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Osseointegration and Cell Response on Titanium Implant Surface Treatments

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Faculdade de Ciências da Saúde

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Dissertação apresentada à
Universidade Fernando Pessoa
como parte dos requisitos para a obtenção do
grau de Mestre em Medicina Dentaria

Resumo

Introdução:

A osteointegração de um implante é essencial para garantir êxito clínico. Vários tratamentos de superfície foram introduzidos para melhorar a osseointegração e a biocompatibilidade de um implante.

Objectivos:

O objectivo de esta revisão bibliográfica é explorar as diferentes propriedades de Titanio e como á modifica-las, depois de introduzir um implante de Titanio num tecido de osso vivo, a osteointrgração, a resposta celular e a biocompatibilidade do implante podem ser afectadas.

Materiais e métodos:

Para esta revisão bibliográfica, 96 artigos foram usados, tombados das seguintes bases de dados: Pubmed, Science direct and Scielo. Através das seguintes palavras chave: "*Surface treatments", "Titanium dental implants", "osseointegration", "anodization", "anodic oxidation", "plasma spraying", "HA coatings", "hydroxyapatite coatings", "acid etching", "grit blasting", "tribocorrosion" AND "biotribocorrosion".*

Conclusão:

Foi concluído que a superfície ideal deve presentear uma rugosidade de superfície e 1-2µm, exibir aumentada hidrofilicidade e apresentar uma grossa camada de óxido. Deve também carecer de contaminastes citototòxicos a fim de promover um ambiente biológico favorável e finalmente, foi demonstrado que uma carga negativa promove resposta celular.

Abstract

Introduction:

The osseointegration of an implant is essential in order to achieve clinical success. Various surface treatments have been introduced in order to achieve a better osseointegration and biocompatibility of the implant.

Objectives:

The objective of this review was to explore the different properties of titanium and how by changing them, after a titanium implant being introduced to living bone tissue, osseointegration, cellular response and biocompatibility of the implant can be affected.

By this it can be understood what to look for in an implant surface, concluding to the ideal surface characteristics a Ti implant should possess.

Materials and Methods:

For this review, they were used 96 articles, retrieved from the following search engines: Pubmed, Science direct and Scielo. Through the following key words: "*Surface treatments", "Titanium dental implants", "osseointegration", "anodization", "anodic oxidation", "plasma spraying", "HA coatings", "hydroxyapatite coatings", "acid etching", "grit blasting", "tribocorrosion" AND "biotribocorrosion".*

Conclusion:

It was concluded that the ideal surface should present a surface roughness of 1-2µm, have increased hydrophilicity and present a thick oxide layer. It should also lack of any cytotoxic contaminants in order to promote a favorable biologic environment and finally, a negative charge has been shown to promote cell response.

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Index of Abbreviations

HA- Hydroxyapatite

PDGF- Platelet-Derived Growth Factor

TGF- Transforming Growth Factor beta

Cp-Ti- Commercially Pure Titanium

TiO_2- Titanium Oxide

RANKL- receptor activator of nuclear factor kappa-B ligand

OPG- osteoprotegerin

TNF-a- Tumor Necrosis Factor alpha

Sa- Surface Roughness

CA- Contact Angle

CaP- Calcium Monophosphide

Al_2O_3- Aluminium Oxide

SiO_2- Silica Oxide

BIC- Bone to Implant Contact

BCP- Biphasic Calcium Phosphate

HCL- Hydrogen Chloride

H_2SO_4- Sulfuric Acid

HNO_3- Nitric Acid

HF- Hydrofluoric Acid

Sdr- Developed Interfacial Area Ratio

Sds- Summit Density

SLA- Sand-blasted, Large grit, Acidetched

AE- Acid Etched

RT – Room temperature

ROS- Reactive Oxygen Species

RNS- Reactive Nitrogen Species

O_2(•-)- Superoxide Ion

OH (•)- Hydroxyl Radical

NO(•)- Nitric Oxide

H2O2- Hydrogen Peroxide

HOCl- Hypochlorous Acid

NO_3- Nitrate

CAT- Catalase

SOD- Superoxide Dismutase

PTTS- Plasma Treated Surface

SIT- Sandblasted and Ion Implanted

PS-Plasma Sprayed

PSIT- Plasma Sprayed and Ion Implanted

SEM- Scanning Electron Microscope

VPS- Vapor Plasma Spraying

APS- Atmospheric Plasma Spraying

I. Introduction

The oral rehabilitation of missing teeth by dental implants is one of the most commonly used surgical procedures nowadays. Beginning in the late 1960s the focused efforts of P.I. Branemark led to the detailed microscopic characterization of interfacial bone formation at machined titanium endosseous implants. This process, of bone formation at the endosseous implant surface, known as osseointegration was considered a positive outcome that was contrasted to fibrous encapsulation, a negative and undesired result that leads to failure of the treatment. Osseointegration gives stability able to resist forces and distribute them uniformly to the bone making possible its loading. So, it is clear that clinical success is dependent on the direct structural and functional connection between ordered, living bone and the surface of a load carrying implant. About 50-80% bone-implant contact is found sufficient to have a clinically successful implant (Lian et al., 2010).

To improve osseointegration and biocompatibility of Ti implants, the nature of the implant itself has to be changed. In order to do that, various surface treatments have been introduced to the market that change the mechanical, physical and chemical properties of titanium.

II. Development

1) Materials and Methods:

The bibliographic review of this study was realized between the months of May, June and July of 2016.

References 1985 to 2015were accepted in this review.

For this review 96 articles were used out of the initial 129 that were selected from abstract reading.

Criteria of inclusion were: indexed articles relevant to the theme of the dissertation, articles written in Greek, English or Portuguese and articles that were of scientific interest.

Criteria of non-inclusion were: articles irrelevant to the theme of dissertation, articles that by further inspection provided no further insight to the theme, and articles that did not provide conclusions.

Keywords used for this review were: "*Surface treatments", "Titanium dental implants", "osseointegration", "anodization", "anodic oxidation", "plasma spraying", "HA coatings", "hydroxyapatite coatings", "acid etching", "grit blasting", "tribocorrosion" AND "biotribocorrosion".*

2) Biology of wound healing following implant placement

Peri-implant osteogenesis can be in distance and in contact from the host bone. In distance osteogenesis, new bone is formed on the surfaces of old bone in the peri-implant site. The bone surfaces provide a population of osteogenic cells that lay down a new matrix until reaching the implant. An essential observation here is that new bone is not forming on the implant, but the latter does become surrounded by bone. Thus, in these circumstances, the implant surface will always be partially obscured from bone by intervening cells. Distance osteogenesis is typical of cortical bone healing. In contact osteogenesis, new bone starts forming directly on the implant surface. Since, by definition, no bone is present on the surface of the implant upon implantation, the implant surface has to become colonized by bone cells before bone matrix formation can begin (Davies J. E., 2008).

Bone matrix is synthesized by only one cell: the osteoblast. Since each osteoblast may become a completely entombed osteocyte, the osteoblast is incapable of migration away from the bone surface, and the only method by which this surface can receive further additions is by the recruitment of more osteogenic cells to the surface, which then differentiate into secretorily active osteoblasts. Bone matrix mineralizes and has no inherent capacity to "grow." Once bone formation has been initiated, the matrix and the cells that have synthesized that matrix have almost no ability to govern the ongoing pattern of bone growth on the implant surface. The only way for new bone to be formed on an implant, is by osteogenic cells to migrate to its surface. Then, if we require that bone "grows" around the implant to establish functional endosseous integration, this too can only be achieved by the continued recruitment around, and migration of osteogenic cells to the implant surface. Thus, it can be concluded that the most important stages of endosseous healing precede bone formation (Davies J. E., 2008).

During surgery, dental implant surfaces interact with blood components from ruptured blood vessels. Within a short period of time, various plasma proteins such as fibrin get adsorbed on the material surface. Fibrinogen is converted to fibrin and the complement and kinin systems become activated. As in fracture healing, the migration of bone cells in peri-implant healing will occur through the fibrin of a blood clot. Since fibrin has the potential to adhere to almost all surfaces, it can be anticipated that the migration of osteogenic cell populations towards the implant surface will occur. However, as the migration of cells through fibrin will cause retraction of the fibrin scaffold, the ability of an implant surface to retain this fibrin scaffold during the phase of wound contraction is critical in determining whether the migrating cells will reach the implant surface. Activation of platelets occurs as a result of interaction of platelets with the implant surface as well as the fibrin scaffold and this leads to thrombus formation and blood clotting (Anil et al., 2011).

