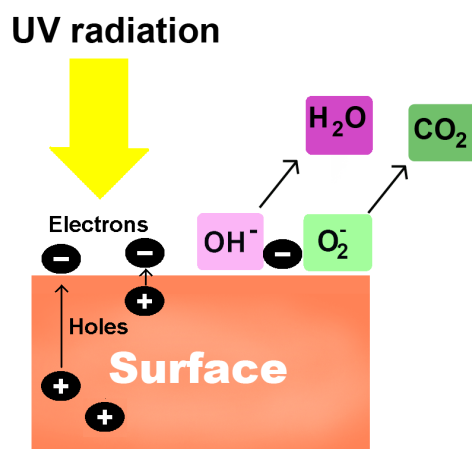


Figure 1.1 - Brief illustration of photocatalytic effect on a surface.

1.3 Earlier done studies similar to the project

There have been a lot of similar studies done on the photocatalytic effect of TiO_2 that support our theory that our titanium surfaces will also show the same effect. In the article by Suketa et al [2] they study the photocatalytic effect of anatase TiO_2 on gram-negative anaerobic bacteria. A pure Ti disc is used, coated with the anatase TiO_2 film and the strong photocatalytic reaction activated by UVA radiation is studied. After 120 minutes illumination of UVA the viability of bacteria on the film was less than 1%. They conclude that the bactericidal effect of photocatalytic activated TiO_2 is of great use for sterilizing contaminated surfaces of dental implants. By degradation of methylene blue they determined the photocatalytic properties of the coated anatase TiO_2 surface. The concentration of methylene blue that was used was 10 ppm and to eliminate reduction in the concentration of methylene blue by absorption during the illumination time, they soaked the specimens for 24 hours in standard methylene blue. After soaking, the specimens were irradiated with UVA radiation at the wavelength 352 nm and intensity of 2 mW/cm^2 . Control discs were also exposed with UVA, with and without aluminum foil. The solution, used during the soaking and exposure, was retrieved and the optical density, OD, was measured at a wavelength of 664 nm.

1.3.1 Effects of degradation of methylene blue

The group of Hermann et al studied both the degradation pathway of methylene blue [3] and the degradation of other types of dyes [4]. During their studies they found that the degradation leads to the conversion of organic carbon into harmless CO₂. They also discovered that when the irradiation period was 1000 min, nitrogen and sulfur heteroatoms turned into inorganic ions such as nitrate and ammonium ions respectively. Their conclusion was that methylene blue can be successfully decolorized and degraded by titania-based photocatalysis at room temperature.

1.3.2 Historical overview of photocatalytic effect on TiO₂

Hashimoto et al [5] describe the possibility of using solar light for photoelectrolysis that was demonstrated in 1969 and also the photocatalytic cleaning effect of TiO₂ coated materials. It is also mentioned that the general way to measure the surface wettability is to evaluate the contact angle, CA, which is the angle between the solid surface and the tangent line of the liquid phase at the interface of the solid-liquid phases. If the surface is exposed by UV radiation the CA decreases. When the CA is almost 0° the surface becomes completely wetted, also called highly or super hydrophilic. They have obtained many experimental results showing that the highly hydrophilic state is not a simple clean surface but it is a structurally changed metastable¹ one. Carbon on the surface is also evaluated in the article, using x-ray photoelectron spectroscopy, XPS. After 60 min of UV irradiation both the CA and the carbon peak of XPS decreased compared to before the irradiation.

1.3.3 Changes in temperature give rise to changes in contact angle

Takeuchi et al [6] found that changing the temperature of Ti samples showed a good correlation with the changes in the contact angle of the water droplet on a TiO₂ surface. As mentioned above, UV radiation affects the surface too. The effects are in the article studied in combination with the amount of hydrocarbons on the surface, measured with XPS. Their results are similar to what was seen by Hashimmote et al [5], the contact angle increase during storage and decreased after UV irradiation. The group of Takeuchi also studied the effect of heating the TiO₂ film, which resulted in changes of the contact angle. An interesting notice is that they evaluate the effect on the

¹Excited state of an atom, molecule or atomic nucleus that has exceptional long lifetime. [7]

contact angle when the surface has other atoms or particles on it. If there are particles on the surface it can cause an error when measuring the angle.

1.3.4 Hydrophilic antibacterial properties

How cells interact with hydrophilic surfaces has been studied by the group of Lifan et al [8]. Based on the observation of cell deformation and FT-IR analysis the fast acting germicidal activity of fresh TiO_2 has been studied. The cell deformation and interaction between cells and TiO_2 coated surfaces depends on oxygen vacancies. How much the cell deforms and interacts are dependent on the wetting properties of TiO_2 . The surface has a higher germicidal effect on gram-negative cell, cells with thin cell wall, than on gram-positive cells, cells with thicker cell wall. When TiO_2 is exposed to air this germicidal effect decreases at the same time as the wetting property decreases.

The group of Li B et al [9] studied the final amount of bacterial adhesion on a TiO_2 surface exposed by UV radiation wavelength, between 254-340 nm, followed by bacteria attachment. It was concluded that the adhesion decreased with up to 40%. Also if the surface was exposed after bacterial adhesion the final amount of attached bacteria was reduced with up to 50%. The difference can be explained by the fact that when exposing the metal before bacterial adhesion the surface becomes more hydrophilic due to the photocatalysis but the bacteria are not affected compared to when the surface and attached cells are exposed. Then the photocatalysis acts on the bacteria-metal oxide surface and can then reduce biofouling by oxidizing the surface polymers of the bacteria and through that decrease their adhesion to the surface.

1.3.5 Photocatalytic activity as antibacterial treatment

If and how the photocatalytic activity could work as antibacterial treatment were studied of the group of Chun et al [10]. Using the dip-coating method and sol-gel², TiO_2 was first coated on the surface of orthodontic wires. By measuring the change of wires weight the bacterial adhesion to the wires were evaluated. Using the dilution agar plate³ method for *Streptococcus mutans*, *S. mutans*, and spectrophotometry for *Porphyromonas gingivalis*, *P. gingivalis*, the antibacterial activity of the surface modified orthodontic wires was demonstrated. Comparing TiO_2 coated and uncoated wires it was only the coated that showed an antiadherent effect against *S. mutans*. The coated

²Also known as chemical solution deposition, a wet-chemical technique

³A disc used to culture microorganisms.

wire had also a bactericidal effect on *S. mutans* and *P. gingivalis* which can cause caries and tooth loss respectively.

1.4 Project outline

This thesis work started with a literature study followed by setting up an experimental plan. The plan included design of a UV exposure device together with an external company. Further more an assay with methylene blue was designed to measure photocatalytic effects of dental implant materials. Characterization of surface properties with respect to hydrophilicity and chemical composition, using contact angle (CA) goniometry and x-ray photoelectron spectroscopy (XPS) respectively, was also done.

2 Background

2.1 Dental implant history

In Elaine Williams book "A Matter of Balance", [11], it can be read that in 1953 the Swedish professor Per-Ingvar Brånemark observed that a piece of titanium embedded in animal bone tissue became firmly integrated and was difficult to remove. After a year of research Brånemark discovered that the human body tolerated titanium and that it could integrate gently with existing bone tissue. Brånemark called the process *osseointegration*.

From the the article of Pye A.D. et al [12] it is written that in 1965 the first patient was treated with the Brånemark system of dental implants, and the implants were introduced on the market. In 1992 the modern ceramics for dentistry were developed and during 2000 the surface treatment material TiUnite was developed and used as a coating to the Brånemark system implants to enhance osseointegration. More about the TiUnite material in section 3.2.3 Today the field of dental implants is expanding quickly. In year 2000 the estimated number of placed dental implants was two million over the world each year. [13]

As written in the book Biomaterials - An introduction, [14], new technologies and biocompatible materials make it possible to develop the healing process and osseointegration. A dental implant is an artificial tooth that consists of inert, biocompatible materials inserted in the maxilla (upper jaw) and/or the mandible (lower jaw). A tooth implant looks like a real tooth and has the same functions. An inserted implant works as a replacement for lost orofacial structures which can be a result of trauma and congenital defects, or for the management of tooth loss.

2.2 Nobel Biocare

Nobel Biocare (NOBN, SIX Swiss Exchange) is the world leader in innovative restorative and esthetic dental solutions. As a complete solutions provider Nobel Biocare offers the most comprehensive range of solutions from tooth to root, for single tooth to fully edentulous indications. The solutions portfolio covers dental implants (including the key brands NobelActive™, Brånemark System®, and NobelReplace™), individualized prosthetics and equipment (NobelProcera™), guided surgery solutions and biomaterials. Nobel Biocare has approximately 2,250 employees and recorded revenue of EUR 581.4 million in 2009. The company is headquartered in Zurich, Switzerland. Production takes place at seven sites located in Canada, Israel, Japan, Sweden, and the US. Nobel Biocare has 35 direct sales organizations. [15]

2.3 Components of common dental implants used today, produced by Nobel Biocare

Nobel Biocare produces and sells dental implants and devices to place them. In their brochure [16] they describe what the implant consists of. Dental implants are built up by different parts, which together and inserted to the jaw, works as an artificial tooth. In Figure 2.1 it can be seen that there are six large parts to consider for a dental implant, veneering material, prosthetic coping, dental implant abutment, prosthetic screw, screw fixture and coating. As mentioned before it is important that the implant looks good and works well. For the best result without implant loosening different materials are used, adapted to what tissue the specific part of the implant will connect to.

Closest to the jawbone, where the implant will be anchored, is the screw fixture. This fixture should consist of a tensile and strong material like titanium. To enhance the integration with bone cells and speed up the fixation of the implant it is possible to cover the surface of the fixture with a coating. Nobel Biocare uses a material called TiUnite, a porous material developed to increase the bone cell adhesion.

As seen in Figure 2.1, inside the screw fixture (the implant) a prosthetic screw is placed. The surface of this screw is covered with a diamond like coating to minimize incidence of tooth loosening. This screw is used to fixate the tooth crown consisting of dental implant abutment, prosthetic coping and veneering material. The dental implant abutment interacts in the interface between smooth tissue and bone tissue, which is the place where for examples bacteria can migrate into the implant and infections can take place. On the top of abutment the prosthetic coping is placed, working as a base for the veneering material. Nobel Biocare offers manufactured single crown copings in zirconia and alumina for optimal strength and esthetic. Finally on the top the veneering material mimic the appearance of natural tooth color and form.

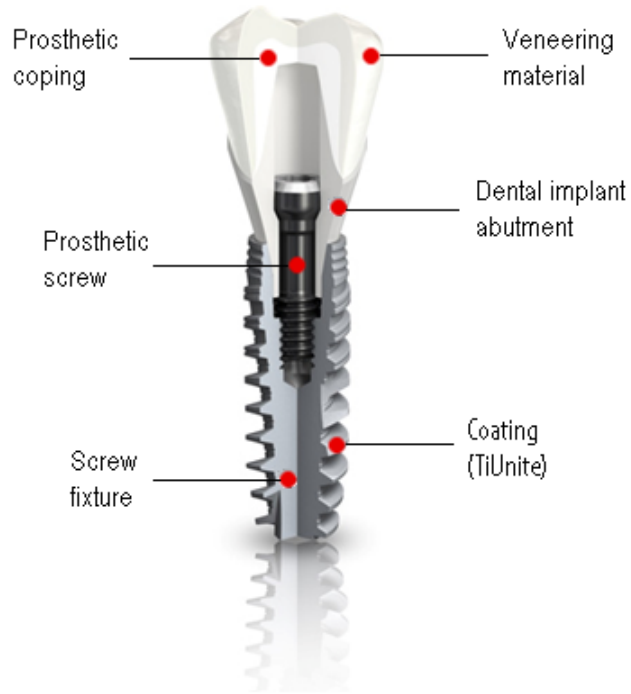


Figure 2.1 - A dental implant with some important parts. Courtesy of Nobel Biocare.

3 Theory

3.1 Biomaterials and biocompatibility

In the biomaterial book of J. Park, Biomaterials - an introduction [14], it is written that a material that can be used to make devices to replace parts or functions in a body in a safe, reliable, economic, and physiologically acceptable manner can be called a biomaterial. A biomaterial can be defined as a synthetic material that replace parts of a living system or functions in intimate contact with living tissue to improve human health. There are three major factors that shows how successful the material or implant is. The properties and biocompatibility of the implant, the health condition of the recipient, and the competency of the surgeon, who both performs the surgery and follows the progress. There are a lot of requirements that needs to be full filled to make the biomaterial accepted by the body. That the material should be biocompatible includes among other things that the material should be nontoxic, nonallergenic, nonimmunogenic, and sometimes no time-dependent degradation. Other requirements are adequate mechanical strength and fatigue life, proper weight and density. The material should be relatively inexpensive, reproducible and easy to fabricate and process for large-scale production.

Park also writes that when an implant is inserted into the body, it will react differently dependent on what material it is. It is fundamentally the same reaction if the body will get a simple splint. If the material is harmless the body will let it be but a part of the healing process will start never the less. When a material, an artificial implant, is accepted by the surrounding tissue and by the body as a whole the material is *biocompatible*. The material that is inserted can be designed to degrade over time, and then the products of degradation need to be harmless to the tissue and organs too. Overall the implant should be compatible with tissues in terms of mechanical, chemical, pharmacological and surface properties.

In a study by Att W. et al [17] it was discovered that when surfaces that have been stored are exposed to UVC the cell attachment capacity increases to a level 50% higher than for new surfaces. If the surface is exposed with UVA instead there will not be any effect at all. Both UVA and UVC will make the surface hydrophilic but it is only UVC that effectively reduces the surface carbon to a level comparable with a new surface. Recent reports suggest that determining the hydrophilicity is effected by hydrocarbons. The more hydrocarbons that attach to the surface, the higher is the contact angle which leads to less hydrophilic. The absorption rate of proteins and attachment of

cells are strongly correlated to the percentage of carbons on TiO_2 . Tests have shown that UV treatment accelerate the establishment of an implant fixation with four times. [17]

When the TiO_2 is exposed with UV an electron is excited from the valence band to the conduction band. The TiO_2 surface is then electropositive which allows both proteins and cells to directly attach to the surface without any help from divalent cations. This is why UV treated titanium surfaces usually are assumed to be cell attractive. [18] In the biomaterial book of Park J. [14] it is written that when a medical device is designed it is important to also think of the time that the implant is going to be inside the human body, where it is located and how it is applied or implanted. This means that which materials and coatings that are used can vary depending on the device application and use.

3.2 Materials used in dental implants

Today there are a lot of materials used in dental implants. Two materials that often are used is titanium-based and zirconia-based materials. [16]

3.2.1 Titanium

Titanium, Ti, is a metallic chemical element that is very strong, easy to machine and it has very low density which results in a very light material. The melting point of Ti is rather high, around 1700° , and the density is 4.5 g/cm^3 , compared to 316 stainless steel with 7.9 g/cm^3 . [14] Titanium is an allotropic material that is built up with hexagonal close-packed, HCP, structures. Below 882°C Ti keeps the HCP structure but over that temperature it changes to body-centered cubic, BCC, structure. By the formation of a solid oxide layer on the surface the material is very resistant to corrosion. This can be very useful in medical applications like prostheses, orthopedic and dental implants. Ti can be alloyed with many other chemical elements like iron, aluminum and vanadium. Ti6Al4V is one alloy that is widely used to manufacture implants. [14]

3.2.2 Titanium dioxide

In the book of biomaterials [14] it is written facts about some materials used in this project among some the titanium dioxide. TiO_2 , naturally occurs as oxidation of titanium. In the nature, TiO_2 can for example be found as the minerals rutile and anatase, two crystal structures that commonly are used

in photocatalysis. Anatase shows higher photocatalytic activity than rutile, meanwhile rutile is the most common and also the most stable form. The two structures are in the article by Linsebigler A.L. et al [19] described as chains of TiO_6 octahedrons where the difference between the two is distortion of each octahedron and assembly pattern of the chains. In Figure 3.1 the unit cell structure of rutile and anatase are illustrated. The differences in lattice structures of the two TiO_2 crystalline forms cause different mass densities and electronic band structures. For rutile the energy gap is at 3.1 eV and for anatase it is at 3.3 eV which corresponds to a wavelength around 365 nm and 380 nm respectively. Anatase is according to the book Photocatalytic Reaction Engineering, [20], the most popular crystalline form of TiO_2 to use in photocatalytic processes given its superior activity compared to rutile. The only photons that can excite and activate TiO_2 are those with wavelengths equal to or less than 388 nm.