Moreover, platelets are a rich source of many growth and differentiation factors which play a key role in the wound healing process by acting as signaling molecules for recruitment and differentiation of the undifferentiated mesenchymal stem cells at the implant surface. Platelet degranulation releases a number of growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-b), together with vasoactive factors such as serotonin and histamine, factors that play an important role in the regulation of the wound-healing cascade, (JY, Gemmel and Davies, 2001)

Absorption of proteins such as fibronectin and vitronectin on the surface of dental implants could promote cell adhesion and osseointegration. During the initial remodeling, a number of immune cells mediate early tissue response followed by migration of phagocyte macrophages. These cells initially remove the necrotic debris created by the drilling process and then undergo physiological changes which lead to expression of cell surface proteins and production of cytokines and pro-inflammatory mediators. This cytokine-regulated cellular recruitment, migration, proliferation and formation of an extracellular matrix on the implant surface can be influenced by the macrophages. These cells express growth factors such as fibroblast growth factor (FGF-1, FGF-2, FGF-4), transforming growth factors, epithelial growth factor as well as bone morphogenetic proteins (BMPs). The end result of this complex cascade is promotion of a wound healing process that includes angiogenesis. (Anil S, 2011)

Thus, as in fracture healing, the migration of osteogenic cells in peri-implant healing will occur through the transient three-dimensional biological matrix formed as a product of the coagulation cascade—the fibrin of the blood clot—and may be both potentiated and directed, either directly or indirectly through knock-on stimulatory events involving leukocytes, (Bromberek et al., 2002) by the release of cytokines, growth factors, and microparticles from platelets activated by contact with the implant surface. Finally, when the osteogenic cells reach the implant surface, they can initiate bone matrix secretion, by osteogenic cells, of the cement line matrix. This is a collagen-free, mineralized interfacial matrix laid down between old bone and new bone (Davies J., 1996).

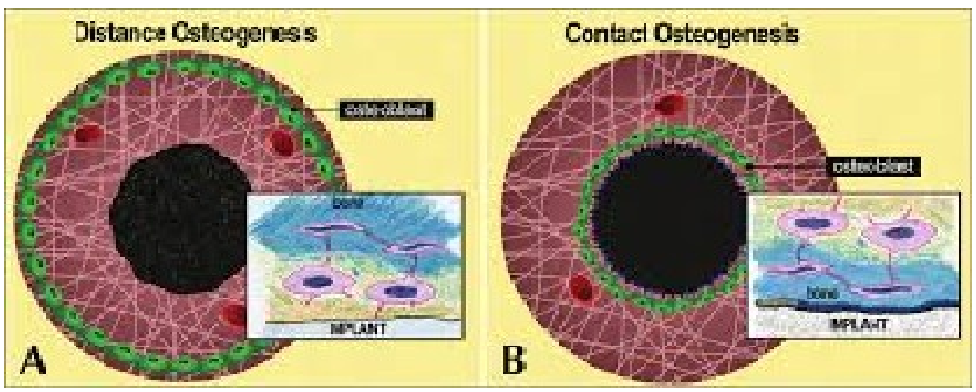

Figure 1: Drawings to show the initiation of distance osteogenesis (A) and contact osteogenesis (B) where differentiating osteogenic cells line either the old bone or implant surface respectively. The insets show the consequences of these two distinctly different patterns of bone formation. In the former the secretorily active osteoblasts, anchored into their extracellular matrix by their cell processes, become trapped between the bone they are forming and the surface of the implant. The only possible outcome is the death of these cells. On the contrary, in contact osteogenesis, de novo bone is formed directly on the implant surface, with the cement line in contact with the implant (insert) and is equivalent to the osteonal interface illustrated in Figure 1 (can be viewed at www.ecf.utoronto.ca/ ~bonehead/ (follow buttons to osteogenesis and osteoconduction).

3) Dental Implant Materials

When a material is inserted into an organism, an interaction between the two is inevitable, leading to three possible outcomes, integration, incorporation, or rejection. The outcome depends on various factors, such as the nature of the material, that can be classified as: bioinert, biotolerated, or bioactive; osteogenic, osteoconductive or osteoinductive. These properties influence the biological response that will be induced pos-implantation (Albrektsson and Johansson, 2001).

Bioinert material, is a material that doesn't induce a foreign body reaction when introduced to the organism and establishes direct contact with the surrounding tissues without interposition of fibrous tissue. A biotolerated material is moderately accepted from the organism; that means that there is no total direct contact and fibrous tissue can be involved in the healing process. Bioactive material, is a material that not only forms a direct contact with the surrounding tissues but also establishes a chemical bond. Generally, these materials present calcium and phosphate ions at their surface, that help establish a chemical bond (Puleo and Nanci, 1999).

An osteogenic material is capable to mobilize cells of the osteoblastic cell line, to a determined place, and promote the formation of bone tissue. An osteoconductive material, guides the formation of bone to its surface when implanted in such tissue by mobilizing osteogenic cells. An osteoinductive material, promotes formation of bony tissue, when implanted out of such tissue, by mineralizing undifferentiated cells, and inducing their differentiation to osteoblastic cells. An ideal material would possess all of the three abovementioned properties (Albrektsson and Johansson, 2001).

One of the most frequently used biomaterials for dental implants is commercially pure titanium (Cp-Ti) and its alloys. An implant is a structure that is surgically inserted in the alveoli and through means of osseointegration establishes a tight connection with the bone. The reason behind its commercial popularity among dentists is its optimal properties, that include: biocompatibility, high corrosion resistance, high mechanical resistance and thermoelectric conduction. Its corrosion resistance and biocompatibility but also its osseointegration are consequence to an oxide layer (TiO_2) with a width of 2-10nm that Ti forms immediately when exposed to air (Bordji, et al., 1996).

The most used Ti alloy is Ti6Al4V. Aluminum is added to increase the mechanical resistance of the implant while Vanadium stabilizes the phase maintaining resistance to corrosion. Commercially pure titanium and Ti6Al4V are materials with a very similar elasticity to the bone and so, forces and tensions are distributed homogenously from the cervical area of the implant to the bone (Anusavice, Shen and Rawls, 2013).

There are 4 grades of commercially pure Ti (1,2,3 and 4) with grade 1 being the purest with a 99.8% of Ti, grade 4 being the most used commercially. Elements that can be found in Cp-Ti are considered impurities that are permitted from American Society for Testing and Materials. Cp-Ti is a light metal with high resistance to corrosion and a good relation between mechanical resistance and resistance to fracture when subjected to loads similar to occlusal forces. Ti6Al4V on the other side presents a higher mechanical resistance and lower elastic moduli (Triplett et al., 2003).

4) Tribocorrosion

The oral cavity is a dynamic environment, that sometimes can be hostile for an implant. Biofilm, acids produced by bacteria or even substances that can be found in saliva such as fluoride and chloride when in contact with the Ti can induce the chemical process known as corrosion to its surface impairing its mechanical resistance (Souza et al., 2013; Souza et al.,2015).

Tribocorrosion is the phenomenon which occurs when two surfaces that are in contact suffer from simultaneous corrosion and degradation. When this happens in a biological environment it is known as biotribocorrosion. Biotribocorrosion of a material is dependent on its chemical and mechanical properties as well as the medium that is inserted into. For example: the oxide layer that protects Ti from corrosion can be damaged by mechanical wear, and in return be susceptible to chemical corrosion (Celis, Pontiaux, and Wegner, 2006; Souza et al., 2015).

The presence of biofilm in a Ti surface causes a decrease of pH and so turns the environment acidic, promoting chemical reactions that result in corrosion. Dental implants are situated in an area where oxygen is scarce or non-existent, so, only anaerobic bacteria can survive in this environment. *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotela intermedia* are examples of anaerobic bacteria that can cause periodontal and periimplantary diseases (Sissons, Wong and Shu, 1998; Barbour et al.,2007; Cruz et al., 2011).

When corrosion or wear occurs metallic particles and ions are released to the surrounding tissues. The immune system recognizes them as foreign objects and an inflammatory response mediated by cytokins occurs in the periimplantary tissues, leading to reabsorption of the bone and periimplantary diseases (Renvert,Persson, 2009; Cruz et al., 2011).

Metallic ions can accumulate in surrounding tissues or can be spread through systemic circulation to organs like the lungs, spleen, lymph nodes and the liver. This accumulation can alter the expression of the receptor activator of nuclear factor kappa-B ligand (RANKL) and to the osteoprotegerin (OPG) in osteoblastic cells, which contribute to the osteoclastic activity of pathologic osseous remodeling (Urban et al., 2000; Triplett et al., 2003).

A study elaborated by Wachi T. et al. (2015) showed that Ti ions with a concentration of 9ppm synergize with the bacteria P. gengivalis-LPS to increase the expression of cytocin ligant2 (CCL2)n RANKL and OPG at the gingival tissues, initiating inflammation and reabsorption of the bone. A concentration of 13ppm is enough to provoke necrosis of the cell.

Ti particles with a size of 10µm or less cause inflammatory responses and are considered cytotoxic, while particles of 1-3µm can be phagocytized by neutrophils or macrophages. Phagocytation of these particles causes an inflammatory response to the tissue, an increase in tumor necrosis factor alpha (TNF-a) and the phagocyte itself undergoes degenerative morphologic changes. TNF-a is a pre-inflammatory cytokin that capable of causing cellular death (Kumazawa et al., 2002).

It is well known that in high concentration metallic particles are harmful for fibroblasts, altering their proliferation and viability. *In vitro* experiments proved that Ti particles with a concentration of 0.001% in a saline solution can increase the proliferation of fibroblasts, but higher concentrations can decrease it. Recent studies showed as well that nanometric TiO_2 particles, that are released during biotribocorrosion, can induce cytotoxicity and genotoxicity. It was shown that in cellular cultures containing these particles, cell viability decreased and mutations increased. A culture of alveoli macrophages showed that when in contact with TiO_2 particles, the cells were under oxidative stress, proving their genocytotoxicity (Maloney et al., 1993; Wang, Sanderson and Wang, 2007; Souza et al., 2015).

But not only the TiO_2 particles can cause damage to the cells, *Vanadium* and *Aluminum* particles have also proven to be cytotoxic, impairing cellular growth, as shown by a study elaborated by Okazaki (2001), Vanadium is potentially genotoxic as well (Manaranche, Hornberger, 2007).

5) Implant surface character

Implant surface character is one implant design factor affecting the rate and extent of osseointegration.

The process of osseointegration is now well described both histologically and at the cellular level. Precisely how much of the implant surface directly contacts bone, how rapidly this bone accrual occurs, and the mechanical nature of the bone/implant connection is influenced by the nature of the implant surface itself (Mendonca 2008).

The surface characteristics of an implant which influence the speed and strength of osseointegration include surface chemistry, topography, wettability, charge, surface energy, crystal structure and crystallinity, roughness, strain hardening, the presence of impurities, thickness of titanium oxide layer, and the presence of metal and non-metal composites (Anil et al., 2011).

5.1 Topography

Implant surface topography refers to macroscopic and microscopic features of the implant surface. Surface topography plays an important role in the osseointegration of titanium implants (Le Guehennec, 2007).

It is not clear whether the height of surface irregularities is more important than the distance between them, and which combination of these factors could improve osseointegration. Although the increase in surface roughness promotes greater mechanical anchorage, the implant bone interface strength will not increase with the continuous increase of surface roughness (Anil et al., 2011).

5.2 Surface Roughness

The surface roughness of the implants can significantly alter the process of osseointegration because the cells react differently to smooth and rough surfaces. Fibroblasts and epithelial cells adhere more strongly to smooth surfaces, whereas osteoblastic proliferation and collagen synthesis are increased on rough surfaces (Boyan et al., 2001). Investigators have demonstrated that while the adhesion of fibroblasts is lesser on rough surfaces, the adhesion and differentiation of osteoblastic cells are enhanced (Wennerberg and Albrektsson, 2000).

Smooth surfaces as well as excessive roughness induce osteoblasts into a fibroblast phenotype. Smooth surfaces induce it because of the lack of space for osteoblasts to grow while excessive roughness induce it due to large spaces between the irregularities. *In vitro* and *in vivo* studies have shown that titanium surface roughness influences a number of events in the behavior of cells in the osteoblastic lineage, including spreading and proliferation, differentiation, and protein synthesis (Sammons et al., 2005; Zhao, 2006).

The organization of the cytoskeleton, special orientation of the cells as well as synthesis and mineralization of the bone matrix are favored by an increased surface roughness. It has been shown that titanium implants with adequate roughness may influence the secondary stability of implants, enhance bone-to-implant contact, and may increase removal torque force (Wennerberg and Albrektsson, 2009).

Based on the average surface roughness (Sa) surfaces with an average Sa≤1 µm are considered smooth and those with a Sa>1µm are considered rough. e.g. Machined Titanium is a smooth surface with Sa values of 0.53 to 0.96 µm (Sykaras et al., 2000).

High roughness results in mechanical interlocking between the implant surface and the bone ingrowth. However, a major risk with high surface roughness may be an increase in peri-implantitis as well as an increase in ionic leakage. A moderate roughness of 1-2µm may limit these two parameters (Wennerberg and Albrektsson, 2000).

The microtopographic profile of dental implants is defined for surface roughness as being in the range of 1-10μm. This range of roughness maximizes the interlocking between mineralized bone and the surface of the implant. A theoretical approach suggested that the ideal surface should be covered with hemispherical pits approximately 1.5μm in depth and 4μm in diameter. Implant surface roughness is divided, depending on the dimension of the measured surface features into macro, micro, and nano-roughness (Anil et al., 2011).

Macro roughness comprises features in the range of millimeters to tens of microns. This scale directly relates to implant geometry, with threaded screw and macro porous surface treatments. Micro roughness is defined as being in the range of 1–10 μm. This range of roughness maximizes the interlocking between mineralized bone and implant surface. Studies supported by some clinical evidence suggest that the micron-level surface topography results in greater accrual of bone at the implant surface. The use of surfaces provided with nanoscale topographies are widely used in recent years. Nanotechnology involves materials that have a nano-sized topography or are composed of nano-sized materials with a size range between 1 and 100 nm. Nanometer roughness plays an important role in the adsorption of proteins, adhesion of osteoblastic cells and thus the rate of osseointegration (Anil et al., 2011).

5.3 Surface Charge

It has been shown that the surface charge of a dental implant affects its osseointegration. A negative charge or a positive charge has been found to be more promising than a neutral charge since a charge on an implant surface promotes hydrophilicity. (Boyan, 1996).

On a negatively charged biomaterial surface, cells proliferate more actively; meanwhile, multiple layers of cells and enlarged colonies of osteoblast-like cells can be also observed. In contrast, cell adhesion and proliferation on positively charged biomaterial were found to be subdued (Yan Guo, Matinlinna and Tang, 2012).

An implant treated with acid etching, using NaOH as the acidic solution, forms a bioactive sodium titanium oxide (sodium titanate) layer, on the titanium surface, that is charged negatively. The negatively charged layer, attracts positively charged calcium ions that begin to accumulate on the biomaterial surface, turning it to a positive charge; hence, the surface starts to attract negatively charged phosphate ions, which react with the calcium ions to form a calcium phosphate (i.e. a type of apatite) layer. This calcium phosphate layer takes an amorphous structure after its formation, and it subsequently transforms into more stable crystalline apatite (Hamouda, et al., 2012; Yan Guo, Matinlinna and Tang, 2012).

A charged implant surface can induce electrical attraction or repulsion between the implant surface and the surrounding chemical species, depending on their polarity. Besides the effect on crystal nucleation, another significant role of Ca_2^+ is to attract cell-adhesion proteins (e.g., integrins, fibronectin, and osteonectin), which are characterized by their capacity to interact with a specific ligand. These proteins significantly affect the attachment, adhesion, and spreading of osteoblasts. Consequently, osteoblasts attach and proliferate on a matrix grown on the bone-like apatite layer formed with Ca_2^+ ions, which may result in faster and stronger bone-to-implant bonding. In contrast, a positively charged implant surface attracts anionic groups which act as antiadhesive molecules, which negatively affect osteoblast adhesion (Ohgaki et al., 2001).

5.4 Surface Energy

Surface charge influences surface energy which is a measure of the extent to which the bonds are unsatisfied at the surface. The surface energy of an implant is an important factor that influences osseointegration. Specifically, studies have shown that a high surface free energy on the implant surface enhances the hydrophilicity of its surface, which in turn promotes the adhesion of blood components such as proteins, as well as osteoblasts. Studies have shown though, that an extremely high surface energy while promoting the adhesion of cells, can hinder their motility and their subsequent functions (Yan Guo, Matinlinna and Tang, 2012; Gittens et al., 2014).

A high surface free energy initiates the absorption of proteins such as fibronectin, which is an extracellular matrix protein responsible for osteoblast cell differentiation, cell-cell interactions and cell-matrix interactions (Jia et al., 2015).

A high surface energy results in a high degree of wettability; thus when an implant is exposed to blood, the entire surface will almost immediately be covered by it, stimulating the blood proteins to attach to the surface, to start the bone healing process, however from a clinical point of view, a recent overview failed to find convincing evidence of the effectiveness of increasing surface energies (Wennerberg, Galli, and Albrektsson, 2011).

Wetting of high and low energy substrates: The energy of the bulk component of a solid substrate is determined by the types of interactions that hold the substrate together. High energy substrates are held together by bonds, while low energy substrates are held together by forces. Covalent, ionic, and metallic bonds are much stronger than forces such as van der Waals and hydrogen bonding. High energy substrates are more easily wet than low energy substrates. In addition, more complete wetting will occur if the substrate has a much higher surface energy than the liquid (De Gennes, 1985).