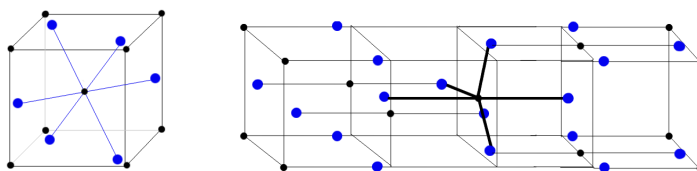


Figure 3.1 - Unit cell structures of TiO_2 . To the left rutile and to the right anatase. Adapted from the article by Amy L. et al, [19]

3.2.3 TiUnite

TiUnite is a material developed in 2000 by Nobel Biocare and it is used as a surface coating of titanium oxide on implants. Nobel Biocare has a brochure [21] describing what the material is and what it is used for. It has been proved that the rough TiUnite surface enhances the speed of osseointegration with the implant by stimulating rapid bone growth. The material is a porous, titanium-oxide implant surface that is manufactured by an anodic oxidation process. To produce TiUnite, Nobel Biocare uses an electrochemical process to induce the natural surface oxide layer on a titanium implant and make it grow in a controlled way.

The surface characteristics of the implants have been studied to a great extent by for example by Sul, Y.T. et al [22]. Looking at the chemical composition of the surface it consists of mainly TiO_2 , but also contaminant C, Na, N and phosphorus. The morphology can be described by duplex oxide structure with an outer porous film with micropores and an inner barrier film

without micropores. On the surface the pore size is up to $4\ \mu\text{m}$. The crystal structure of TiUnite consists of both anatase and rutile.

3.2.4 Zirconium dioxide

In the biomaterial book [14] it is also written some about zirconium dioxide, also called Zirconia, ZrO_2 . It is a white crystalline oxide of the chemical element zirconium. The material is allotropic and has a high density, refractive index, fracture toughness and hardness, and low thermal conductivity. It is possible to use ZrO_2 up to a temperature of 2400°C . Some properties of zirconia are as good as or sometimes even better than properties of alumina ceramics. One example where zirconia is much better than alumina is fracture toughness. When a crack occurs in the material small particles of partially stabilized ZrO_2 are dispersed in the materials and starts a phase transformation that operates to prevent the propagation of cracks. This increases the fracture toughness. The high biocompatibility properties make the material very interesting for implants.

Yttrium-stabilized zirconia is a material that has been used to produce the femoral head of total hip joint prostheses and this material has two advantages over the alumina. The first one is the finer grain size and a well-controlled microstructure, the other one is higher fracture strength and toughness due to the phase transformation toughening process. [14]

3.3 Photocatalytic effect

3.3.1 Ultraviolet radiation

Ultraviolet, UV, radiation refers to electromagnetic radiation with shorter wavelength than the visible light, in the 200-400 nm wavelength range. The wavelengths emitted from the sun are separated into three types of UV; UVA, UVB and UVC, see Figure 3.2.

The ozone layer separates the UV radiation and only lets wavelengths above 290 nm through, which means that UVA and part of the UVB are the only UV radiations that reaches earth. This can though be dependent on how thick the ozone layer is. UVA is not harmful to the biological molecules but UVC is dangerous, UVB is somewhere between. In Figure 3.2 UVA, UVB and UVC are illustrated in a spectrum. [23]

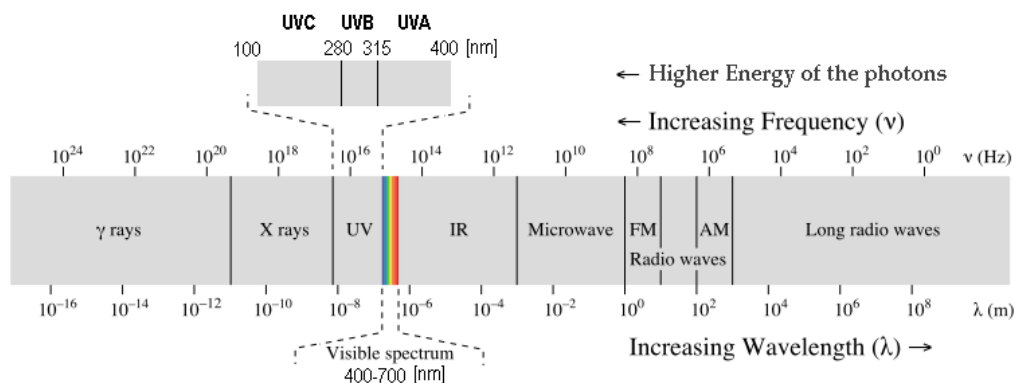


Figure 3.2 - Electromagnetic spectra showing the UVA, UVB and UVC radiation and their wavelengths. Adapted from Wikipedia. <http://schools-wikipedia.org/images/204/20443.png.htm>. The image was uploaded 5 August 2007 and available 28 June 2010.

In Table 3.1 the wavelength of the different UV radiation can be seen. In Equation 3.1 it is shown how the energy of each wavelength is calculated and the results can also be seen in Table 3.1.

Table 3.1 - Table over UV radiation, its wavelengths and energy.

UV radiation	λ [nm]	E_{UV} [eV]
UVA	315-400 nm	3.10-3.94 eV
UVB	280-315 nm	3.94-4.43 eV
UVC	100-280 nm	4.43-12.4 eV

$$E = \frac{h \cdot c}{\lambda} \quad (3.1)$$

where $h = 4.1356692 \cdot 10^{-15}$ eVs is Planck's constant, $c = 299\,792\,458$ m/s the speed of light and λ the wavelength of the radiation. [20] [24]

3.3.2 Quantification of the photocatalytic effect (Methylene blue assay)

Methylene blue, MB, is a heterocyclic aromatic chemical compound, a thiazine dye used for several medical purposes. Using MB in small doses it acts as a reducing agent whereas in larger doses it acts as an oxidizing agent. One property is dose-dependent oxidation or reduction properties. [25] Methylene blue is a cationic dye and because of that it is favored to adsorb on negatively

charged surfaces at high pH's. [4] Degradation of methylene blue has earlier been used to measure the photocatalytic effect. When for example titanium dioxide is exposed with UV radiation the material let out free radicals. If the material is in a methylene blue solution during the exposure time the free radicals will react with methylene blue that will be degraded. The photocatalytic effect can visually be seen with the human eye, when MB degrades the blue color of the solution will be lighter. For a more detailed degradation pathway of methylene blue see the article of the group Houas et al [3] and the group Lachheb et al [4].

3.3.3 Photocatalytic activity, organic breakdown and other effects of UV treatment on surfaces

The reaction mechanism of the photocatalytic process can in brief be described as follows. When aqueous TiO_2 is illuminated with irradiation energy greater than the band gap energy, E_{bg} , of the semiconductor valence band holes, h_{vb}^+ , and conduction band electrons, e_{cb}^- , are generated. For a brief illustration see Figure 3.3. In the case of anatase TiO_2 $h\nu > E_{bg}$ where $E_{bg} = 3.2\text{eV}$.

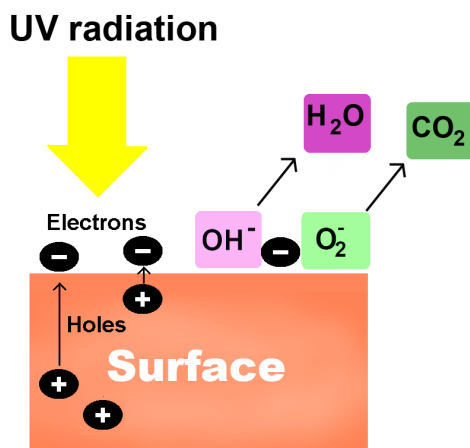
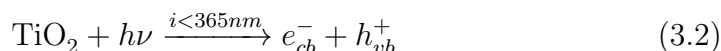
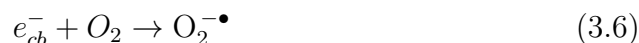
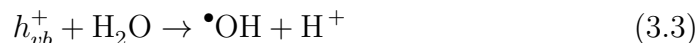


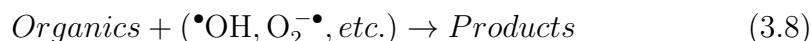
Figure 3.3 - Brief illustration of photocatalytic effect on a surface.



The valence band holes and conduction band electrons that are photogenerated can be recombined, liberate heat or they can move to the surface of TiO_2 . If the electrons and holes move to the surface they can react with species that are adsorbed onto the catalyst surface.



Hydroxyl radicals, $\bullet\text{OH}$ that are produced in (3.3) and (3.4) together with other oxidants, e.g. superoxide radical anion, $\text{O}_2^{-\bullet}$ that is produced in (3.6) can further mineralize organic compounds to the end products water and CO_2 [26].



An artificial UV radiation source can start the photocatalytic process. Usually the artificial UV sources are made of different metals and noble gases. The energy range of mercury emission spectra can usually induce photochemical reactions. The crystalline form of TiO_2 anatase is a metal oxide semiconductor and those have been found to be the most suitable photocatalysts because of their photocorrosion resistance and wide band gap. To activate the photocatalytic effect of TiO_2 , the irradiation needs to be equal to or below 388 nm because the band gap of TiO_2 . It is also possible for natural solar light to activate TiO_2 if the activation spectrum overlaps with the solar spectrum. There is only 4-5% of the sunlight with 300-400 nm that reaches the earth's surface and this amount can be used to power photocatalytic reactors. [20]

3.4 Characterization of biomaterial surface properties

3.4.1 Hydrophilicity

Hydrophilicity and hydrophobicity describes the ability of a surface to become wetted by water. When applying a droplet on a hydrophobic surface the water will retain the droplet shape. If the surface is hydrophilic instead the water droplet will float out, see Figure 3.4.

Hydrophilicity and hydrophobicity can be related to the surface energy. Surface energy can be expressed like the energy cost/unit area to cleave a

crystal. This means that the surface energy is related to the cohesive energy of the solid, which is the energy gained by arranging the atoms in a crystalline state, and the areal density of surface bonds that are created during cleavage. [27]

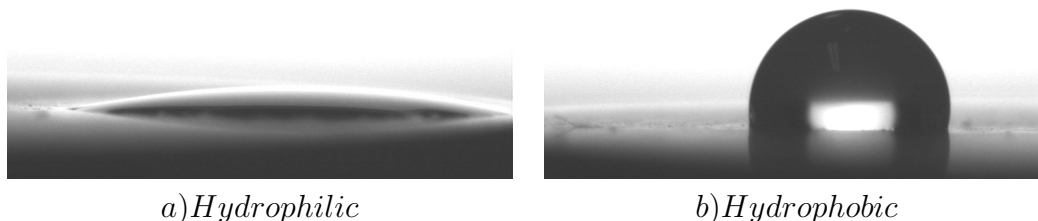


Figure 3.4 - *Hydrophilic and hydrophobic surfaces illustrated with water drops.*

3.4.2 Catalytic activity

According to the book "Chemical principles" by Atkins, P. and Jones, L., [28], a catalyst is a substance that increases the rate of chemical reactions without being consumed itself. The catalyst can speed up both the forward and the reverse reaction rates so that the reactions reaches equilibrium but it can not affect the composition at equilibrium. In the book "Elements of chemical reaction engineering" by Scott Fogler, H. [29] it is also mentioned that by promoting another molecular path or mechanism for the reaction the catalyst can change the reaction rate. At room temperature the gaseous hydrogen and oxygen are virtually inert but react rapidly when exposed to platinum.

There are two different catalysis, *homogeneous* and *heterogeneous*. In the homogeneous catalysis the catalyst is in solution with at least one of the reactants. An example is the process for manufacturing normal isobutylaldehyde, with propylene, carbon monoxide, and hydrogen as the reactants and a liquid-phase cobalt complex as the catalyst. The heterogeneous catalyst on the other hand involves more than one phase. Usually the catalyst is a solid and the reactants and products are in liquid or gaseous form. An example of that is the production of benzene, manufactured from the dehydrogenation of cyclohexane using platinum-on-alumina as the catalyst. The heterogeneous catalytic reaction occurs at or very near the fluid-solid interface which means that a large interfacial area can be helpful in the process.

3.4.3 Chemical composition

The highly hydrophilic conversion when the Ti surfaces are exposed to UV has been shown to be a cause of photogenerated holes, not the electrons. In the bulk of TiO_2 photogenerated holes are produced, that diffuse to the surface where they are trapped at lattice oxygen sites. The trapped holes will react with the adsorbed organics and water on the surface. When reacting with water, OH radicals are produced. There will also be a small part of the holes that reacts with TiO_2 and together with water molecules at the titanium site it will break down bonds between the lattice titanium and oxygen ions. When the water molecules coordinate they release a proton as a charge compensation which leads to that a new OH group forms. This process increases the OH groups on the surface, see Figure 3.5. But OH groups produced by UV irradiation are shown to be thermodynamically less stable than the initial OH groups. This means that a TiO_2 surface that is covered with UV irradiation produced OH groups will have a higher surface energy than a surface covered with initial OH groups. [5] A high surface energy can be coupled to hydrophilic and a low energy a less hydrophilic or hydrophobic surface. [27]

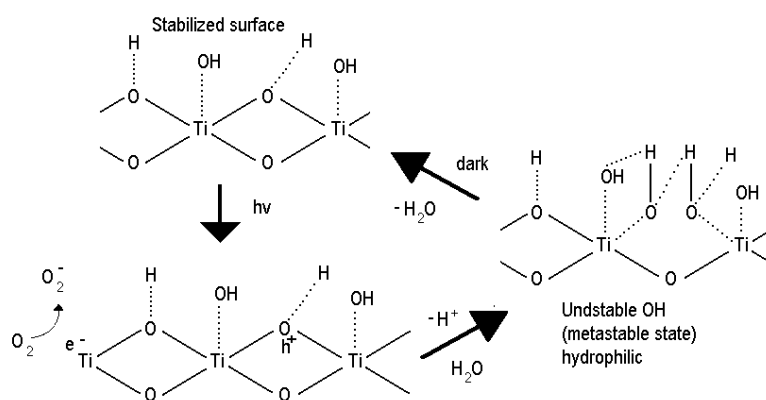


Figure 3.5 - Schematic illustration of reversible changes in amount of OH groups on TiO_2 surface exposed by UV radiation in the dark. Adapted from an article of Balaur E. [30]

4 Experimental techniques

In this project there are one technique used to expose the discs to UV radiation and some techniques that are used to evaluate different effects of the exposure. The techniques are shortly described below.

4.1 UV-radiation device

A UV radiation exposure source was developed and created by an external company. The device is able to expose the titanium discs with different wavelengths and intensity for different time periods. Both UVA and UVC light sources were built with LED technique and with different wavelengths. To be able to further adjust the intensity there were also lamps with different numbers of LED's. The exposure source can be seen in Figure 4.1.

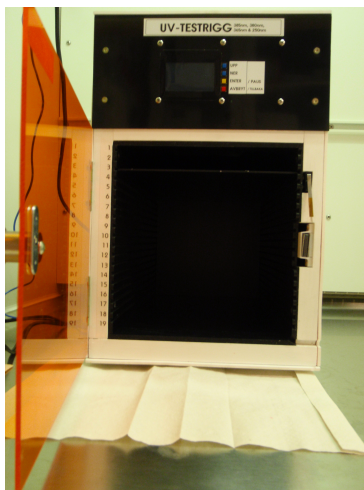


Figure 4.1 - The UV light exposure device.

The different lamps were created to make it possible to investigate which lamp with what wavelength that could activate the photocatalytic activity of the titanium based surfaces. It was also of interest to explore if different intensity could influence the result. Different LED lamps were created with four different wavelengths and two different numbers of LED's. The four different wavelengths were 365 nm, 380 nm and 385 nm which are UVA light. As well as 255 nm, which is UVC light. During the methylene blue experiment all four lamps were used, but during the contact angle and XPS experiments only one lamp was used, the LED lamp with a wavelength of 365 nm. All lamps and their intensity are presented in Table 4.1 and in Figure 4.2 there

is an illustration of one of the LED lamps. Figure 4.2 shows the LED with the wavelength of 365 nm, the other LED lamps look similar.

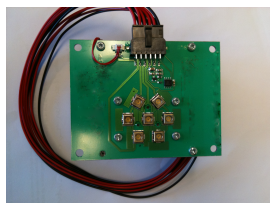


Figure 4.2 - LED lamp with the wavelength 365 nm. All other used LED lamps look similar.

Table 4.1 - Comparison of the different lamps and their intensity.

UV radiation	365 nm Blackray	365 nm LED	380 nm LED	385 nm LED
UV irradiance [$\mu W/cm^2$]	5750	11700	7900	6300
Distance between lamp and disc [cm]	15.5	4	4	4

A UV light measuring instrument, Delta OHM HD2302.0 LightMeter, was obtained to measure the intensity of the light. A light sensor measured the intensity of different wavelengths. To be able to adjust the intensity more than with the two different LED combinations the UV exposure box had different levels to place the tray with the discs on, see Figure 4.3.