5.5 Wettability

Wettability is influenced by the surface energy of an implant and is one of the most important factors for bone to implant contact. It governs the degree of contact that the surface of the implant will have with the physiological environment. It can be described in general as the ability of a solid surface to reduce the surface tension of a liquid in contact with it such that it spreads over the surface and wets it (Kilpadi and Lemons, 1994; Zhao, Liu, & Ding, 2005). Wettability and surface energy influence the adsorption of proteins, and increase adhesion of osteoblasts on the implant surface. An increased wettability enhances the biocompatibility of an implant, promoting interactions between the implant and the biological environment (Sartoretto et al., 2015). A hydrophilic surface is better for blood coagulation than a hydrophobic surface. The expressions of bone-specific differentiation factors for osteoblasts are higher on hydrophilic surfaces. Consequently, dental implants manufacturers have developed high hydrophilic and rough implant surfaces which in turn exhibited better osseointegration than implants with smooth surfaces (Anil et al., 2011).

Contact angle (CA): A way to experimentally determine wetting is to look at the contact angle (θ)

If $\theta=0$ the liquid completely wets the substrate.

If $0<\theta<90$, high wetting occurs.

If $90<\theta<180$, low wetting occurs.

If >180, the liquid does not wet the substrate at all.

Water CAs very close to 0 are termed as superhydrophilic and above 150 degrees as superhydrophobic (Gittens et al., 2014).

The Young Equation relates the contact angle to interfacial energy:

$$\cos\theta = (\gamma sv - \gamma sl) / \gamma lv$$

where ***γsv*** the interfacial energy between the solid and gas phases, ***γsl*** the interfacial energy between the substrate and the liquid, γlv is the interfacial energy between the liquid and gas phases, and θ is the contact angle between the solid-gas and the solid-liquid interface.

As seen from Young's equation, the contact angle is directly coupled to the surface energy, where a surface with high surface energy has a low contact angle and is easily wetted in contrast to a low-energy surface with a high contact angle.

5.6 Surface Chemistry

Surface chemistry can be roughly defined as the study of chemical reactions at interfaces. It is closely related to surface engineering, which aims at modifying the chemical composition of a surface by incorporation of selected elements or functional groups that produce various desired effects or improvements in the properties of the surface or interface. Surface chemistry involves adhesion of proteins, bacteria, and cells on implants. Surface chemistry has the potential to alter ionic interactions, protein adsorption, and cellular activity at the implant surface (Schliephake et al., 2005). These modifications may subsequently influence conformational changes in the structures and interactive natures of adsorbed proteins and cells. Furthermore, within the complexities of an *in vivo* environment containing multiple protein and cellular interactions, these alterations may differentially regulate biologic events. Modifications to the implant surface chemistry may lead to alterations in the structure of adsorbed proteins and have cascading effects that may ultimately be evident at the clinical level (Anil et al., 2011).

Surface chemistry is highly dependent on surface topography and vice versa, since a treatment that produces a certain topography will change the chemistry of the implant's surface. While overlooked oftenly, surface chemistry plays an important role in the absorption of proteins, the cell attachment, the behavior of the attached cells as well as biocompatibility of the implant itself. While topography kept constant, changes in surface chemistry can alter the biocompatibility of an implant enough to change it from cytotoxic to fully cytocompatible (Cassinelli et al., 2003; Thevenot, Hu and Tang, 2008)

In vivo evidence has supported the use of alterations in surface chemistry to enhance osseointegration (Sartoletto et al., 2015).

5.7 Atomic Composition

Atomic composition plays an important role in osseointegration since cellular connections are established initially in an atomic level through chemical components present at the surface, including O2 and Ti. Titanium is a very reactive material, forming an oxide layer immediately after contacting O2. Because of this layer the maximum percentage of Ti in its surface is 33%, with the rest 66% being oxygen. Realistically though, as described by many authors, Ti implants present a percentage of Ti less than 20% due to contamination with hydrocarbons and carbides (Morra et al., 2003).

Machined implants are the ones to contain more contaminants due to the oils and lubricants used during production and polishing processes.

Even chemical sterilization can increase the percentage of contaminants found in the implant surface, leading many companies to use radiations as the sterilization process (Morra and Cassinelli, 1997; Sakai et al., 1998; Morra et al., 2003).

Machined implants are found to contain a toxic element, Pb. Probably this element contaminates machined surfaces through the machines used to create this type of surface. Elements such as nitrogen, fluoride, aluminum, silicon, phosphorus and calcium have been found in plasma treated and acid surfaces, though in very small quantities. Nitrogen and fluoride can be found in surfaces that were treated with acids, due to the contact with the acids themselves, while nitrogen can be explained in Plasma treated surfaces from the Ti particles that are projected, nitrogen could bond to these particles. All these particles have the potential to inhibit mineralization of the bone, but their low concentration, as well as the oxide layer limit this possibility (Smith et al., 1991; Morra et al., 2003).

6) Methods of surface modifications of implants

It has been shown that the possibility of osseointegration failure due to fibrous connective tissue development between implant surface and bone is increased when the latter has not undergone any treatment (Le Guehennec et al., 2007, Von Wilmowsky et al., 2014). The importance of interface in the microscopic and ultramicroscopic structure between implant-bone and implant-soft tissues needs to be stressed. In order to amplify and accelerate osseointegration, various implant modifications have been presented seeking to higher bone to implant contact.

The methods employed for surface modifications of implants can be broadly classified into 3 types-mechanical; chemical; and physical. These different methods can be employed to change the implant surface chemistry, morphology, and structure. The main objective of these techniques is to improve the bio-mechanical properties of the implant such as stimulation of bone formation to enhance osseointegration, removal of surface contaminants, and improvement of wear and corrosion resistance (Anil et al., 2011).

The mechanical methods include grinding, blasting, machining, and polishing. These procedures involving physical treatment generally result in rough or smooth surfaces which can enhance the adhesion, proliferation, and differentiation of cells. The physical methods of implant surface modification include plasma spraying, sputtering, and ion deposition. Plasma spraying includes atmospheric plasma spraying and vacuum plasma spraying. This is used for creating titanium and CaP coatings on the surfaces of titanium implants. Sputtering has been used to deposit thin films on implant surfaces to improve their biocompatibility, biological activity, and mechanical properties such as wear resistance and corrosion resistance. Methods of surface modification of titanium and its alloys by chemical treatment are based on chemical reactions occurring at the interface between titanium and a solution. The chemical methods of implant surface modifications include chemical treatment with acids or alkali, hydrogen peroxide treatment, sol-gel, chemical vapor deposition, and anodization. Chemical surface modification of titanium has been widely applied to alter surface roughness and composition and enhance wettability/surface energy (Bagno and Di Bello, 2004; Anil et al., 2011).

6.1 Grit Blasting/Sand Blasting

Blasting is explained as the use of abrasive particles against another material under high pressure in order to make it smoother, remove contaminants, or to roughen the surface. Titanium surfaces can be grit blasted with hard ceramic/metallic particles in order to roughen them. The particles are projected through a nozzle at high velocities by means of compressed air to the titanium implant surface and depending on the size and shape of the ceramic particle, which is polyhedral with sharp corners (Barriuso et al., 2014), and on the velocity of the blasting, erosion and material tearing of the titanium surface, is inflicted. The result is different surface roughness levels that can be produced on the implant's surface. The blasting material should be chemically stable, biocompatible and should not hamper the osseointegration of the titanium implants. The most common particles that are used are Alumina (Al_2O_3), titanium oxide (TiO_2) and calcium phosphate ($Ca_2P_2O_7$) (Parekh, Shetty and Tabassum, 2012).

Sand blasting, besides roughening the surface to increase the surface area, it also is a method that cleans surface contaminants and produces beneficial surface compressive residual stress. As a result, such treated surfaces demonstrate higher surface energy, indicating higher surface chemical and physical activities and enhancing fatigue strength as well as fatigue life (Oshida and Daly, 1990).

Topographic variations of the order of 10nm and less may become important because microroughness on this scale length consists of material defects such as grain boundaries, steps and vacancies, which are known to be active sites for absorption and thus may influence the bonding of biomolecules to the implant surface. There is evidence that surface roughness on a micron scale allows cellular adhesion that alters the overall tissue response to biomaterials. Microrough surfaces allow early better adhesion of mineral ions or atoms, biomolecules and cells form stronger fixation of bone or connective tissue, result in a thinner tissue-reaction layer with inflammatory cells decreased or absent, and prevent microorganism adhesion and plaque accumulation, when compared with the smooth surfaces (Oshida, 2007).

Alumina (Al_2O_3) or silica (SiO_2) particles are most frequently used as a blasting media, but because alumina oxide is insoluble in acid, its residues may have a negative effect on bone formation, principally caused by the release of cytotoxic silicon or aluminum ions in the peri-implant tissue (Aliofkhazraei, 2015).

Titanium oxide is also used for blasting titanium dental implants. Titanium oxide particles with an average size of 25μm produce a moderately rough surface in the 1–2μm range on dental implants. An experimental study using microimplants in humans has shown a significant improvement for bone-to-implant contact (BIC) for the TiO_2 blasted implants in comparison with machined surfaces. Other experimental studies confirmed the increase in BIC for titanium grit-blasted surfaces. Other studies have reported high clinical success rates for titanium grit-blasted implants, up to 10 years after implantation. Comparative clinical studies gave higher marginal bone levels and survival rates for TiO_2 grit-blasted implants than for machined turned implants. Wennerberg, Albrektsson and Laussma, (1996) demonstrated with a rabbit model that grit-blasting with TiO_2 or Al_2O_3 particles gave similar values of bone–implant contact, but drastically increased the biomechanical fixation of the implants when compared to smooth titanium. These studies have shown that the torque force increased with the surface roughness of the implants while comparable values in bone apposition were observed.

Using biphasic calcium phosphate (BPC) particles to roughen a surface by means of grit blasting, has been found to produce a more biocompatible surface, when compared to TiO_2 and Al_2O_3 surfaces, and with an average surface roughness of 1.57μm. These particles have been proven to not cause cytotoxicity to mouse osteoblastic cells of the MC3T3-E1 cell line. BCP treated implants have been proven also to promote an earlier cell differentiation and bone apposition when compared to alumina grit-blasted and machined surfaces. These particles can be also removed by means of acid-etching, providing so, a surface free of contaminants (Subramani and Ahmed, 2012; Basiuk and

Basiuk, 2015)

The particles that are projected with grit-blasting, while roughening the implant's surface, have been found to impair the mechanical performance of the implant. The projected particles can cause notch-like superficial defects, evidence of erosion and material tearing. Fine cracks can also be observed around particles that are firmly attached to the craters they created in the metal. These observations are evidence of a reduced long term mechanical performance. Grit blasted surfaces were found to have a reduced endurance limit of 25% when compared to polished surfaces (Shemtov-Yonan, Rittel, and Dogoroy, 2014)

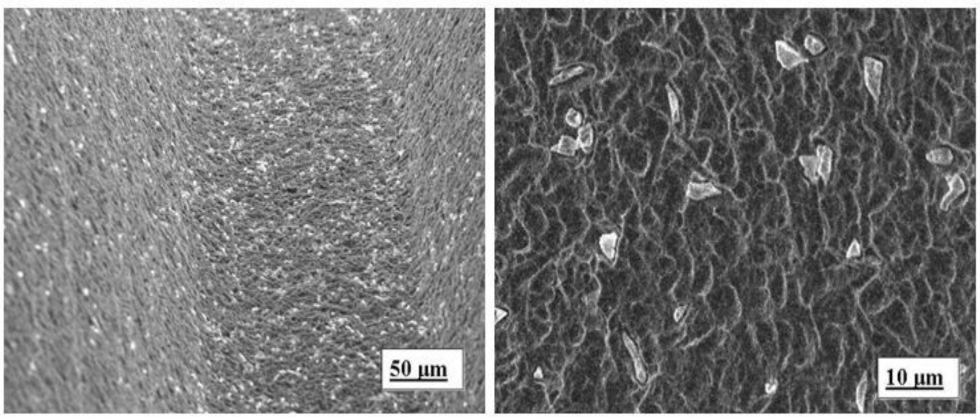

Figure 2: Particles of Titanium (left) and aluminium (right) encrusted in a sandblasted titanium surface (can be viewed at: http://www.intechopen.com/books/implant-dentistry-a-rapidly-evolving-practice/factors-affecting-the-success-of-dental-implants)

6.2 Acid Etching

Chemical etching with strong acids such as HCl, H_2SO_4, HNO_3 and HF is another method for roughening the surface of dental implants. Acid etching of titanium removes the oxide layer and parts of the underlying material producing micro pits on the implant surface with sizes ranging from 0.5 to 3µm and larger pits of approximately 6 to 10µm in diameter depending on the acid concentration, the acid solution, temperature and treatment time. The micro pits that are formed result in an enlarged active surface area that subsequently increases the retention and biomechanical interlocking between implant and bone as well as enhancing osteoblast activity with quicker formation of bone at the interface. This yields low surface energy and reduces the possibility of contamination since no particles are encrusted in the surface, having a positive effect on bone apposition, a higher percentage of contact surface area when comparing with a grit blasted surface and strong implant anchorage. This type of surface not only facilitates retention of osteogenic cells, but also allows them to migrate towards the implant surface (Wong et al., 1995; Juodzbalys, Sapragoniene, Wennerberg, 2003; Cho and Park, 2003).

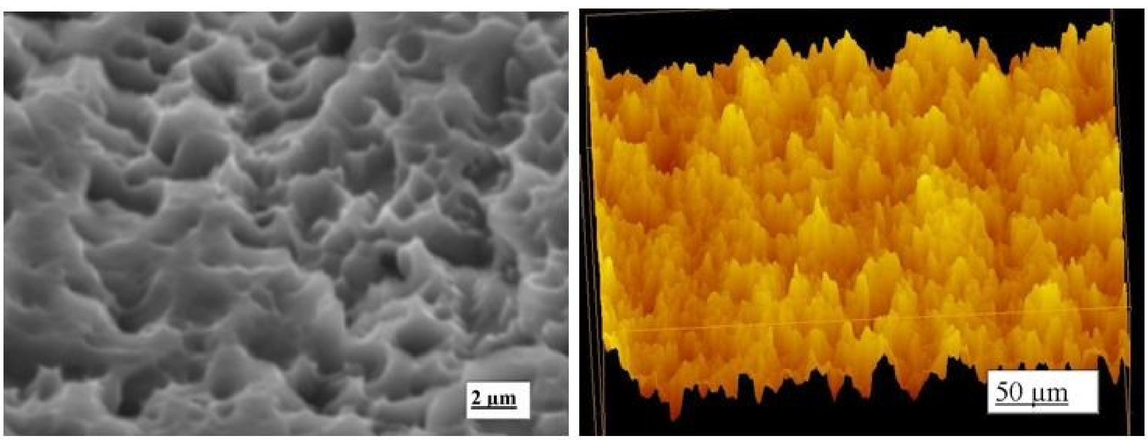

Figure 3: Microtopography of an acid etched Titanium dental implant (can be viewed at: http://www.intechopen.com/books/implant-dentistry-a-rapidly-evolving-practice/factors-affecting-the-success-of-dental-implants)

Each manufacturer has its own acid etching method regarding concentration, time and temperature for treating implant surfaces.

Etching time and etching temperature

A recent study made by Bruno Ramos Chrcanovic, Ann Wennerberg et al. (2015) evaluated how etching time and temperature influence the roughness parameters of a specific acid solution (HCl/ H_2SO_4). After treating the surface of 30 Ti discs with the same solution but with different etching temperatures and times it was observed that irregularities on the surface of the discs became deeper with increased etching temperature and increased in size and depth with increasing etching time. The increase in temperature changed the irregularity pattern from linear grooves with sharp edges to micro-pits and finally to deeper valleys, removing the grooves produced by the polishing process. An etching temperature of 60 or 90°C will provide a moderate roughness only after at least 15 minutes of etching time. But an etching temperature of 120°C is too high when the etching time is of 15' or more as the irregularities that the treatment produced were visible with the naked eye and in some sites peeled when scratched.

When comparing etching time and etching temperature it can be clearly observed from this study that temperature plays a more important role in the variance of the surface roughness parameters. Specifically, it was observed that 43.2% of the variance in Sa (mean roughness) is being explained by the temperature and 11.5% by the time. The numbers for Sdr and Sds were 34.6% and 31.3%, respectively for the temperature and 10.9% and 0.06% for the time (Chrcanovic, Wennerberg and Martins, 2015).

Acid Etching roughness and topography

Acid etching produces a minimally rough surface of Sa values around 0.5-3μm, depending on the acid solution, acid time and temperature, the bulk material and surface microstructure. Due to the presence of hydrogen ions in the acid, there is a speculation of a hybrid layer. The oxide layer has been found to be amorphous and with a thickness of 10nm (Sul, Johansson and Albrektsson, 2006)

Various studies have shown that this surface treatment provides a better osseointegration when compared to as-machined implants. It was found that bone to implant contact was higher 12 months after implantation and a higher removal torque after 1,2 and 3 months (Ballo et al., 2011).

In order for the acidic solutions to change the properties of titanium and roughen its surface they have to come in direct contact with it. But before attacking the metallic titanium, the acids must first dissolve the protective titanium oxide layer. During the course of the corrosion process of titanium, native hydrogen ions (H^+) are released. These small ions diffuse rapidly into the metal because the latter is left without its dense protective oxide layer; the sub-surface is therefore enriched with hydrogen (Aronsson et al., 2001). When saturation in hydrogen is reached, titanium hydride is formed. Titanium hydride may be biologically important because a hydride layer is much better suited as a template for binding biomolecules chemically into a titanium surface (Videm et al., 2008). GIRXD analysis have been shown that Ti hydride is present in Ti implants treated with acid etching.