There are two parameters that can be changed during the UV exposure. How many percents of the lamp that should radiate and for how long time it should be on. To use the UV exposure device there are safety requirements that have to be fulfilled, the front door and the lid on the top have to be closed. If they are opened, it is not possible to start the exposure. The front door consists of an orange plexiglas which will stop the dangerous UV radiation to pass.



Figure 4.3 - Inside the UV light exposure device there are a scale of steps, with different highs from the UV source, where the discs can be placed.

4.2 Contact angle to measure hydrophilicity

The contact angle measuring instrument used in this project is a DSA1 v 1.80, Drop Shape Analysis from KRÜSS. The measuring instrument is used together with a computer with a Drop Shape Analysis, DSA, software. The device consists of a sample table, a needle with water⁴ positioned above the table, a lamp and a camera which takes photos from the side, see Figure 4.4. The DSA is connected to a computer that collects all data with a specific software.

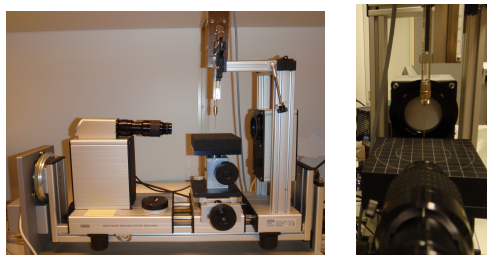


Figure 4.4 - The Drop Shape Analysis device. To the left an image of the device from the side. To the right an image of the "camera view" of the device.

⁴CAS no. 7732-18-5

The DSA software can be used to measure the contact angle. Using the DSA software the arrangement of the sample and the drop in the image can be described. In the program it is possible to choose what drop type the study will be made on. This has to be decided before the program can start measure. There are two different drop types to choose between, the sessile drop and captive double. Which one that is best depends on the used measuring setup. Typical for sessile drops are drops lying on a solid surface and forms a characteristic contact angle with the surface, see Figure 4.5 a). If the surface has very high energy the contact angle that forms is hard to measure. Sometimes the sample is immersed in a liquid, and then the classical and best method is the captive bubble method. Compared to the sessile drop on the surface, an air bubble, surrounded by a liquid phase, is deposited beneath the solid sample, see Figure 4.5 b). For this project we choose the sessile drop method because we wanted to look at a flat surface without high energy.

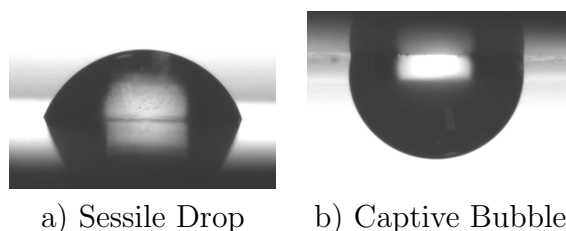


Figure 4.5 - *Different drop shapes. To the left a sessile drop and to the right a captive drop.*

When the drop type is chosen the user drops a drop on the sample surface and use the program to measure on it. First a baseline has to be placed in the image. It is a line that represents the surface from where the program should start to measure. Then one method out of the four methods Circle, Conic section, Polynomial and Young-Laplace needs to be chosen. The title of each method is indicative of its mechanism of action.

The requirements of the Circle method, CIR, are only fulfilled when looking at very small contact angles and drop volumes. It is assumed to look at a drop shape in form of a circular arc. Conic section method, also called *Tangent method 1* or T-1, assumes an elliptical drop shape and when measuring the method fits a general conic section equation to the drop shape. The angle between the baseline and the tangent at the conic section curve at the three-phase contact point becomes the contact angle, see Figure 4.6. The third method Polynomial method, also called *Tangent method 2* or T-2,

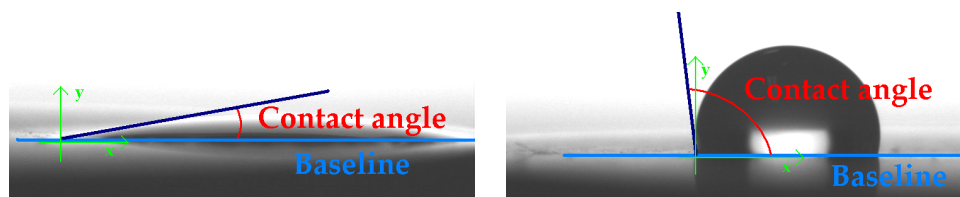


Figure 4.6 - An illustration of how the contact angles are measured at different surfaces. To the left a hydrophilic surface, and to the right a hydrophobic surface.

only evaluates the phase contact region. For the contour shape there are no geometrical requirements but the polynomial method adapts itself to a curve that can fit the three phase contact point. The last method, Young-Laplace or L-Y, is very useful for symmetrical drop shapes that not are affected of sample tilting or contact with the deposition needle. It is an iteration method that looks at the characteristic drop shape under the influence of gravity. The surface tension is also possible to measure with this method. When the method is chosen it is used by the program to measure the drop contact angle. [31] In Table 4.2 all the methods are summarized [31], but in this project only two methods are used, T-1 and CIR.

4.3 Methylene blue assay to measure photocatalytic effect

This experiment is a method to study photocatalytic effect. As can be read in section 3.3.2 methylene blue is an organic element that will degrade when there is a photocatalytic effect. When radicals are produced they react and degrade MB.

The material that is supposed to be tested is placed in a methylene blue solution that will be exposed to UV radiation. If the color of MB is reduced there is a photocatalytic effect. It is also possible to irradiate the solution with the material and then see if there is any effect after exposure. To see if the MB has degraded, the color changes to a lighter tone, the absorbance is measured by a spectrophotometer. The spectrophotometer measures the absorbance at 665 nm. As can be read in section 3.3.2, MB has a maximum wavelength, λ_{max} , at 660 nm where it is easiest to measure. The measurements here are done at 665 nm because it is the closest wavelength that the filter in the spectrophotometer has.

Table 4.2 - Table over what method to use in what situation when measuring contact angle. [31]

Summery of contact angle measuring methods				
	Circle	Conic section	Polynomial	Young-Laplace
Measuring rang				
0-20 °	x			
10-100 °		x	x	x
100-180 °			x	x
Drop weight (volume+density)				
Low	x	x	x	x
High		x	x	x
Very high			x	x
Deposition				
Static (contour without needle)	x	x	x	x
Dynamic (contour with needle)		x	x	
Contour shape				
Symmetrical	x	x	x	x
Slightly asymmetrical		x	x	
Very asymmetrical			x	

4.4 X-ray photoelectron spectroscopy to measure surface chemical composition

X-ray photoelectron spectroscopy, XPS, also known as Electron Spectroscopy for Chemical Analysis, ESCA, was developed in the middle of 1960s by Kai Siegbahn together with his research group at the University of Uppsala in Sweden. For this device Siegbahn was awarded the Nobel Prize for Physics in 1981. [32] XPS is one of the most used techniques for analyzing surface chemicals. [33] It is possible to explore the first few atomic layers and assign chemical states of the detected atoms. [32] During the analysis of the surface there is usually no altering of the components, which gives a good result and makes it possible to measure the sample again. Using XPS it is possible to look at the elemental composition, empirical formula, chemical state and electronic state of elements on and within a material. [33]

Using XPS requires ultra high vacuum and a beam of monoenergetic soft x-rays are irradiating the material while the kinetic energy and number of electrons that escape are measured. The x-rays may penetrate rather deep into the sample but the depth of ejected electrons that will escape is limited. Therefore it is only possible to measure from 1 to 10 nm depth into the material. [33] A spectrum of the number of detected electrons per energy interval versus their kinetic energy is obtained as a plot. [32]

The sample is irradiated with monoenergetic soft x-rays that usually are Mg $K\alpha$ 1253.6 eV or Al $K\alpha$ 1486.6 eV. As mentioned the penetrating power in a solid of these photons is limited to the order of 1-10 μm . The photons interact with atoms on the surface causing electrons to be emitted by the photoelectric effect. The measured kinetic energies of the emitted electrons are given in Equation 4.1. [32]

$$E_k = h\nu - BE - \Phi_s \quad (4.1)$$

where $h\nu$ is the energy of the photon, BE the binding energy of the atomic orbital from which the electron originates and Φ_s is the spectrometer work function. The binding energy can be described as the energy difference between the initial and final states after the photoelectron has left the atom. There is a variety of possible final states of ions from each type of atom which gives the emitted electrons a variety of kinetic energies and binding energies. This can be explained by differences in the chemical potential and polarizability of the atom or compound. Because each element has a unique set of binding energies it is possible to identify and determine the concentration of the elements on the surface. Using these chemical shifts it is possible to identify the chemical state of the material. [32] A brief illustration of how XPS works can be seen in Figure 4.7. The resulting energy spectra, called survey spectra, exhibit resonance peaks that are characteristic of the electronic structure for atoms at the surface of the sample.

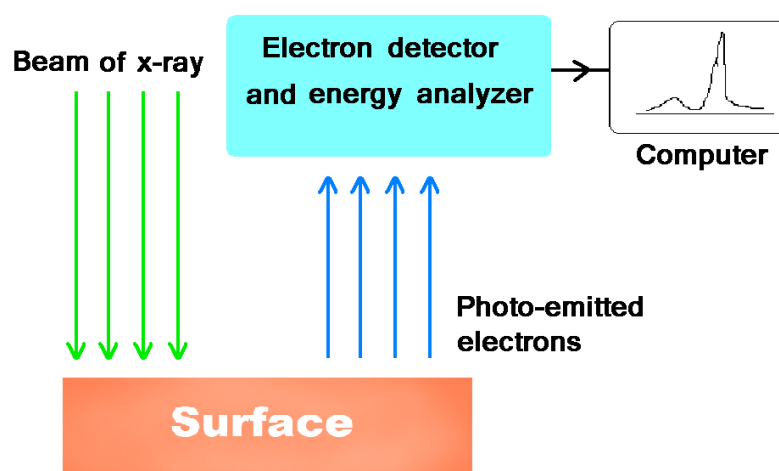


Figure 4.7 - A brief illustration of how the XPS is working.

5 Materials and methods

The materials and methods used in this project are described in more detail below. There were four materials studied, machined titanium, TiO_2 , TiUnite and zirconium and all materials were shaped as discs.

5.1 Surface materials

5.1.1 Machined Ti discs

Nobel Biocare cut a titanium stick with a lathe into thin discs that here are called Ti machined. The surface was shiny but had circle marks/tracks from the lathe and only covered with native TiO_2 . The surface has a smooth appearance with ordered grooves and ridges [34]. The roughness (Ra) is of $0.3\ \mu$, measured by light interferometry [35].

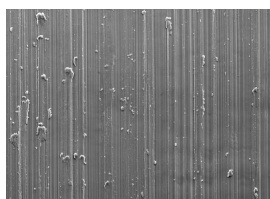


Figure 5.1 - SEM image of a machined Ti surface, approximately μm scale. Courtesy of Nobel Biocare.

The implants that were used from the beginning by Nobel Biocare were made of machined titanium. Every new material that is discovered is compared to machined titanium to see if it is better otherwise it is not worth to use. Hence the machine discs are used as a reference to all other measurements.

5.1.2 TiO_2 discs

Machined titanium discs was sent to an external company where they were coated with nano TiO_2 . From the beginning it is the same production process as for machined surfaces and after that the discs are coated with a suspension of titanium dioxide followed by heat treatment. The surface structure is rough with pores in nm range. The TiO_2 coating consists of mostly anatase, a crystalline form of Ti. [34].

5.1.3 TiUnite discs

TiUnite discs consist of mainly O, Ti, C and phosphorus. The coating are crystalline clusters of anatase, embedded in amorphous TiO_2 . TiUnite is

Nobel Biocares own specific coating which gives a gray, matte and porous surface on the disc. The porous texture has open pores in μm scale.

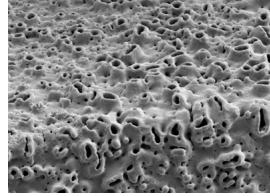


Figure 5.2 - SEM image of a TiUnites porous surface, approximately μm scale. Courtesy of Nobel Biocare.

5.1.4 Zirconia discs

Zirconia discs are in the same size as the other discs, 15 x 1.7 mm. The surface is white, rough and matte. More about the material Zirconia in section 3.2.4.

5.1.5 Polished machined Ti discs

The polished discs are the original machined Ti discs in section 5.1.1, with the same dimensions (15 x 1.7 mm) but the top surface is polished by hand with abrasive papers of different fineness to make the machined Ti surface a bit smoother. More about the polished discs in section 5.4.1.

An overview of all discs and their dimensions can be seen in Table 5.1.

Table 5.1 - Dimensions of the used discs.

Material	Diameter, D [mm]	Thickness, d [mm]
TiUnite	15	1.7
TiUnite small	6	1.7
TiO ₂	15	1
TiO ₂ small	6	1.7
Machined Ti	15	1.7
Machined Ti small	6	1.7
Polished Machined Ti	6	1.7

5.2 Cleaning of surfaces

The process used is ultrasonic baths with different steps. It is developed to make the discs as clean as possible and in a shape as good as when they are new. A large box is filled with tap water. Inside the box there is a row with three beakers filled with 1 l water⁵ and detergent. The first bowl has 10 μ l of detergent and the rest is water. The ultrasonic cleaner is heated up to $\geq 60^\circ$ and the temperature is verified with a thermometer. When the temperature is reached the discs are placed in a samplerack that are immersed in the first beaker for 10 minutes. In order to ensure effective cleaning it is important to separate the discs from each other, no overlapping. When 10 minutes has passed the disc rack are moved to the second and third beaker for 10 minutes each.

When the discs are cleaned in the ultra sound bath they are gently removed from the disc rack and placed in a pre-cleaned glass vial, containing 70% ethanol solution, and the glass vial are closed with a top cover. The vial is labeled with content and date. [36]

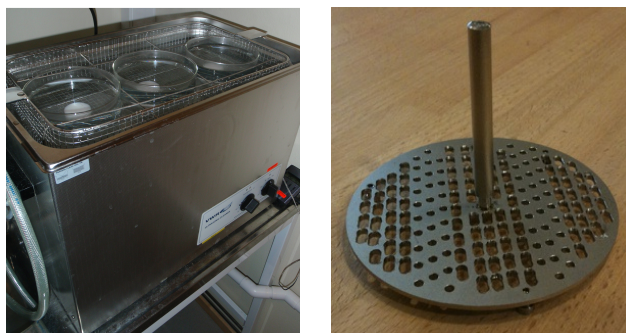


Figure 5.3 - Devices used to wash the discs. To the left the ultra sound washer with a length of approximately 70 cm, and to the right a discrack with a size of approximately 10 cm in diameter.

5.3 Storage

In all the experiments; hydrophilicity, photocatalytic effect and chemical composition, the discs of different materials were stored in different envi-

⁵Purified and deionized water, cleaned by a purification system manufactured by Millipore Corporation. If nothing else is mentioned all in the project used water is purified and deionized.

ronments for different times. There were three different storage media; air, plastic and ethanol, and three storage times 0 h, 48 h and 168 h were used. The discs stored in air were placed on a metallic plate that stood in a laboratory, without any cover, at room temperature and normal laboratory air exchange. Discs stored in plastic were placed in plastic bags, Grippie LDPE polyester, with ziplock. When the bag were closed, the extra air inside were squeezed out by hand. And discs stored in ethanol were placed in clear glass vials pre cleaned to EPA level 2, polypropylene cap with 0.030" PTEE liner, and filled with 70% ethanol.

Before storage the discs were cleaned at Nobel Biocare, described in section 5.2. After cleaning and transport to the storage place at Chalmers, the discs were washed out with water, dried with N_2 and placed in the storage environment. One exception, the discs that were stored in ethanol, they were never dried, they stayed in the glass vial after the cleaning and when they were studied they were washed out and dried. The stored discs were placed in a room with room temperature.

5.4 Contact angle

The contact angle experiments were divided into 3 parts, CA1, CA2 and CA3. The differences between the experiments were how the discs were treated before or during the storage. In all cases the discs were first cleaned, see section 5.2 and transported in glass vials with 70% EtOH from Nobel Biocare to Chalmers. Before all measurements started all discs were taken out of the EtOH, washed in water and dried with N_2 . All contact angle measurements, on all discs, were measured by the Drop Shape Analysis device, see section 4.2. The storage process is the one described in section 5.3. All discs used in CA1, CA2 and CA3 had the same dimension, 15 mm in diameter times 1.7 mm in thickness.

In the case of CA1 the discs were stored for 3 different time intervals before measuring the CA. In CA2 the discs were first stored for 168 h and then exposed to UV radiation for four different time intervals. And in case CA3 the discs were first exposed to UV for 24 h, after that they were stored for same time intervals as in case CA1.