Each acid etching treatment produces a unique surface topography with distinct surface properties that are dependent on various parameters, such as acid mixture composition, time of the treatment, temperature and prior treatments. A weak acid might not affect the morphology and low etching temperature might produce small micropits, for whereas a combination of strong acids in high concentration, high etching temperatures and treated for a considerable amount of time will likely produce a rough surface with numerous micro pits (Chrcanovic and Martins, 2014).

6.3 Grit blasting and Acid Etching

Soaking in acidic solutions implants that were previously grit blasted serves many purposes. The acid solution reduces the highest peaks, smoothening the irregularities caused by the blasting particles, reducing the average surface roughness to typically between 1-2µm. The acid serves as well as a removal of blasted particles, removing so contaminants, and creates a titanium hybrid intermediate to the implant and the titanium oxide layer (Conforto et al., 2004).

By rinsing the SLA implant in a nitrogen atmosphere and storing in saline solution until installation, the amount of carbon contamination could be reduced, improving the hydrophilicity of the implant surface (Rupp et al., 2006). The result of this procedure is a new hydrophilic surface (*SLActive*). Several studies have shown that *SLActive* implants achieve a better bone contact, earlier stability and reduce the healing time from 12 to 6 weeks when compared to SLA implants (Buser et al., 2004; Schwarz et al., 2007).

SLA implants have shown in *in vivo* tests as well as in *in vitro* tests a superior and faster osseointegration when compared with other surfaces, especially in the initial healing period, this could be explained to the higher production of cytokines and growth factors that were observed by Kieswetter et al., (1996).

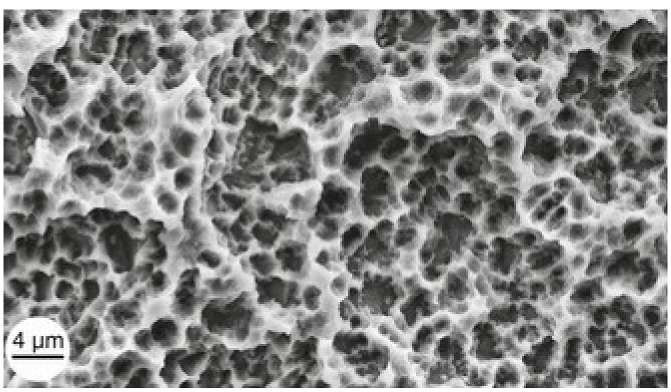

Figure 4: **SEM image of SLA/modSLA surface on c.p. titanium (can be viewd at:http://pocketdentistry.com/sandblasted-and-acid-etched-implant-surfaces-with-or-without-high-surface-free-energy-experimental-and-clinical-background/)**

Sand blasted and acid etched surfaces have a hydrophobic surface and the new *SLActive* implants have a hydrophilic surface which shows a stronger bone response. These have a Sa of 1.75µm and Sdr of 143%, which is indicative of the high density of the peaks than are seen in the SLA implants. The different etching processes also may lead to the formation of Titanium hydrides (TiH_2, TiH_3, TiH_4 or a combination of them) and the replacement of hydride by oxygen results in the slow transformation of the implant surface, resulting in nanometer sized particles of titanium on the surface. The nano roughness may be important in the protein adhesion, immediately after the implant placement. Sand blasting and etching can increase the rate and amount of the bone formation. The alkaline phosphatase specific activity was enhanced and osteocalcin production, the latent transforming growth factor beta and prostaglandin E2, all which were involved in the bone formation were found to be increased (Garg, Bedi and Garg, 2012).

M. Herrero-Climent et al. (2013) performed studies *in vitro* and *in vivo* with 4 different implant treatments. One control group (as-machined), one acid etched group (0.35M HF acid, 15'', RT), a grit blasted (Al particles 600µm in size, 0.25Mpa pressure) and a grit blasted and acid etched group. The *in vitro* results showed a high surface roughness for the grit blasted and SLA groups (average roughness Ra= 4.74 and 4.23 respectively) and a moderate for the AE group (Ra=1.69). It was found that the roughest surfaces showed the highest number of adhered cells, with the Gblasted and Gblast+AE surfaces showing almost double figures in comparison with the control and the AE group, which in turn, did not improve the cell adhesion. Ti samples were implanted into white rabbits and retrieved after 1, 3 and 10 weeks of implantation in order to test osseointegration *in vivo*. After histological analysis it was found that only Gblast and Gblast+AE samples presented new immature bone at the periimplant area. The other groups presented only the originally-machined bone during surgery which was in contact with threads of the implants. This bone provided good initial stability. Results from this study confirm that an increase in roughness translates to a higher cell adhesion, and that acceleration of osseointegration at short-terms of implantation can be achieved by Gblast and Gblast+AE implants. Roughness and topographical features are the most relevant of surface properties for the biological response.

6.4 Plasma Spraying

Biomaterial is any material, substance or combination of substances, of natural or synthetic source that interacts with biological systems to stimulate the growth or replace any tissue or organ for any period of time (Binyamin et al., 2006). Titanium is the most widely used biomaterial in the manufacture of implants for several uses, especially as bone replacement. This is because it has showed excellent mechanical properties as well as resistance to corrosion and biocompatibility (Tsaryk et al., 2007).

Though biocompatible, exposure to Ti in a living system has been linked with increased H2O2 and other reactive oxygen species (ROS). It is well known that ROS are now appreciated to play several important roles in a number of biological processes and regulate cell physiology and function. ROS are a heterogeneous chemical class that includes radicals, such as superoxide ion O_2(•-), OH (•) and NO(•), and non-radicals, such as H_2O_2, singlet oxygen ((1)O_2), HOCl, and NO_3 (-) (Vara, Pula, 2014). Despite their physiological roles, ROS can also damage several biomolecules and all aerobic organisms have developed defenses against them, these include antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase, which regulate the oxidative stress of the cells. Unbalanced ROS and reactive nitrogen species (RNS) generation can cause lipid peroxidation, protein oxidation and DNA damage, which could potentially lead to genotoxicity (Freires de Queiroz, et al., 2014).

Different surface treatments show different genotoxicity, one that has gained attention due to its lower genotoxic and cytotoxic results is plasma spraying.

Plasma is obtained through an electric gas discharge, applying a potential difference between two electrodes inserted into a chamber at pressures below 100Pa. The ions produced are accelerated on the cathodically polarized electrode doing several effects such as the creation of defects on the surface (Alves et al., 2005).

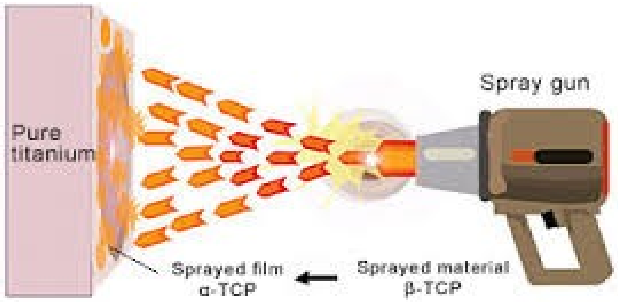

Figure 5: A spraying raw material powder is passed through the plasma flame for spraying on pure titanium as a base material.

Argon plasma spraying produces a surface with good mechanical properties without changing its chemical composition. It was shown as well from studies made by Freires de Queiroz et al., (2013) that argon plasma spraying induces lower genotoxicity than an untreated surface. For this study Ti disks were bombarded with pure argon atmosphere, using a plasma energy source, generating a plasma treated surface (PTTS). The treatment produced an uneven surface with a roughness of (Ra) 0.11μm. PTTS were found to be more hydrophilic when compared to control. Cytotoxic assays showed that though the cells on both surfaces (control and PTTS) were under oxidative stress conditions, as H_2O_2 production was higher when compared to the negative control, H_2O_2 levels of the untreated surface were significantly higher and that decreased the viability of cells on those implants as was confirmed by viability assays. Results showed that despite the PTTS surfaces inducing oxidative stress to the adhered cells, the higher hydrophilicity of those surfaces protected partially the cells from suffering DNA damage, thus proving to be less cytotoxic than untreated surfaces.

A decrease of corrosion resistance of the plasma treated surfaces indicated a thin oxide layer film and propensity of oxygen evolution reaction, causing the increase of superoxide anion radicals at the extracellular medium that in turn stimulate the release of SOD3. SOD3 catalyzes the dismutation of superoxide anion radicals into H_2O_2 and O, in turn H_2O_2 crosses the cellular membrane and increases intracellular ROS levels. This doesn't happen in untreated surfaces, the increased stress on these surfaces was attributed to their low hydrophilicity, and to the higher DNA damage when compared to PTTS (Freires de Queiroz et al., 2013).