5.4.1 Contact angle - storage, CA1

During the CA1 experiment the surface wettability was studied for three different time intervals, 0, 48 and 168 hours. When the discs were dried they

were divided into groups of how long they were supposed to be stored and in what medium. Except for the discs that were stored for 0 h, they were measured directly after they were dried.

In CA1 there were four different discs; machined, TiUnite, TiO₂ and polished machined, stored in three different mediums; air, EtOH, and plastic, with exception to the polished machined discs. There were only 6 polished machined discs. These were used to compare the influence of surface shape. The machined Ti discs have circular patterns from the lathe and by polishing the surface with abrasive paper the patterns will be irregular and totally random. This might affect the contact angle compared to when the patterns are circular. The 6 discs were divided into two time groups, three that were measured after 0 h and three measured after 168 h. The discs were first cleaned according to the cleaning process in section 5.2 and then stored according to the storage process in section 5.3. All discs were stored in groups together with them of same material/coating and that should be measured on the same time to avoid being affected. In Table 5.2 a summary of all used discs can be found.

Table 5.2 - Table of the discs in the experiment CA1.

CA1	Air			EtOH			Plastic		
	0h	48h	168h	0	48h	168h	0h	48h	168h
TiO ₂	3	3	3	3	3	3	3	3	3
TiU	3	3	3	3	3	3	3	3	3
TiM	3	3	3	3	3	3	3	3	3
TiM _P	0	0	0	3	0	3	0	0	0

When the discs had been stored for decided time one disc at time was placed on the working table of the contact angle device and three drops were dropped and analyzed on the surface of each disc. In the software DSA the baseline was placed on the best position and the contact angle was measured with three different techniques, T-1, CIR and L-Y, which are mentioned in section 4.2. Which of the techniques that makes the best fit is estimated by the human eye, and noted in the CA data collection file.

5.4.2 Contact angle - UV exposure on stored discs, CA2

In the CA1 study it was established that storage in plastic bag gave most effect on the contact angle measurements. Because of lack of time to store discs in all medium the remaining tests were limited to storage of discs in

plastic bags only. In CA2 the discs of three different materials, machined, TiUnite and TiO₂, were first cleaned according to the cleaning process in section 5.2 and stored in different plastic bags for 168 h. A summary of numbers of discs for CA2 can be seen in Table 5.3. After storage the discs were exposed to UV radiation from a LED UV lamp, at 365 nm, in the UV exposure device in section 4.2 for 10 minutes, 1, 6 and 24 hours. The UV radiation lamp was placed 3 cm above the discs and had an intensity of 10700 $\mu W/cm^2$. After the discs were irradiated the contact angle was measured with the DSA device.

Table 5.3 - Table of the discs in the experiment CA2.

CA2	Plastic			
	10min	1h	6h	24
TiO ₂	3	3	3	3
TiU	3	3	3	3
TiM	3	3	3	3

5.4.3 Contact angle - UV exposure before storing discs, CA3

In the case of CA3 three different discs; machined, TiUntite and TiO₂, were exposed to UV radiation from a LED UV lamp, at 365 nm, for 24 hours. As in the case of CA2, the irradiation source was placed 3 cm from the sample with an intensity of 10700 $\mu W/cm^2$. After irradiation the discs were stored for three different time intervals, 0, 48 and 168 hours in three different plastic bags, one for each time to measure. How many discs that were used are summarized in Table 5.4. After specific storage time the contact angle were measured with the DSA device.

Table 5.4 - Table of the amount used discs in the experiment CA3.

CA3	Plastic		
	1h	6h	24
TiO ₂	3	3	3
TiU	3	3	3
TiM	3	3	3

A short summary of all experiments can be seen in Table 5.5.

Table 5.5 - Descriptions of the three contact angle experiments, CA1, CA2 and CA3. A more detailed description can be seen in section 5.4.1 - 5.4.3

Experiment	Description
CA1	Discs stored for 0, 48 and 168h
CA2	Discs stored for 168h, than UV exposed for 10 min, 1, 6 and 24h
CA3	Discs UV exposed for 24h and stored for 0, 48 and 168h

5.5 Methylene blue - photocatalytic effect

Cleaned discs, see section 5.2, were placed in 24-well cell culture plate⁶ and soaked in 0.05% Methylene blue⁷ for 24 hours prior the experiment. This was done to eliminate reduction of methylene blue in the final assay due to absorption by the discs surfaces. After 24 hours of soaking the methylene blue solution was removed, wells and discs were thereafter gently washed with water. Discs were put in the wells and covered with 400 μl of 10 ppm methylene blue. The 12-well plate was then covered with a UV transparent plastic film to avoid evaporation during the experiment.

Three wells with discs and one well with only methylene blue solution were illuminated with UV radiation for 24 hours while an equal setup was covered from UV exposure by aluminum foil in order to work as negative controls. 300 μl samples were taken at four time points: 0, 1, 6 and 24 hours, the optical density (OD) at 665 nm was measured (Bio-Rad, iMark microplate reader model 680) and the samples were thereafter put back in their original wells. Each surface was run at three replicates.

In Equation 5.1 the absorbance, A , also called optical density, OD, is defined. I is the transmitted light intensity, which is the intensity of the light with the wavelength λ that passes through the sample. I_0 is the intensity of the light before it enters the sample. The higher optical density the harder it is for the light to pass through the sample, the light is absorbed. When measuring the OD of the methylene blue a high value corresponds to a small degradation of methylene blue. A lower value indicates more degradation and more photocatalytic effect.

$$A_\lambda = \log_{10}(I_0/I) \quad (5.1)$$

⁶Costar 24-well flat bottom polystyrene #3526, well diameter 15.6 mm

⁷Sigma-Aldrich #319112

UV light sources tested were Blak-Ray 365 nm, 380 nm LED, 365 nm LED and 255 nm LED. UV radiation intensity and transparency of plastic cover film was measured before start of experiment with the UV-radiation device in section 4.1. The distance from the Blak-Ray lamp to the discs were 15.5 cm and for the LED 4 cm.

5.6 X-ray photoelectron spectroscopy - Hydrocarbons vs storage and UV exposure

The XPS experiment was divided into three parts where the first part consisted of studying cleaned discs, the second of studying discs stored for 168h and the third of studying UV exposed discs. All discs used in this study were cleaned by the cleaning process in section 5.2. In the first measurement discs of dimension 15 mm x 1.7 mm were used, and because of the size there was only room for three discs at the same time. The samples were fixed on the samplerack that was placed in the pressure chamber. Using the large discs (15x1.7 mm) they were fixed in a temporary way that made the surfaces not totally horizontal. During the following two studies smaller discs were used, with dimension 6 mm x 1.7 mm. These discs were small enough to fix nine on one samplerack special made for this test, see Figure 5.4.



Figure 5.4 - A specific rack for the XPS study where the discs are placed on. The diameter of one disc is 6 mm and of the rack around 30 mm.

Three measurements were done. The first one on new cleaned large discs (15 x 1.7 mm), the second one on small discs (6 x 1.7 mm) stored for 168 hours and the third one on small discs (6 x 1.7 mm) that first were stored and then exposed to UV radiation from a LED UV lamp, at 365 nm, for 24 hours. The irradiation source was placed 3 cm from the samples with an intensity of $10700 \mu\text{W}/\text{cm}^2$. The discs were cleaned according to the process in section 5.2 and transported from there to Chalmers in a vial with 70% EtOH, then washed with water and dried with N_2 before the first test was made. Discs were placed on the samplerack, placed in the pressure chamber and measurements on the samples were taken. Two test-points were made on each disc.

On the TiO₂ discs one of the test-points was taken in the middle and the other one close to the edge. The second test was made after the discs were first cleaned and stored for 168 h and then exposed to UV radiation for 24 h. Same as in test one, two test points were made on each disc. The result of the measurements are summarized in survey spectrum, which is a plot over counts/second, c/s, vs the binding energy. In the spectrum it is possible to analyze which elements that can be found on the surface.

6 Results

All results are presented under each category headline, *Contact angle*, *Methylene blue* and *XPS*. Standard deviations are inserted as error bars in each diagram. All diagrams in sections 6.1 and 6.2 have help lines drawn between the data points as a guide for the eye.

6.1 Hydrophilicity (measured by contact angle)

The contact angle was measured with a drop shape analysis device where the angle between the surface and a water droplet was measured. Three different studies have been done called CA1, CA2 and CA3. They will be described below each headline. All contact angles have been measured with three different methods, T-1, CIR and L-Y to be able to compare the results and choose the best method. More about the methods, see section 4.2. When the method has placed the baseline, suggested a curve fit and measured the contact angle, it is only the human eye that can tell which of the methods that did the best fit.

As been mentioned before there were three measurements on three drops on each discs. It was not all results that seemed to follow the trend or that gave any result at all. So from the three results on each disc a mean was calculated. There was one exception, the TiO_2 coated discs, where the coating was assumed to not be totally homogeneous. The only contact angles that were comparable to other discs were the value of the drop in the middle, drop 2. This does not give a mean for each TiO_2 disc, but one value on each disc.

6.1.1 Cleaned and stored discs (CA1)

In CA1 the contact angle was studied for four different surfaces, TiO_2 , TiU-nite, machined and polished machined discs, that were stored during three different times, 0h, 48h and 168h. The discs were stored in three different media; air, ethanol and in a plastic bag. After each storage time the contact angle was measured. The results are illustrated in the graphs below where Figure 6.1 shows the change of contact angle during storage time in air. Figure 6.2 shows the results of the change of contact angle due to storage time in ethanol and Figure 6.3 the results of discs stored in a plastic bag. As can be seen the polished machined disc are only studied in the case of storage in ethanol, that is because of the limited number of discs.

TiUnite does not show any difference in CA, except for a small change when the discs had been stored in a plastic bag, CA1. Machine and TiO₂ discs stored in air are quite similar. The CA increases with time from 10-15° to around 40°. Discs stored in EtOH are according to the results cleaner after 168h storage than new cleaned discs. The contact angle changes from 10-15° to 20-25° after 48h storage and after 168h the contact angle decreases to 0-8°. Discs stored in plastic bags show an increase in the contact angle for both machined and TiO₂ discs from 10-15° to 60-70°. Even the CA for TiUnite discs increases very little.

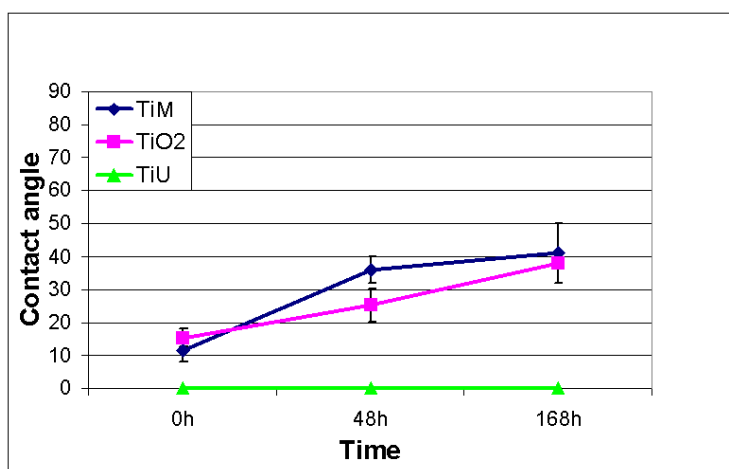


Figure 6.1 - Comparison of contact angles for discs stored in air for different times.

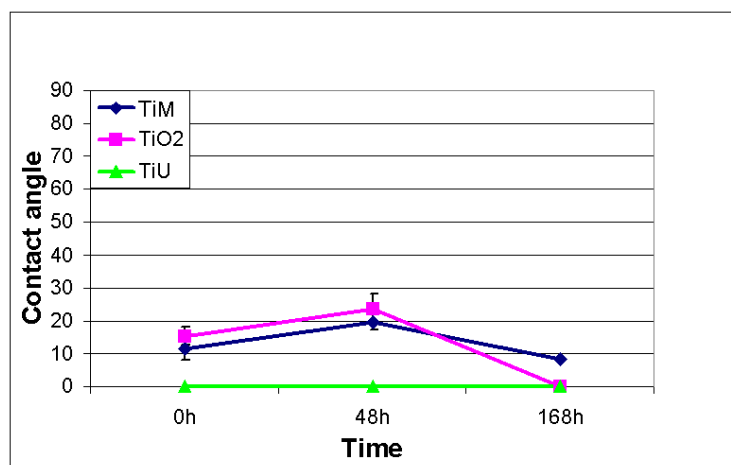


Figure 6.2 - Comparison of contact angles for discs stored in EtOH for different times.

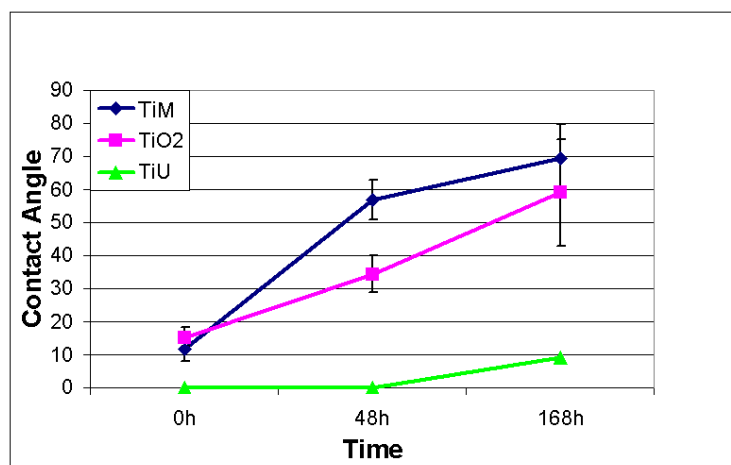


Figure 6.3 - Comparison of contact angles for discs stored in plastic for different times.

Comparing the machined discs it can be established that storage in the plastic bag causes the largest contact angle. Air increases it rather much too but EtOH decrease the angle to a smaller angle than from the beginning. Comparing the TiO_2 discs the result is similar as for machined discs, except that the CA does not increase that much for TiO_2 .

In Figure 6.4 the results of only M discs are illustrated, during the storage time 0-168h, in the different medium; air, EtOH and plastic bag, from the experiment CA1. It can be seen that storage in plastic bag has the largest negative affect on the contact angle, which increases from around 10° to 70° . Storage in air also results in a larger contact angle. After 168h it is around 40° while storage in EtOH decreases the contact angle to $< 10^\circ$, smaller than from the beginning.

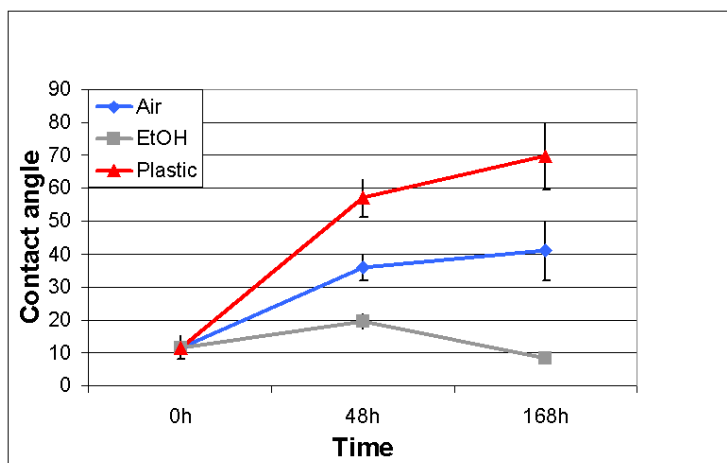


Figure 6.4 - Comparison of contact angles on M discs stored in different mediums.

In Figure 6.5 the results of only TiO_2 discs are illustrated, during the storage time 0-168h, in the different medium; air, EtOH and plastic bag from the experiment CA1. After 48h the contact angle is similar for all storage medium. Compared to the case of stored M discs it can be seen that the contact angle for TiO_2 increases most when being stored for 168h in a plastic bag, from around 15° to 60° . Similar to the results for M in EtOH, the contact angle decreases after 168h, down to around 0° .

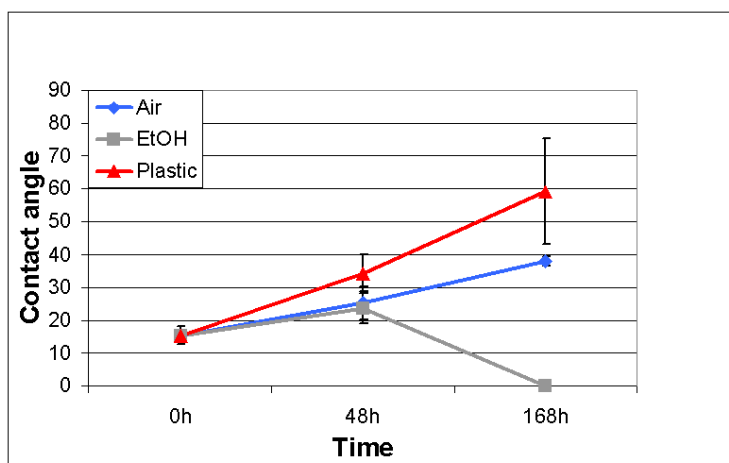


Figure 6.5 - Comparison of contact angles on TiO_2 discs stored in different mediums.