It has been shown that a high oxidative stress increases the activity of antioxidant enzymes (Mates, 2000; Husain et al., 2003). Another study though showed that it didn’t affect, or reduced it slightly their activity, but increased the gene expression of them, leading to an excessive consumption or degradation of those enzymes and in succession adhered cells can't cope with the accumulation of ROS resulting to oxidative damage of the cell (Lino-dos-Santos et al., 2011).

A thick oxide layer is important in Ti implants because it improves corrosion resistance, avoiding the release of particles, ions and unstable ions during the electrochemical process of corrosion and oxidative stress, improving the biocompatibility (Tavares et al., 2009)

Yang, Ong and Tian, (2002) performed *in vivo* tests on beagles with 4 different surface treatments (sandblasted Ti, PS porous Ti, sandblasted and ion implanted (SIT) and PS and ion implanted (PSIT). After retrieving the implants in different time periods (2, 4 and 8 weeks) it was found that after 2 weeks there was already newly formed bone around the implant and at 8 weeks the new bone was identical to the preexisting bone and concentrated on the Haversian canal, indicating a complete healing of the drilled bone area. At a higher magnification the newly formed bone was observed to grow within the pores of PSIT 100-200μm, suggesting mechanical interlocking between the implant and the bone. It was also observed that when Ti concentration was decreased, Calcium and Phosphate concentrations increased, confirming the presence of a Ca-P layer at the PSIT implant. That layer increased from 4-6μm to 30μm in a period of 4 weeks (from 4th week to 8th week). A significant increase in the concentration of Ca and P in the SIT and PSIT implant was observed at the implant surface from 4 to 8 weeks suggesting a tendency for gradual mineralization to occur into the pores of the implants. Fracture tests performed on implant-bone blocks proved that the implants were tightly connected to the bone and mechanical interlocking was successful. TiO_2 was present in PSIT, confirming a thick oxide layer. The porous surface provided an increased contact surface that benefited implant fixation but at the same time did not result in an increased release of Ti ions that could harm the cells. From these results it was concluded that ion implantation helps plasma sprayed implants to improve osseointegration by means of favoring the mineralization process when compared to a PS treatment.

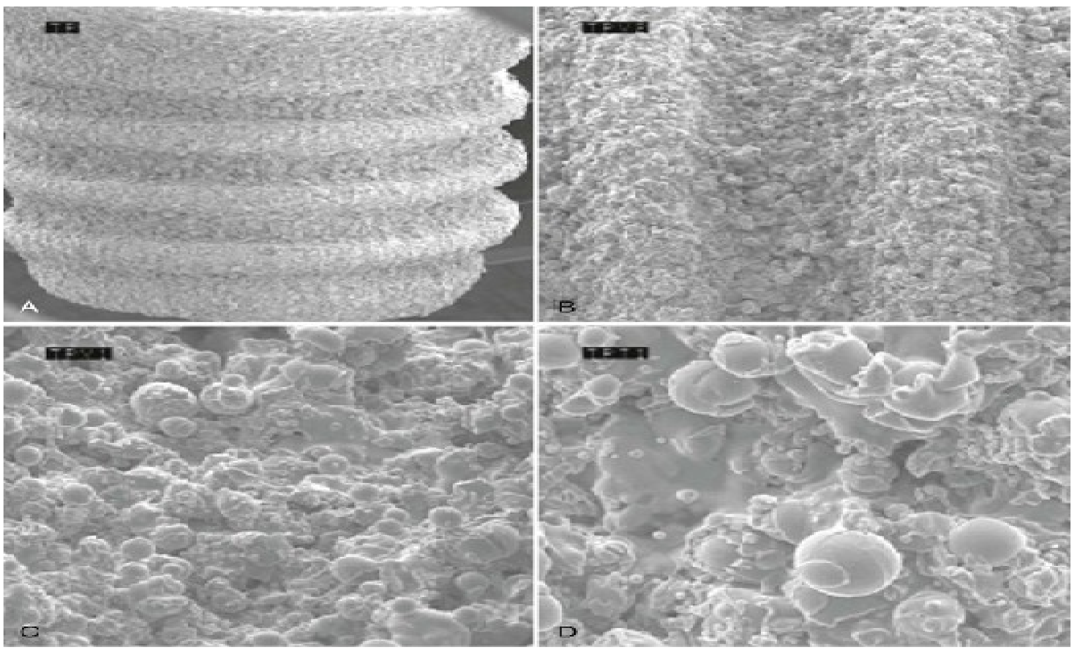

Figure 6: Scanning electron microscope (SEM) image of titanium plasma–sprayed surface implant with rough surface characteristics. A, **Implant with titanium plasma–sprayed surface (×40).** B, **notably complex macrotopography on titanium plasma–sprayed surface (×100).** C, **Titanium plasma–sprayed surface with 1- to 25-µm particles (×500).** D, **Titanium plasma–sprayed surface with 1- to 25-µm particles (×1000). (image can be viewed at: http://pocketdentistry.com/71-periimplant-anatomy-biology-and-function/)**

6.5 Hydroxyapatite Coatings

A plasma spraying gun can be used in order to obtain a coat. Through a powder feeder Titanium or HA particles can be fed to the plasma spraying gun and projected at very high temperatures and speeds onto the substrate which is positioned at a controlled distance, roughening the implant's surface while at the same time forming a coat, with a composition expected to be similar to the initial powder (Denmati et al., 2011).

An HA coat has been reported to improve the osseointegration of a cementless metallic implant. HA is chemically similar to the bone, and is a source of calcium and phosphate to the bone-HA interface. Osteoblasts form osteoid directly on the HA surface coating, suggesting that the bone-implant interface is bonded both chemically and biologically to the HA (Goodman et al., 2013).

Traditionally, HA coatings have been thought of as osteoconductive. However, calcium phosphate biomaterials with certain 3-dimentional geometries have been shown to bind endogenous bone morphogenetic proteins, and therefore some have designated these materials with osteoinductive properties (Le Geros, 2002).

In studies performed in canine models, formation of new bone was found even at distances of 400μm from the HA surface, suggesting the gradient effect of osseoconductive properties of HA. Huang et al., (2015) evaluated the effects of HA coating in Ti implants on the *in vivo* biological performance of porous Ti alloy (Ti6Al4V). Vapor plasma spraying (VPS) is reported to produce a higher crystallinity than the conventional atmospheric plasma spraying (APS), VPS was used to produce HA coated and uncoated implants for this study. Implants were retrieved after 2 or 4 months pos-implantation. Analysis showed that the HA coating was scattered in the internal area of the pores and some interior regions were not covered, nevertheless bone formation was superior to that in the uncoated group at both time intervals. Reconstructed 3D images showed that newly formed bone was distributed into the peripheral region of the Ti at both time points, however histological analysis showed that immature bone penetrated into the core area of the coated surface at 4 months while at this time period the uncoated implant was filled with fibrous tissue at the same area, which may impede new bone ingrowth. Under higher magnification it was observed that newly generated bone was tightly connected to the HA coat of the HA-TI implant, without interposition of non-bone tissue, but the uncoated surface showed fibrous tissue, indirect bone-implant contact and gaps. It was observed as well that in the coated substrate numerous osteoblasts were distributed in a linear fashion along the exterior surface of the immature bone, which indicates a favorable bone formation. Based on these findings it was concluded that HA-coated implants provide a better osseointegration, bone-implant integration abilities and bone ingrowth than the uncoated one. Such an anchoring effect could improve the fixation strength of the implant, which may reduce the risk of surface coating delamination.

The bioresorption of HA coatings is still a matter of controversy. The two main methods of resorption include one that is solution mediated (dissolution), and another that is cell mediated via phagocytosis. The HA coatings undergo variable resorption which is dictated by numerous chemical, biological and mechanical factors including the composition and physico-chemical properties of the coating, the anatomical location, and whether micromotion is present at the interface with bone. Increased crystallinity appears to slow resorption of HA, and decrease bone ingrowth (Goodmanet al., 2013).

During the clinical use of HA-coated implants, failure may occur at the coating-substrate interface. Hydroxyapatite coatings delaminate from the Ti because of an insufficient adhesion to its surface. Compressive stresses as well as mechanical and thermal mismatch between Ti and hydroxyapatite decrease the adhesion as well (Yang, 2011).

In order to strengthen the interface bonding and reduce stress between the Ti substrate and the HA, a titanium bond coat (Cp-Ti) can be used. The bond coat was found to increase adhesion at such level that failure test analysis after push out tests showed that failure occurred between the bone itself and not at the implant-HA interface or HA-bone interface. This strong fixation of the HA coat to the implant was attributed to the rough surface of the plasma sprayed Cp-Ti, which provided high mechanical bonding with the

HA. Another important factor was the lower compressive residual stress in HA coating, after applying the Cp-Ti bond (Yang and Yang, 2013).