In Figure 6.6 results from the comparison of M discs and M-polished discs are illustrated. When the discs are newly cleaned it can be seen that the M-polished disc has a larger contact angle compared to the M disc. After 168h storage in EtOH instead the contact angle is smaller for the polished disc than for the M, even smaller than the contact angle for newly cleaned M discs.

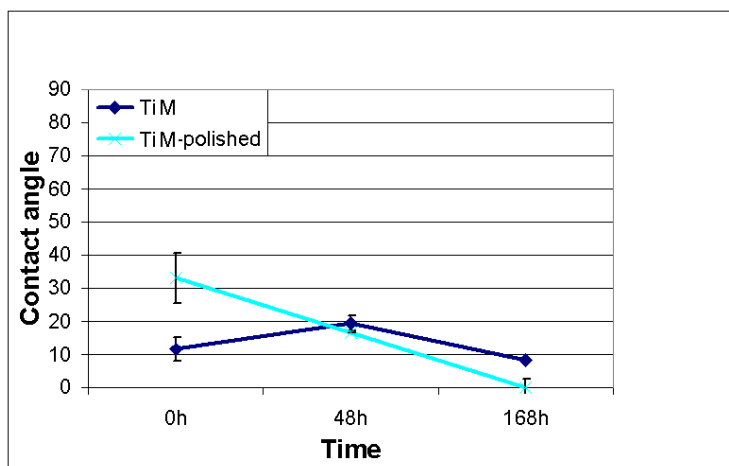


Figure 6.6 - Comparison of contact angles on two different stored M discs, polished and not polished.

6.1.2 Cleaned, stored and UV exposed discs (CA2)

In CA2 the contact angle was studied for three different surfaces, TiO_2 , TiUnite and M discs that first were cleaned and stored in a plastic bag for 168 h and then exposed to UV radiation from a LED UV lamp, with wavelength 365 nm and intensity of $10700 \mu\text{W}/\text{cm}^2$. The three discs were exposed at four different time intervals, 10 min, 1h, 6h and 24h, during four different experiments. Directly after exposure the contact angles were measured and the results were summarized and are illustrated in Figure 6.7.

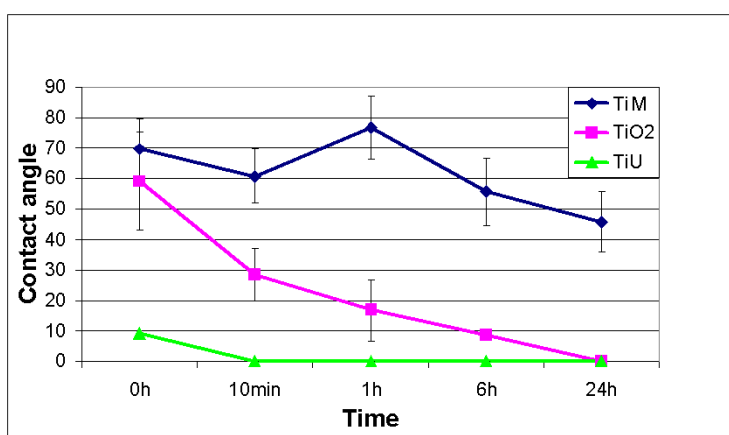


Figure 6.7 - Comparison of contact angles on M, TiO_2 and TiU discs stored for 168h in plastic bags and exposed to UV light for different times.

6.1.3 Cleaned, UV exposed and stored discs (CA3)

In CA3 the contact angle was studied of three different surfaces, TiO_2 , TiUnite and machined discs that first were cleaned and exposed to UV radiation from a LED UV lamp, with wavelength 365 nm and intensity of $10700 \mu\text{W}/\text{cm}^2$. After irradiation the discs were stored in a plastic bag for three different times, 0h, 48h and 168h. After storage the contact angles were measured and the results are illustrated in Figure 6.8.

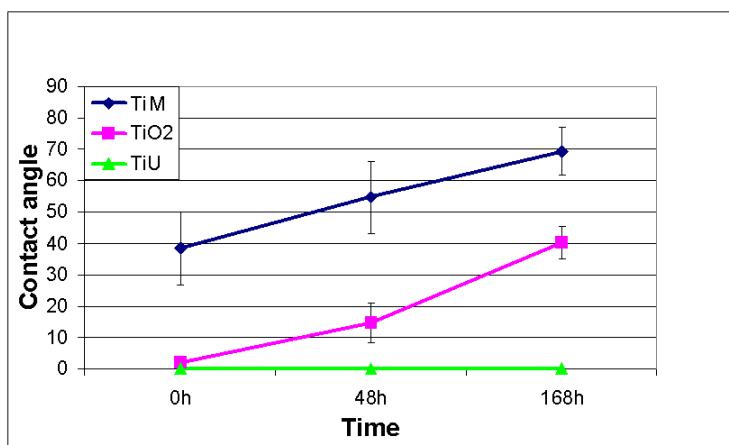


Figure 6.8 - Comparison of contact angles on *M*, TiO_2 and *TiU*, exposed to UV light for different times and then stored for 168h in plastic bags.

6.1.4 Comparison

In the experiment CA1 the discs were cleaned and then, in one case, stored in a plastic for 0, 48 and 168 h, and in CA3 the discs were first cleaned, UV exposed and then stored a plastic bag for the same time interval. By comparing these two experiments it is possible to see if there is any remaining effect of the UV exposure. The results for the machined discs are illustrated in Figure 6.9, and the results for the TiO_2 discs are illustrated in Figure 6.10. In Figure 6.11 the results of all experiments CA1, CA2 and CA3, where the discs have been stored for 168 h. In this figure there are also results from storage in air and ethanol.

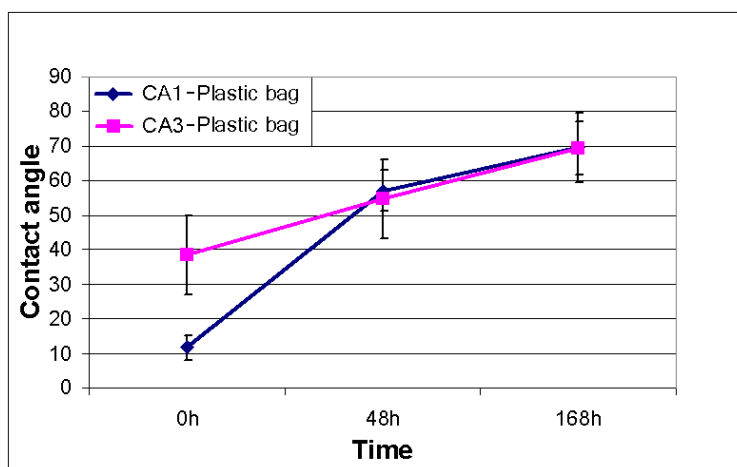


Figure 6.9 - Comparison of results of stored M discs in plastic bags in the experiments CA1 and CA3.

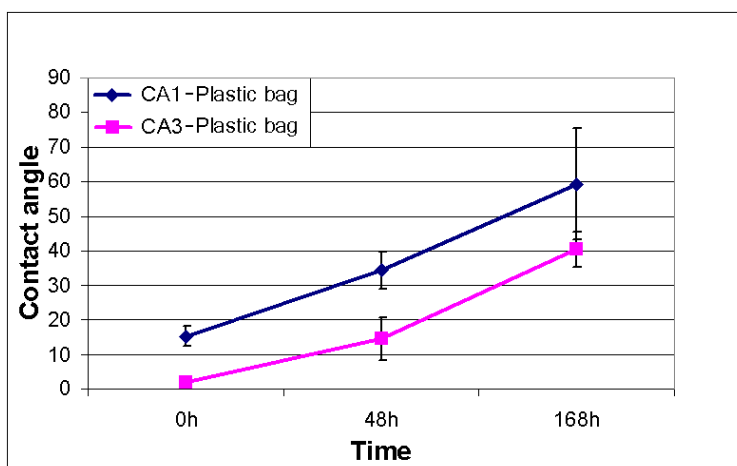


Figure 6.10 - Comparison of results of stored TiO₂ discs in plastic bags in the experiments CA1 and CA3.

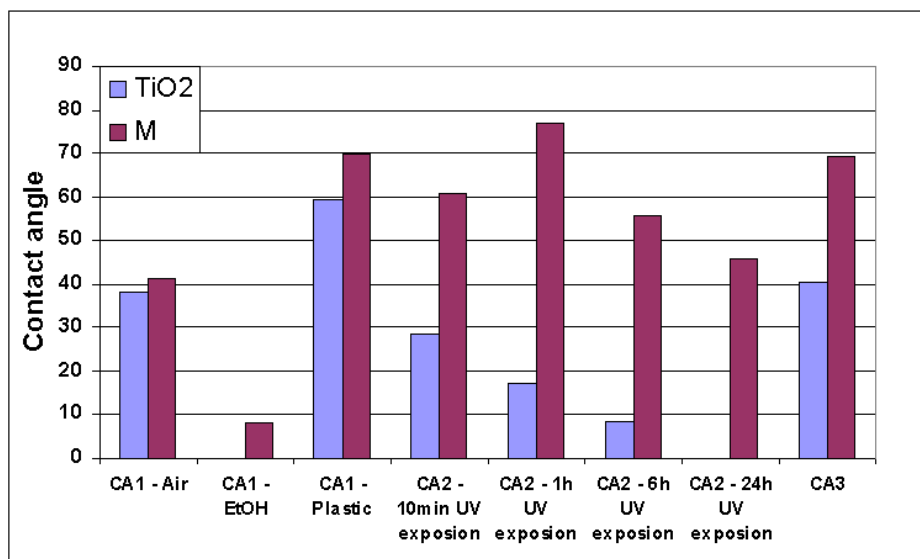


Figure 6.11 - Comparison of results in CA1, CA2 and CA3 after 168h storage and UV exposure time when the experiment concluded.

6.2 Photocatalytic effect (measured by methylene blue assay)

The experiment consists of discs placed in a 24-well cell culture plate covered with a methylene blue solution and soaked for 24 h before they were exposed with different UV sources, black ray radiation and UV radiation from LED lamps with three different wavelengths, 365 nm, 380 nm and 385 nm. Three wells with discs and one well with only methylene blue solution were irradiated for 24 hours while an equal setup of wells was covered by aluminum foil during the UV exposure in order to work as negative controls. 300 μ l samples were taken at four time points: 0, 1, 6 and 24 hours. Figures 6.12 - 6.15 shows the results from the study where the 24-well plate was exposed to UV radiation from 4 different UV lamps; black ray with the wavelength of 365 nm, UV-LED lamps with the wavelength 365 nm, 380 nm and 385 nm. All Figures consists of two images, a) and b). In a) the plate were exposed to UV radiation and in b) the wells were covered with Al foil during the UV radiation time.

6.2.1 Methylene blue assay, using UV - Black Ray 365 nm

In Figure 6.12 a) it can be seen that TiO_2 has the smallest optical density, $\text{OD} \approx 0.2$, compared to the other. The machined and TiUnite discs are also activated at that wavelength and decreases the optical density, $\text{OD} \approx 0.7$ - 0.9 . Zirconia and the control stay rather constant at an OD around 1.6. In b) it can be seen that all materials stay at a rather constant OD around 1.6.

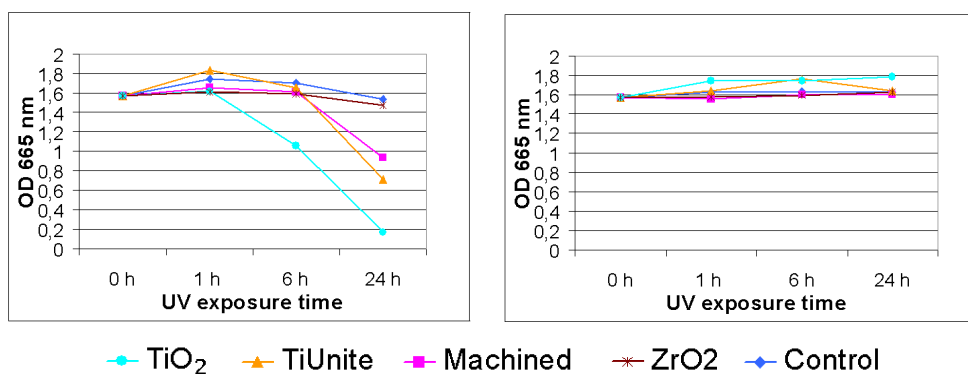


Figure 6.12 - The change in photocatalytic effect measured as optical density on machined titanium, TiUnite, TiO_2 and zirconia discs exposed to 365 nm Black Ray source. To the left the samples were UV exposed, to the right the samples were covered with Al foil.

6.2.2 Methylene blue assay, using UV - LED 365 nm

In Figure 6.13 a) it can be seen that TiO_2 still has a very small optical density, $\text{OD} < 0.1$ but in this case TiUnite shows a similar result and has an $\text{OD} \approx 0.2$. It can also be seen that the OD for all materials has decreased a little. In b) the OD are still constant for all materials.

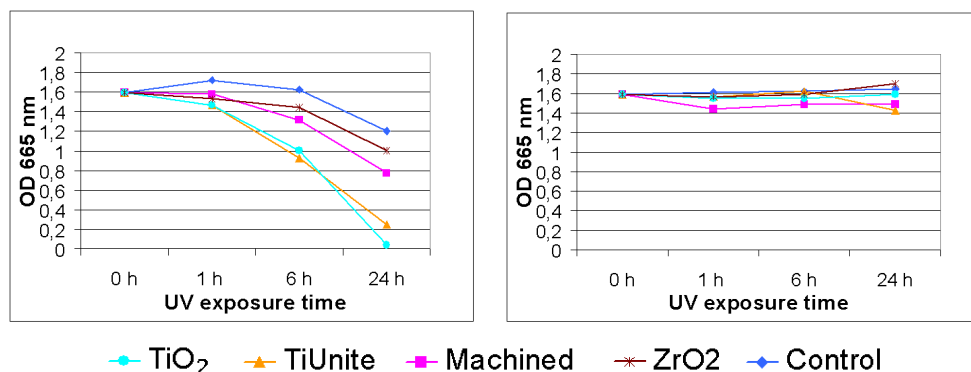


Figure 6.13 - The change in photocatalytic effect measured as optical density on machined titanium, TiUnite, TiO₂ and zirconia discs exposed to 365 nm LED source. To the left the samples were UV exposed, to the right the samples were covered with Al foil.

6.2.3 Methylene blue assay, using UV - LED 380 nm

In Figure 6.14 a) it can be seen that OD for TiO₂ has increased a little and that both TiO₂ and TiUnite now have the same small optical density, OD ≈ 0.2. It can also be seen that all the other materials OD has decreased a bit more compared to the case of 365 nm. In b) the OD for all materials are still rather constant but TiUnite has a small dip after 24h.

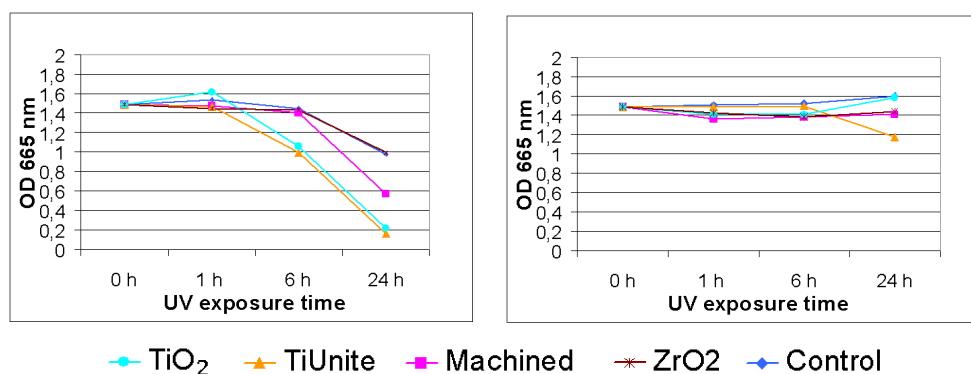


Figure 6.14 - The change in photocatalytic effect measured as optical density on machined titanium, TiUnite, TiO₂ and zirconia discs exposed to 380 nm LED source. To the left the samples were UV exposed, to the right the samples were covered with Al foil.

6.2.4 Methylene blue assay, using UV - LED 385 nm

In Figure 6.15 a) the OD for TiUnite stays around 0.2 but that TiO_2 increases a little bit more meanwhile the other decreases a little more compared to the case of 380 nm. In b) the OD for all materials is still rather constant but TiUnite has a larger dip after 24h.

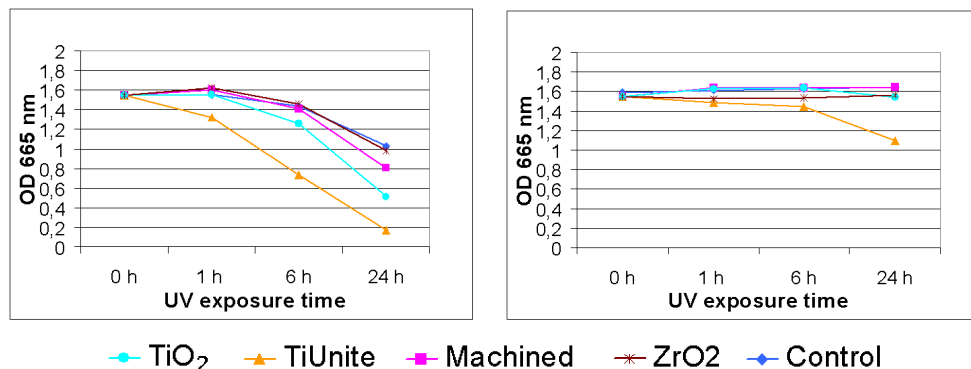


Figure 6.15 - The change in photocatalytic effect measured as optical density on machined titanium, TiUnite, TiO_2 and zirconia discs exposed to 385 nm LED source. To the left the samples were UV exposed, to the right the samples were covered with Al foil.

6.2.5 Comparison

In all results it can be seen that TiO_2 and TiUnite are the two samples that are most effected of the UV radiation. In the first figure, 6.12, TiO_2 shows the highest effect and in the last figure, 6.15, it is TiUnite. Machined and ZrO_2 does not react as much as TiO_2 and TiUnite, but it can be seen that there is a clear effect of changing the wavelength. All controls, the right figure in each pair, does not react at the UV radiation.

6.3 Chemical composition (measured by XPS)

6.3.1 Survey spectra of chemical composition on different disc surfaces

In Figure 6.16 the survey spectra of cleaned machined titanium, TiO_2 and TiUnite discs are illustrated. The three discs have similar amount of carbon, C1s, titanium, $\text{Ti}2p_{3/2}$ and oxygen, OKLL, but comparing other elements there is a difference between the machined titanium disc and the other two, TiO_2 and TiUnite. On the surface of the machined disc there can be found cobalt, Co, fluorine, F, zirconium, Zr, nitrogen, N, and phosphorus, P, in different chemical compositions.

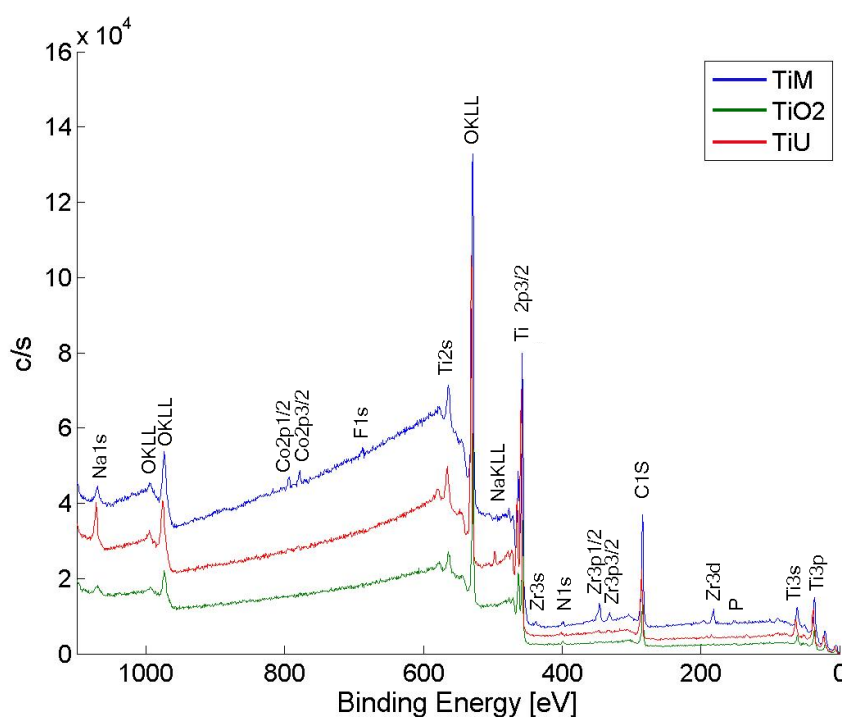


Figure 6.16 - Survey spectra of cleaned machined, TiO_2 and TiUnite discs.

In Table 6.1 all elements that can be found in the survey spectra in Figure 6.16 summarized, and also the elements that can be found in the case of stored and UV exposed discs. It can be seen that the amount of the different elements on the machined titanium disc are similar independent of the treatment with an exception that F and Zr, which are bold and italic in the table, disappear when the disc is stored and does not come back after UV exposure. There are more changes on the TiO_2 and TiUnite disc, particular looking at the

carbon peak that increases after storage and decreases to more or less the starting-point. Because of background and other disturbance it can be hard to exactly tell how much and how the amount change.

Table 6.1 - Table of materials on the different surfaces, machined, TiO_2 and $TiUnite$. The table is built up with marks "S", "W", "vW", + and -. "S" stands for strong, "W" is weak and "vW" is very weak. The "+" sign means that the peak has increased after being stored or UV exposed and "-" that the peak has decreased after being stored or UV exposed.

Elements on the disc surfaces									
Materials	Machined			TiO_2			TiUnite		
	Cle	Sto	Uve	Cle	Sto	Uve	Cle	Sto	Uve
Ti2p3/2	S	S	S	S	S	S+	S	S	S+
Ti2s	W	W	W	W	vW	W+	W	W-	W+
Ti3s	W	W	W	W	vW	vW	W	vW-	vW+
Ti3p	W	W	W	W	vW	W+	W	vW-	vW+
OKLL(545ev)	S	S	S-	S	S	S+	S	S	S
OKLL(1000eV)	vW	vW	vW	vW	vW		vW	vW	vW
OKLL(990eV)	W	W	W	W	W	W+	W	W-	vW+
C1s	S	S	S	W	S+	W-	W	S+	W-
Na1s	W	W	W	vW	vW	vW+	W	W	W+
NaKLL							vW	vW	vW+
Co2p1/2	vW	vW	vW						
Co2p3/2	vW	vW	vW						
F1s	vW								
Zr3s	vW								
Zr3p1/2	W						vW		
Zr3p3/2	vW						vW		
Zr3d	W						vW	W	
N1s	vW	vW	W	vW	vW	vW	vW	vW	vW
P	vW							W	

The survey spectra of stored and UV exposed discs can be seen in Appendix A, 11, Figure 11.1 and 11.2.

The three most interesting elements to study is carbon, titanium and oxygen, because they are in the largest amount on the surface. It is possible to analyze all peaks closer which has been done for the Ti and C peaks. In Figure 6.17 the carbon peaks on the three discs, machined titanium, TiO_2 and $TiUnite$ are plotted. It can be seen that the two carbon peaks for stored TiO_2 differ

a bit from the peaks of cleaned and the UV exposed. The stored machined disc has the highest carbon peak at 288 eV which is double bounded carbon to oxygen. In all the other cases the peaks are similar with the highest peak at 285[eV].

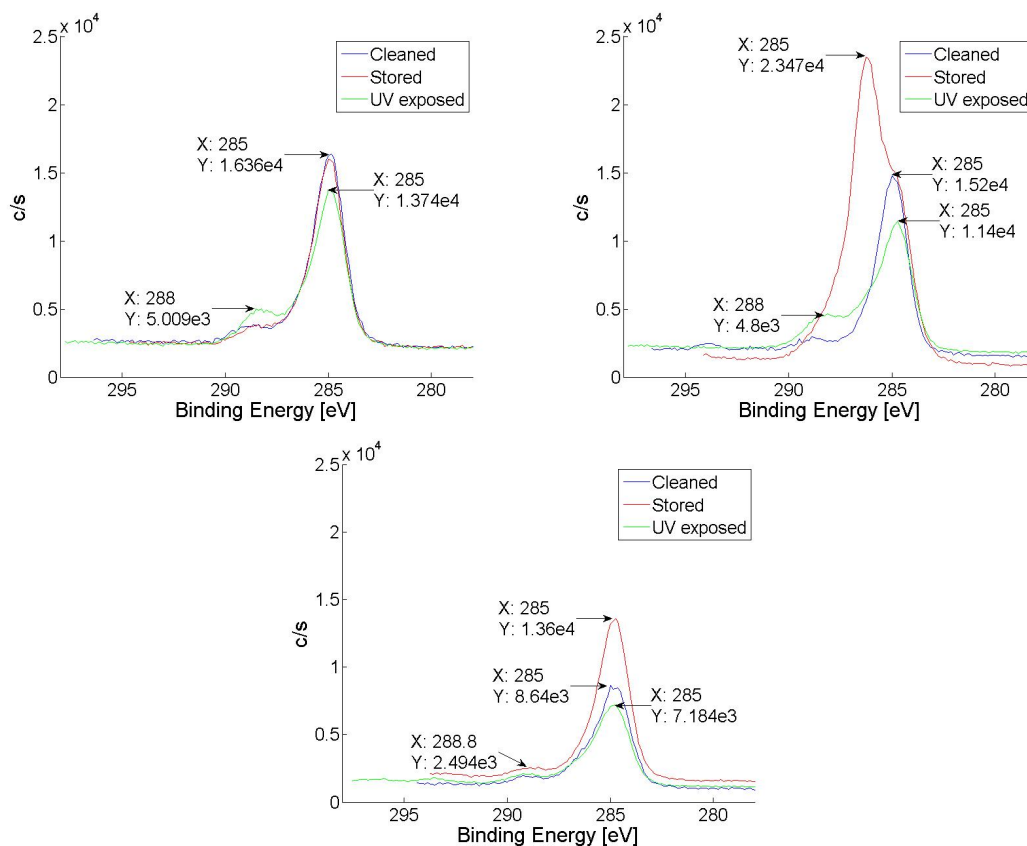


Figure 6.17 - Carbon peak on stored, cleaned and UV exposed discs. Top left the machined titanium disc, top right the TiO_2 disc and middle bottom the TiUnite disc.

Looking at the titanium peaks in Figure 6.18 they are all very similar for all materials, TiO_2 , machined titanium and TiUnite. The highest peak is around 454[eV] and the smallest peak at 459.9[eV].

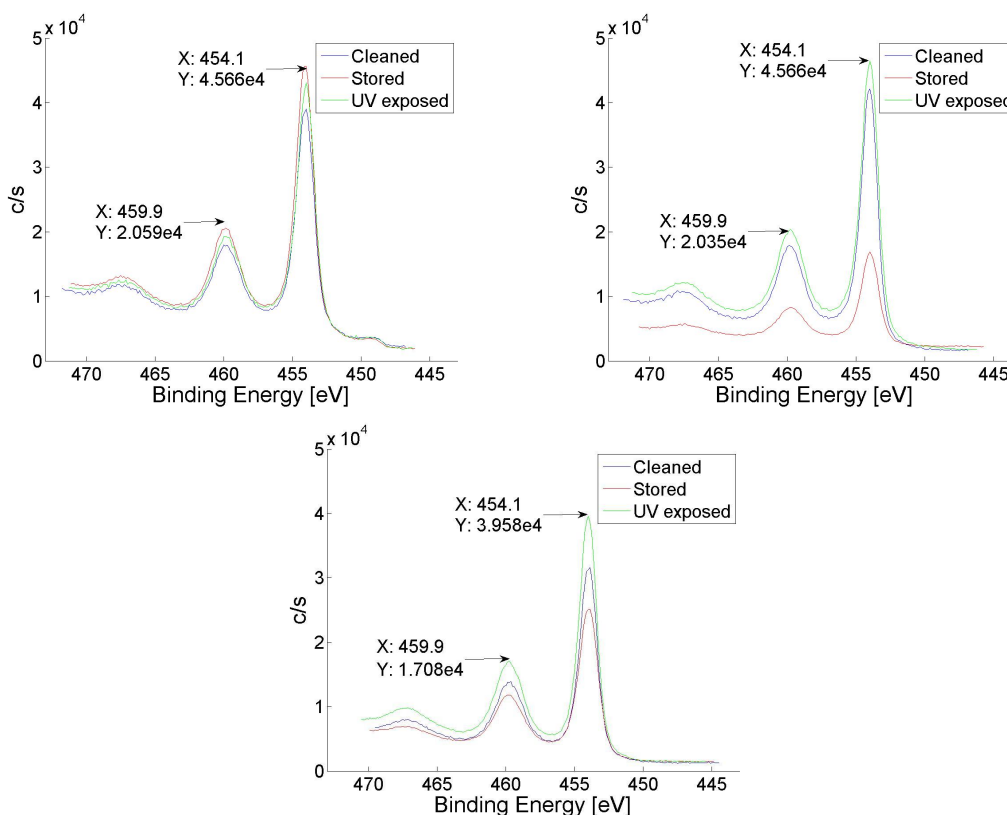


Figure 6.18 - Titanium peak on stored, cleaned and UV exposed discs. Top left the machined titanium disc, top right the TiO_2 disc and middle bottom the TiUnite disc.

6.3.2 Atomic-% of elements on different disc surfaces

In Figures 6.19, 6.20 and 6.21 the surface composition of C, O and Ti are illustrated in a graph showing atomic % versus different scenarios; cleaned discs, stored discs and cleaned and UV exposed discs.

The Figure 6.19 illustrates the surface composition of machined discs, TiM, where there is most C (~ 45 atomic%) and least Ti (~ 20 atomic%). The amount of the elements (C, O and Ti) in the different cases are quite the same for the all scenarios (cleaned, stored and UV exposed). Figure 6.20 illustrates the surface composition of TiUnite discs, TiU, where the C amount has increase a bit compared to TiM, and both O and Ti has decreased some %. In Figure 6.21 the surface composition of TiO_2 discs, TiO, is illustrated. For the case of stored discs the amount of C and O has change place compared

to TiM and TiU, else they have the similar amount. In Figure 6.20 it can be seen that when the TiUnit disc is exposed to UV radiation the carbon amount decreases on the surface. This can be seen in Figure 6.21 too, where the Ti surface become more visible when the carbons decreases, which increases the amount of Ti. It was also shown that amount of O increased on the two surfaces TiUnite and TiO₂, when they were exposed.

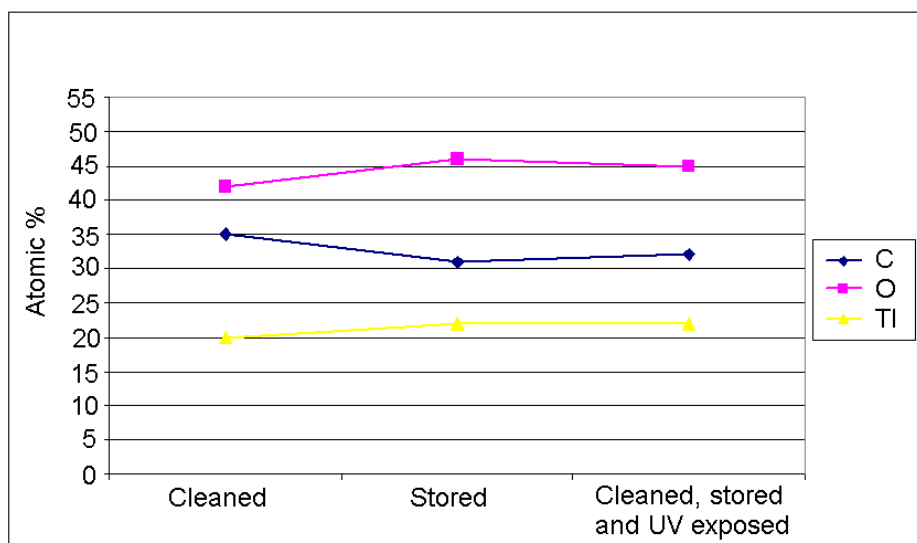


Figure 6.19 - XPS results of amount atomic % of C, O and Ti on the surface of M discs.

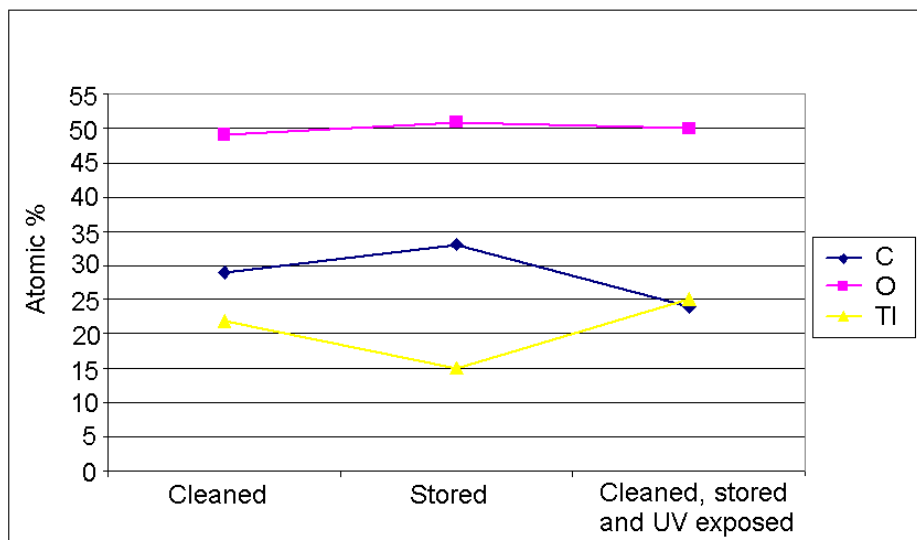


Figure 6.20 - XPS results of amount atomic % of C, O and Ti on the surface of TiU discs.

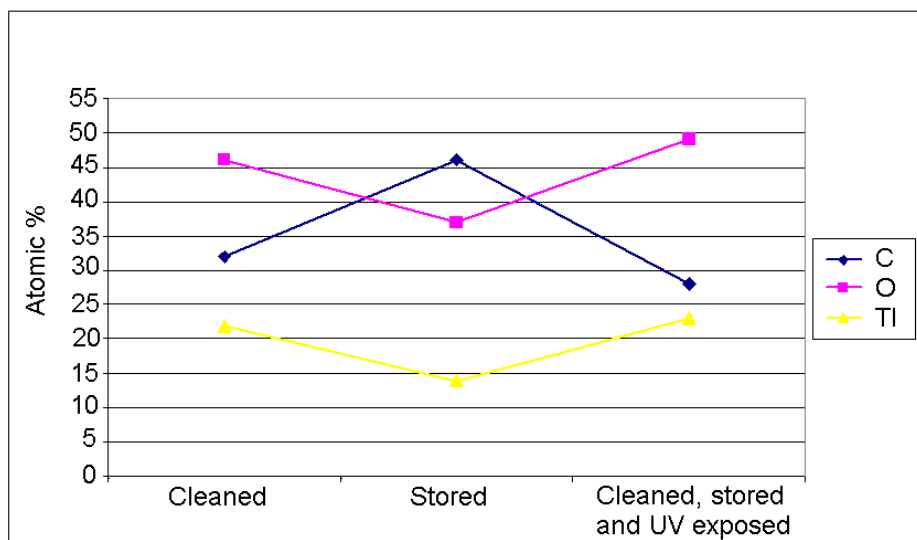


Figure 6.21 - XPS results of amount atomic % of C, O and Ti on the surface of TiO discs.

6.3.3 Comparison

The chemical composition on the three materials are similar in all cases. For the machined titanium disc there are very small changes when the disc is stored and UV exposed. Zr and F are the elements that disappear, the other ones are similar the whole time. There are larger differences when looking at the TiO₂ and TiUnite discs. After storage the carbon peak increases a lot on both discs and after UV exposure the peaks decreases again.

When studying the atomic % it can be seen that for TiM there is no noticeable change but for TiU and TiO the carbon top increases after storage and decreases again after UV exposure.

7 Discussion

7.1 Hydrophilicity (measured by contact angle)

The contact angle study was used to measure how clean the surfaces are after being stored in different medium for different times. By measuring the CA between the surface and a water drop the angel becomes a measurement of how dirty the surface is since atoms on the surface will affect the contact angle. As can be read in many articles, for example [5], it is possible to make the surface cleaner by exposing it to UV radiation of a specific wavelength. In all CA experiments, CA1 (cleaned and stored), CA2 (cleaned, stored and UV exposed) and CA3 (cleaned, UV exposed and stored), the goal is to first see how dirty a disc becomes when it is stored, CA1, and then try to reach a “cleaner” one by illuminating the disc with UV radiation, CA2 and CA3.

Errors that can affect the results could be that the contact angle measurement does not give a correct water volume of the drop or it measures with the wrong method for that case. In this experiment it is assumed that the surfaces and the distribution of dirt are homogenous but in reality this is obviously not the case. This can be seen in the results of the method T-1, which gives two different angels on the right and the left side of the drop. As the group Takeuchi et al [6] concludes, the contact angle will change with the room temperature. The temperature can also affect the volume of the drop. If it is too warm the drop can evaporate faster and the measurement will be incorrect. There are a lot of cases when the drop shape contributes to errors in the measurements, some cases are illustrated in Figure 7.1. In a) the drop is very small which can make it hard for the program to measure. In b) the drop shape is deformed and it floats out irregular, not in a circular. In c) the drop is placed too close to the edge which can restrict the drop to stretch out. In d) two drops are placed too close to each other which can give the same effect as in case c), the drop is restricted. In e) there is dirt on the surface which also can restrict the drop. In f) the drop probably have been restricted somehow, because the angles to the right and left are not similar. During all contact angle experiments there were three methods used; T-1, CIR and L-Y. All three methods measured the contact angle on each drop and the laboratory assistant verified each time which of the methods that did the best fit. By comparing the results L-Y was deleted because of very few god fits. T-1 was used most frequently because measuring two different angles and had most good fits. When T-1 did not give a good fit CIR was used instead.

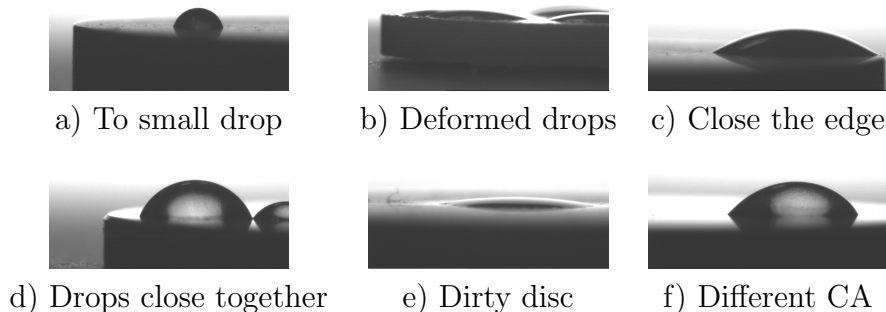


Figure 7.1 - *Hydrophilic and hydrophobic surfaces illustrated by water drops.*

7.1.1 Cleaned and stored discs (CA1)

Comparing all results of the contact angle experiment 1, the trend is that the discs become less clean during the storage time with one exception, TiUnite. In the results of all contact angle experiments, TiUnite discs do not show any large increases in contact angle compared to the other materials. There may be several reasons for this trend and it will be discussed further later on. All the other discs had increasing contact angles the longer time they were stored.

As can be seen in Figure 6.1, where the discs are stored in air for 0, 48 and 168 hours, the results of stored machined and TiO₂ discs are similar. Both materials give rise to contact angles that increase with time and end up at similar values. Looking at Figure 6.3 the results for machined and TiO₂ are similar to but the CA is larger for plastic storage than in air. This can be related to the cleanliness of the surfaces. By storing the material in a plastic bag it will be less clean than if it is stored in air. This can depend on many factors, for example if the room the discs were stored in had very good air conditioning that keeps the small dirt particles away. The plastic bags on the other hand may release hydrocarbons which also affects the CA. Even the TiUnite disc shows a small change in CA when it is stored for 168 h in a plastic bag.

The results in Figure 6.2, where the discs are stored in ethanol, are very interesting because they do not behave as expected. In this graph three materials are compared, TiUnite, M and TiO₂. As can be seen all materials, except TiUnite, starts at a contact angle $>10^\circ$. All materials change after 48 h, from around 20° to $< 10^\circ$, which means the discs are cleaner after 168 h storage in EtOH compared to when they were newly cleaned. One reason

why this happens can be that the cleaning process not is enough; there is still dirt on the surface that let go during storage in EtOH.

In both Figure 6.1 and Figure 6.2 the M and TiO₂ discs follow each other in change of contact angle and as can be seen the error bars cross each other in almost all points. This indicates that it is not correct to conclude any of the materials to be better than the other. One exception is storage in air, after 48h, where the error bars do not cross each other. There the TiO₂ has a smaller contact angle. In the case of storage in EtOH the error bars crosses each other in both points, 0h and 168h, but not after 48h storage. Here the TiO₂ has a larger margin to be better than in storage in EtOH and air.

In Figure 6.4 and Figure 6.5 it can be seen that storage in plastic results in the largest contact angles, and that storage in EtOH results in the smallest contact angles after 168h. It can also be seen that in both figures the error bars increases with the contact angle.

In Figure 6.6 a comparison of the surfaces of M discs and polished M discs can be seen. One reason why the polished M disc starts at a very high contact angle can depend on that particles from the polishing stay on the surface during the cleaning process and they let go when it is stored in EtOH. After 168h storage in EtOH the polished M disc is cleaner than the M disc. This can be explained by the reason mentioned in section 5.4.1, that the circular patterns from the lathe have been blurred and the drop is not restricted to float out on the polished M compared to the M disc.

Comparing all storage medium it can be seen in all figures that storage in plastics bag give the discs the largest contact angle, hence become less hydrophilic. Storing discs in air did not give rise to small contact angles either but storage in EtOH can make the discs even cleaner than when the discs are newly cleaned according to the contact angle results.

7.1.2 Cleaned, stored and UV exposed discs (CA2)

As can be seen in Figure 6.7 the trend shows that the contact angle decreases after exposure to UV radiation. There is one exception, the M disc after 1h UV exposure, where the contact angle increases. This can be an error in the measurement because all other results tend to decrease. The error bars are rather large for all contact angles larger than 10° but they only cross each other in the start point of M and TiO₂. It can be concluded that it is possible to decrease the contact angle with exposure of UV radiation and that it has

a larger effect on the TiO_2 discs compared to the M discs.

7.1.3 Cleaned, UV exposed and stored discs (CA3)

In Figure 6.8 it can be seen that the contact angle tends to increase with storage time. As can be seen in the comparison of M in Figure 6.9 the contact angle is smaller in the experiment CA1, where the discs are stored, compared to in experiment CA3, where the discs are exposed to UV radiation for 24h before storage. This can be related to that the M disc does not have a surface as reactive as the TiO_2 which may have affected the M discs differently during the exposure time, it became dirtier, as in the case of storage in air. TiO_2 on the other hand, in Figure 6.10, that has the photocatalytic effect, has a smaller contact angle after being irradiated for 24h before storage in CA3 compared to when the discs were stored in CA1. It can be concluded that it has a small effect to expose the discs before storage, but it does not seem to be a lasting effect. In this figure it can also be seen that the error bars increase with increased contact angle.

7.1.4 Comparison

In Figure 6.9 and 6.10 the results of stored machined and TiO_2 discs in experiment CA1 and CA3 are compared. The machined discs, that do not react in the same manner as TiO_2 when being exposed to UV radiation, are ending in the same contact angle of 70° . It can also be seen that in CA1 and CA3 the machined discs do not have the same contact angle at the start, which means that the UV exposure probably has affected the surface. Looking at TiO_2 instead, the results from CA1 and CA3 follows each other except a shift of $\sim 10^\circ$. In this graph it can also be seen that TiO_2 starts at smaller angle in the case of UV irradiation before storage than storage directly after cleaning.

As been mentioned in all graphs TiUnite disc does not give any changes in contact angle, with one exception in CA1, after 168 h storage in a plastic bag, but apart from that there is no difference. Possible explanations for that can be the porous surface that can adsorb parts of the water drop applied on the surface and because of that the drop floats out. The surface of TiUnite is a bit rough, which can obstruct the possibility to measure the contact angle on existing drops. In section 3.2.3 it is mentioned that TiUnite consists of both rutile and anatase which can be a reason why the discs may not react as much to UV irradiation.

7.2 Photocatalytic effect (measured by methylene blue)

As can be seen in all figures from Figure 6.12 - 6.15, discs made of M, TiO₂ and TiUnite have a photocatalytic effect in all cases using different lamps and wavelength. In Figure 6.12 the TiO₂ surface shows the largest effect which can be expected since TiO₂ consists of most anatase, which is easiest activated at a wavelength around 365 nm. TiUnite consist of mostly rutile, but also anatase, and show an activation but not as high as TiO₂. The photocatalytic effect of TiUnite increases with the wavelength of the UV radiation meanwhile the photocatalytic effect of TiO₂ decreases a little. With increasing wavelength ZrO₂ follows the control steadily and do not increase that much. This can be explained due to that the photocatalytic effect of ZrO₂ will be activated by a wavelength of 255 nm or lower.

In the part of the 24-well plate that was covered with alumina foil, the OD stays constant at 1.5 except TiUnite control discs that actually decrease with increasing wavelength. This is not expected and there is no good explanation for why this is happening. One speculation is that while letting the discs soak for 24h in the methylene blue solution before being exposed to UV radiation, the TiUnite surface may soak less than the others, and during exposure time it soaks more.

Things that can give errors in the measuring are that the wells can be illuminated with different intensity because of placement of the plate under the lamps. It may also be too little MB solution that covers the disc surfaces in the wells, which can result in a lower photocatalytic effect.

7.2.1 Comparison

It can over all be seen that TiO₂ and TiUnite are the material that show the most photocatalytic effect when they are UV exposed. This is as mentioned the natural way, because these two materials consists of anatase, which can give rise to photocatalytic effect. In the beginning it is TiO₂ which has the larges effect, because of the wavelength. In the end the photocatalytic effect of TiUnite passes TiO₂ because the energy of the light with wavelengths larger than 380[nm] is lower than the optimal energy that affects TiO₂.

7.3 Chemical composition (measured by XPS)

Analyzing the survey spectra in Figure 6.16 and the Figures in Appendix A 11.1 and 11.2 it can be seen that they have similar composition of tita-

nium, carbon and oxygen, but looking at the other elements it differs. In the Table 6.1 it is easy to see that the carbon peak increases for the TiO₂ and TiUnite discs after storage, but not as noticeable for the machined discs. The decrease of carbon on the TiO₂ and TiUnite surfaces shows that the photocatalytic effect works with UV exposure. On the machined disc, where fluorine and zirconium can be found when the disc is cleaned, it can be seen that these two elements disappear after storage. One reason for that can be that other dirt particles on the surface covers these elements. Why zirconium can be found on machined titanium and TiUnite from the beginning can be because they are produced with the same lathe as zirconium discs. Fluorine can come from the water that they are washed in. Other changes of elements on the surface are probably connected to each other and the larger increase of carbon. When carbon increases something else would decrease.

In Figure 6.19 to 6.20 it can be seen that the amount of titanium of the same chemical composition can be found on all discs, treated in different ways. This can have an effect on particles that arrive and attach to the surface. For the particle all surfaces look similar in a chemical composition view.

As can be seen in Figure 6.19 the M discs are not affected as much by the UV exposure compared to TiUnite and TiO₂ in Figure 6.20 and 6.21 respectively. When the TiUnite disc is exposed to UV radiation the carbon amount decreases on the surface and the Ti surface become more visible which increases the amount of Ti. In the case of TiO₂ exposed to UV radiation, the amount of C decreases a lot meanwhile the amount of both O and Ti increase.

7.3.1 Comparison

In both the survey spectrum and the figures of atomic % it can be seen that the amount of carbon on TiO₂ and TiUnite increases when the discs are stored and that the amount decreases again after UV exposure. That the amount increases is a natural effect of storage and that it decreases is based on the photocatalytic effect. As the results in the methylene blue experiment it was mostly these two materials, TiO₂ and TiUnite, that had the photocatalytic effect, which confirm the reasonable reason that the photocatalytic effect decreases the carbon amount.

8 Conclusion

It can be concluded that the photocatalytic effect could be activated for both TiUnite and TiO_2 discs by exposing them to UV radiation. Both the commercial and the LED UV radiation device works very well and have similar effect. The machined surface showed a small photocatalytic effect that could only be observed in the methylene blue experiments.

Storage of the different discs increased the contact angle in all cases except storage in EtOH for 168h, where the contact angle decreased instead. The largest contact angle was observed when the discs were stored in plastic bags. The amount of carbon increased with storage time, on the TiUnite discs and the TiO_2 discs, according to the results from the XPS experiment. However these carbons could be removed by exposing the discs to UV light.

It can be seen that UV radiation does not give rise to a high photocatalytic effect of the machined titanium discs. Studying the results from the methylene blue experiments it can be concluded that M discs reacts on the irradiation, but not as much as TiUnite and TiO_2 . By studying the changes in contact angle it can be seen that the M discs become dirtier during storage time with the exception for storage in EtOH and UV exposure does not give rise to a large decrease of the contact angle.

The TiUnite discs show a photocatalytic effect in the methylene blue experiments and it can be concluded that it increases with increasing wavelength. There is no effect shown of storing newly cleaned TiUnite discs for 168h, with the exception of a small change in contact angle after storage in plastic bag. It can also be concluded that adsorbed carbons could be removed with UV exposure and hence expose more of the titanium surface.

Analyzing the chemical composition it can be seen that the surfaces consists of mostly the same composition of elements but of different intensity and amount. It can also be seen that particles attached to the surface, "see" the same composition of titanium on all discs independent of treatment. UV exposure of TiO_2 changes both the surface atomic composition and activates the photocatalytic effect. Studying the methylene blue degradation the OD decreases when TiO_2 is irradiated. Best activation effect can be seen by UV radiation of wavelength 365 nm, but there is also an activation at increasing wavelengths. It can also be concluded that the surface is negatively charged and that the amount of carbon increased when the surface was stored, while it could be decreased when it was exposed to UV radiation.

When reviewing the methylene blue experiment it can be concluded that the custom built UV device construction with LED lamps can activate the photocatalytic effect as efficiently as the commercially available Black-Ray UV lamp. This can be seen by comparing the methylene blue assay results at 365 nm. The photocatalytic effect could be obtained for the TiUnite and TiO₂ surfaces at all tested wavelengths, hence the LED lamps of all wavelengths have a desired effect.

The use of UV radiation to activate the photocatalytic activity of TiUnite and TiO₂ surfaces were shown to be successful. Exposing these surfaces to UV radiation could give rise to materials with improved properties, used in dental applications, that could potentially increase the success rate of dental implants.

Summarizing results from all methods used in this project, contact angle measurement, degradation of methylene blue and x-ray photoelectron spectroscopy, they have been conclusive and all of them have ended up with similar results about the effect of cleaning, storage, and UV exposure. The results from the XPS experiment have strengthened the other results about the hydrophilicity and the photocatalytic effect.

9 Future perspectives

Materials coated with TiO_2 have been used for self-cleaning, anti-bacterial and/or anti-fogging functions based on the photo-induced decomposition reaction and the photo-induced hydrophilicity. These functions are obtained without using any chemicals, only nature's own resources such as sunlight and rainwater. This means that these TiO_2 coated materials can be classified as being environmentally friendly. [5]

For the future of this project it would be interesting to explore and test the difference in contact angle when using all different wavelengths. So far only one wavelength has been tested. Interesting question to answer would be; Will there be any difference in contact angles for the different exposure times? Or maybe there will be a difference when just changing the intensity of the used wavelength.

The next step is to do all experiments with UVC radiation to see if there are any differences in photocatalytic effects and also try to identify if there are any changes in biocompatibility. This can be studied in other ways than what have been done in this project, in vitro testing with cells or antimicrobial test are two examples.

Something else that is of interest is to study the effect of storage in EtOH. Does it always make the discs "cleaner"? The TiO_2 disc got a contact angle as low as close to 0, but can the contact angle on the M discs become even cleaner while storing for a longer time than 168h? Would they still be cleaner after an extended time of storage in EtOH or does the effect change? It was also seen in the experiment CA2 that a longer exposure time gave a smaller contact angle. Can this effect be preserved for longer times if the irradiation time is longer?

To better relate the results obtained in this study to the different surface features of the materials used, methods to study the charge and the roughness of the surface could be used. Such methods could be SEM, TEM and AFM that can give images with high magnification and resolution. There is also a technique other than XPS that could be used to study the surface charge called a Kelvin test. This method measures the changes in work function in a material/surface. An electrode is placed on the surface and the resistance capacitance is measured.

10 Acknowledgements

First I would like to thank Jenny Fäldt och Fredrik Osla, my supervisors at Nobel Biocare, for letting me do my master thesis together with them in their group. They have helped me a lot during these months. I would also like to thank Sofia Svedhem at Chalmers University of Technology for being my examiner and support during this work. In the end of the project Fredrik went on another important mission which gave Karoline Brattås the role as my supervisor. She did a great job helping me with my report before handing it in and thank you Karoline. During my time at Nobel Biocare and Chalmers I only met kind and helpful people that I of course also would like to thank.

In the beginning of my master thesis, when I started to write my report I had an interesting discussion with a very good friend Mikael Andersson, about in what program I should write. He had thousands of reasons why I should chose to write in Latex and nothing else. First I was suspicious, but today I am glad I listened to the advice. Not only for the exceptional help Mikael have given me but also because I think it was easy to handle when you knew how to. Thank you very much Mikael, for guiding me in my choice of writing program and of course for all other support that you have given me both during this master thesis work and else. I would also really like to say thank you to some other very important people that have been there for me and supported me in everything, my wonderful family and friends; Therese, Laila and Per Klang, Morgan and Marita Örn, John Berntsson and Andreas Carlsson. And of course Every one who has been there for me when I have needed it! Thank you all, very much!

Summarizing I would like to say that I have had a lot of fun, learnt enormously much and also met a lot of good, interesting and important people that have given me a developed interest for the subject I have been working with and also a wider perspective.

References

- [1] Norowski, PA Jr, Bumgardner JD. Biomaterial and antibiotic strategies for peri-implantitis: a review. *J Biomed Mater Res B Appl Biomater.* 2009; 88(2): 530-43.
- [2] Suketa, N. Sawase, T. Kitaura, H. Naito, M. Baba, K. Nakayama, K. Wennerberg, A. Atsuta, M. An Antibacterial Surface on Dental Implants, Based on the Photocatalytic Bactericidal Effect. *Clinical Implant Dentistry and Related Reseach.* 2005; 7(2): 105-111.
- [3] Houas, A. Lachheb, H. Ksibi, M. Elaloui, E. Guillard, C. Herrmann, J-M. Photocatalytic degradation pathway of methylene blue in water. *Applied Catalysis B: Environmental.* 2001; 31: 145-157
- [4] Lachheb, H. Puzent, E. Houas, A. Ksibi, M. Elaloui, E. Guillard, C. Herrmann, J-M. Photocatalytic degradation of various types of dyes (Alizarin S, Creocsein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania. *Applied Catalysis B: Environmental.* 2002; 39: 75-90
- [5] Hashimoto, K. Irie, H. Fujishima, A. TiO_2 Photocatalysis: A Historical Overview and Future Prospects. *Japanese Journal of Applied Physics.* 2005; 44(12): 8269-8285.
- [6] Takeuchi, M. Sakamoto, K. Matra, G. Coluccia S. Anpo, M. Mechanism of Photoinduced Superhydrophilicity on the TiO_2 Photocatalyst Surface. *The journal of physical chemistry B.* 2005; 109(32): 15422-15428
- [7] Jones, R. A. L. *Soft Condensed Matter.* New York: Oxford University Press, 2007.
- [8] Lifen, L. Barford, J. King Lun, Yeung. Non-UV germicidal activity of fresh TiO_2 and Ag/TiO_2 . *Journal of Environmental Sciences.* 2009; 21(5): 700-706.
- [9] Li, B. Logan, BE. The impact of ultraviolet light on bacterial adhesion to glass and metal oxide-coated surface. *Colloids Surfaces B: Biointerfaces.* 2005; 41(2-3): 153-161.
- [10] Chun, MJ. Shim, E. Kho, EH. Park, KJ. Jung, J. Kim, JM. Kim, B. Lee, KH. Cho, DL. Bai, DH. Lee, SI. Hwang, HS. Ohk, SH. Surface modification of orthodontic wires with photocatalytic titanium oxide for

- its antiadherent and antibacterial properties. *Angle Orthod.* 2007; 77(3): 483-488.
- [11] Williams, E. *A Matter of Balance*. Göteborg: Novum Grafiska AB, 1992.
- [12] Pye, A.D. Lockhart, D.E.A. Dawson, M.P. Murray, C.A. Smith, A.J. A review of dental implants and infection. *Journal of Hospital Infection* 2009; 72, 104-110.
- [13] Klinge, B., Hultin, M. & Berglundh, T. (2005). Peri-implantitis. *Dent Clin North Am* 49, 661-676, vii-viii
- [14] Park, J. Lakes, R.S. *Biomaterials - An introduction*, Third Edition. New York: Springer Science + Business Media, 2007.
- [15] Nobel Biocare Annual report 2009. Received from Nobel Biocare.
- [16] Broschyr about Nobel, *World leader in innovative restorative and esthetic dental solutions*, 2009. Received from Nobel Biocare.
- [17] Att, W. Hori, N. Iwasa, F. Yamada, M. Ueno, T. Ogawa, T. The effect of UV-photofunctionalization on the time-related bioactivity of titanium and chromium-cobalt alloys. *Biomaterials.* 2009; 30(26): 4268-76.
- [18] Iwasa, F. Hori, N. Ueno, T. Minamikawa, H. Yamada, M. Ogawa, T. Enhancement of osteoblast adhesion to UV-photofunctionalized titanium via an electrostatic mechanism. *Biomaterials.* 2010; 31(10): 2717-2127.
- [19] Linsebigler, A.L. Lu, G. Yates, J.T. *Photocatalysis on TiO₂ Surfaces: Principles, Mechanisms, and Selected Results*. American Chemical Society. 1995; 95: 735-758.
- [20] de Lasa, H. Serrano, B. Miguel S. *Photocatalytic Reaction Engineering*. New York: Springer Science + Business Media, Inc, 2005.
- [21] Broschyr about Nobel, *TiUnite®- 10 years of enhanced osseointegration*, 2009. Received from Nobel Biocare.
- [22] Sul, Y.T. Byon, E. Wennerberg, A. Surface Characteristics of Electrochemically Oxidized Implants and Acid-Etched Implants: Surface chemistry, morphology, pore configurations, oxide thickness, crystal structure, and roughness. *The international Journal of Oral & Maxillofacial Implants.* 2008; 23(4): 631-640.

-
- [23] Boeker, E. van Grondelle, R. Environmental Physics Second Edition. West Sussex: John Wiley & Sons, Inc, 1999.
- [24] Nordling, C. Osterman, J. Physics Handbook for Science and Engineering, 8th edition. Lund: Studentlitteratur AB, 2006.
- [25] Dart, R. C. Medical toxicology, third edition. Philadelphia: Lippincott Williams & Wilkins, 2004.
- [26] Yang L, Yu LE, Ray MB. Degradation of paracetamol in aqueous solutions by TiO₂ photocatalysis. Water Research. 2008; 42(13): 3480-3488.
- [27] Surface oxides on titanium: Preparation, characterization and biomaterials. Jukka Laysmaa, Department of Physics, Göteborg 1991.
- [28] Atkins, P. and Jones, L. Chemical Principles, the quest for insight, third edition. New York: Freeman and company, 2004.
- [29] Scott Fogler, H. Elements of Chemical Reaction Engineering, third edition. Upper Saddle River, New Jersey: Prentice Hall PTR, 1999.
- [30] Balaur, E. Macak, J.M. Tsuchiya H. Schmuki P. Wetting behaviour of layers of TiO₂ nanotubes with different diameters. Journal of Materials Chemistry. 2005; 15 : 4488-4491.
- [31] Practical Contact angle measurement (4). Technical Note TN314e; Page 1; F. Thomsen, 05/2008; Measuring with method - but which one? Received from KRUSS.
- [32] Moulder, J.F. Stickle, W.F. Sobol, P.E. Bomben, K.D. Handbook of X-ray Photoelectron Spectroscopy; A reference book of standard spectra for identification and interpretation of XPS Data. Minnesota: Perkin-Elmer Coporation Physical Electronics Division, 1992.
- [33] Czichos, H. Handbook of Materials Measurement Methods. New York: Springer Science & Business Media, Inc, 2006.
- [34] Omar, IV.O. Lennerås, M. Suska, F. Emanuelsson, L. Hall, J. Palmquist, A. Thomsen, P. Interfacial gene expression and stability of oxidized and machined titanium implants, In Manuscript, 2010.
- [35] Omar, O. Svensson, S. Zoric, N. Lennerås, M. Suska, F. Wigren, S. Hall, J. Nannmark, U. Thomsen, P. In vivo gene expression in response to anodically oxidized versus machined titanium implants. Journal of Biomedical Matererials Research, part A. 2010; 92(4): 1552-1566.

-
- [36] 8.2. Standard Operating Procedure, TiUnite process, final cleaning and packaging of implants and components for *in vivo* and *in vitro* use produced at PW. Nobel Biocare, document no. 81529, approved by Johansson U. 2010-05-05. Received from Nobel Biocare.

11 Text/Appendix A

11.1 Results of XPS measurement

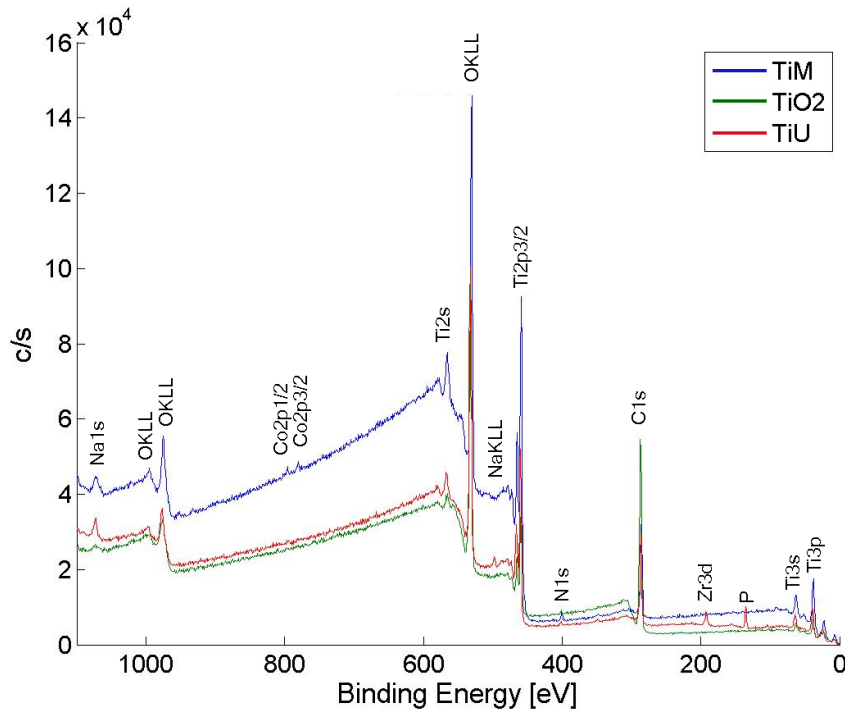


Figure 11.1 - Survey spectra of stored machined, TiO₂ and TiUnite discs.

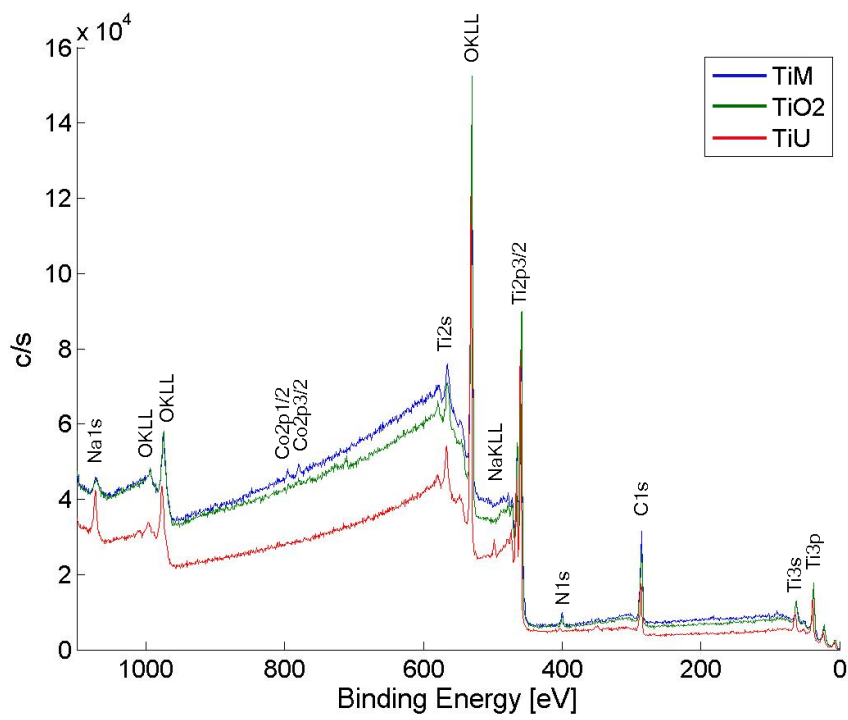


Figure 11.2 - Survey spectra of UV exposed machined, TiO_2 and $TiUnite$ discs.