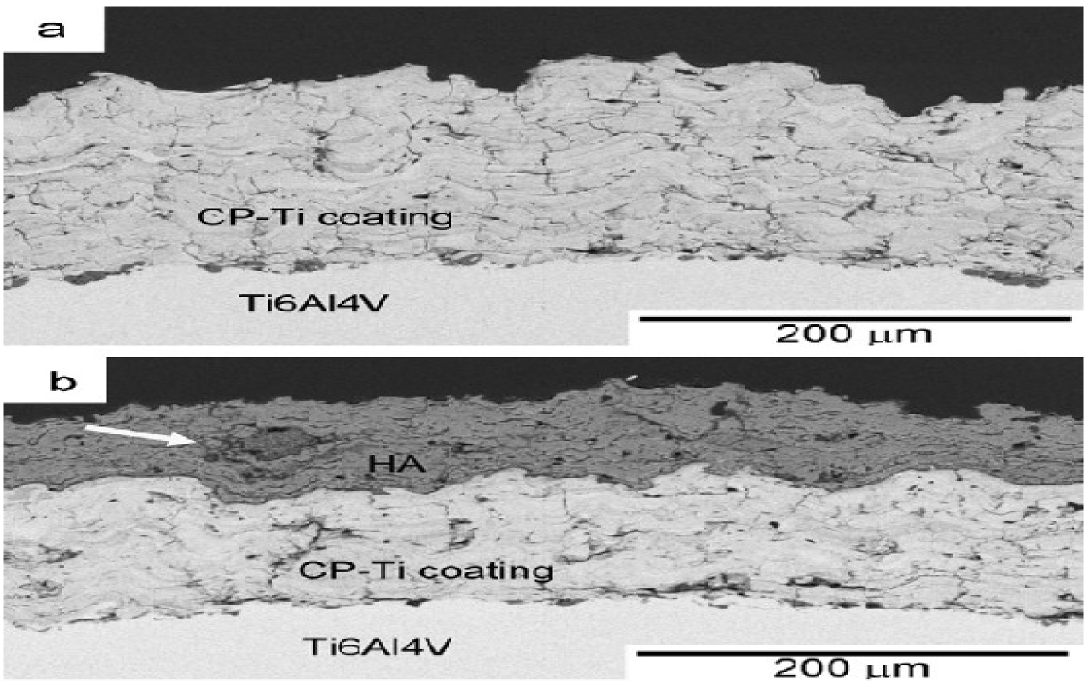

Figure 7: Cross-sectional BEI images of the (a) Cp-Ti and (b) HA coating on the Cp-Ti. (Image retrieved from the article Yang and Yang, 2013).

6.6 Anodic Oxidation

Anodized surface implants are implants which are placed as anodes in galvanic cells, with an acid (phosphoric or sulfuric usually) as the electrolyte, and current is passed through them. Immediately an oxide layer begins to grow from the native state of 5nm to approximately 10.000nm. Thin pathways are revealed in the exterior of the oxide barrier before true nanopores form. By continuing the process of anodization, propagation of individual pathways through the oxide barrier occurs, with their heads increasing in width. Finally, a porous structure is formed by cylindrical cells, situated close to each other, with each of them containing a pore at the center, separated from Ti with an oxide barrier (Wennerberg and Albrektsson, 2010).

The Sa of *TiUnite* is reported to be 1.1µm and its Sdr 37%. Another study reported the thickest *TiUnite* surface oxide coating of 2µm with anodic oxidation, with the implant having a rough surface with a pore size distribution of 0.06-12µm, showing the presence of micro and nano pores. According to Hall and Lausmaa, (2010) There is 5% phosphorus in the surface layer in the form of phosphates. At the implant surface, there is amorphous TiO_2 and the crystalline grains which are present in the amorphous matrix are of anatase TiO_2, although few spots could originate from thermodynamically more stable rutile (Jamer et al., 2008).

The formation of pores is directly related to the anodization variables (electrolyte concentration, anodization regime, etc.)

The main chemical reactions that occur during anodization are:

At the interface Ti/Ti oxide $\quad Ti \leftrightarrow Ti_2^+ + 2e^-$

At the interface Ti oxide/electrolyte $\quad 2H_2O \leftrightarrow 2O_2- + 4H^+$

$2H_2O \leftrightarrow O_2 + 4H^+ + 4e^-$

At both interfaces $\quad Ti_2^+ + 2O_2^- \leftrightarrow TiO_2 + 2e^-$

Hydrogen ions can also migrate to the cathode, where they capture electrons given from the anode, transforming in gaseous hydrogen, completing the circuit.

Oxide anodization of Ti in a solution of H_2SO_4 with a concentration of 1M, turned the surfaces porous in voltages between 90-180V. Electric discharge occurs when voltage is greater than 105V. Porosity and size of the pores increase by increasing voltage from 155V to 180V. They also increase by increasing concentration of the H_2SO_4 from 0.5M to 1M, but further increase in concentration, at least till 3M, hasn't shown any changes in porosity and pore size. Rutile and anatase phases can be observed in H_2SO_4 anodized surfaces, these phases are necessary for formation of apatite. It was concluded that oxidative anodization with H_2SO_4 as solution is a process that efficiently improves bioactivity of Ti, making immediate loading possible (Yang et al., 2004).

Adhesion and function of fibroblasts has shown to be improved in oxidized surfaces when compared to non-anodized. A study (Oh et al., 2006) showed 300-400% acceleration of adhesion for osteoblast cells inside layers of nanoporous Ti implants.

It was demonstrated that nanoporous layers improved endothelial cell mobility and increased intercellular interaction because of the nanoscale disposition. Contact area of this type of implants surface was found to be $78.3 \pm 33.3\%$ in comparison to only $22.7 \pm 24.7\%$ for Gblasted surfaces. Nanoporous TiO_2 surfaces create bone interlocking so strong that fracture tests showed that the fracture site was not at the interface bone-implant, but within the bone itself (Bjursten et al., 2010).

Osteoblastic cells grown in a porous layer are distributed better and extend more filopodia that interconnect neighboring cells. The reason behind this, is that nanoporous Ti not only provides a greater surface area and roughness, but also forms a configuration of interconnected cells (Oh et al., 2006).

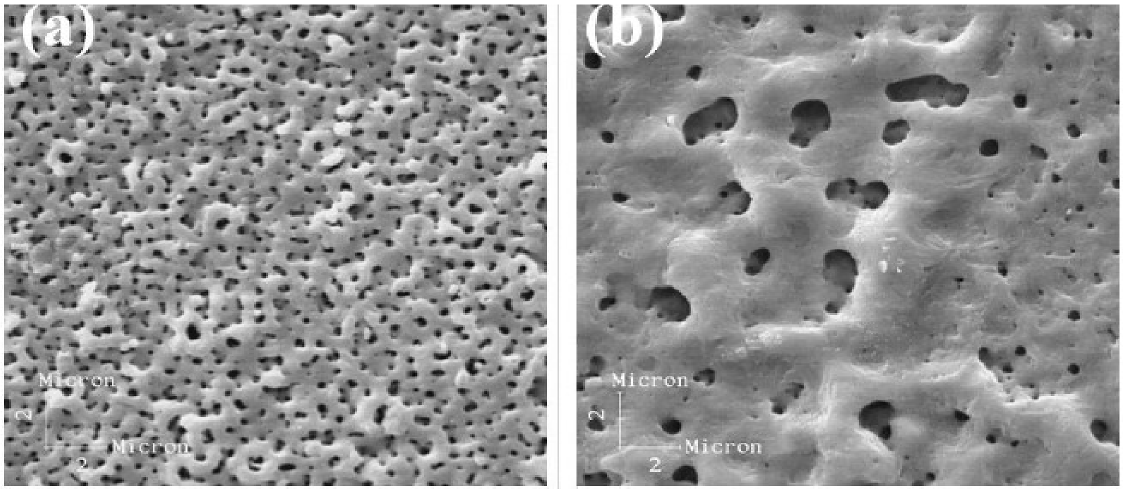

Figure 8: SEM micrographies (5000x) of the Ti anodic films produced in (a) 1.0M H2SO4/150V, and (b) 0.5M Na2SO4/100V (image can be viewed at: http://www.scielo.br/img/revistas/rmat/v12n1/a18fig01.jpg)

7) Discussion

Colocation of dental implants as a method to substitute missing teeth is one of the most common surgical procedures nowadays. Titanium and its alloy Ti6Al4V, due to its biocompatibility and excellent mechanical properties, has been the material of choice for most implantologists and oral surgeons.

Surface properties of Ti implants are very important, affecting its biocompatibility, osseointegration and cellular response, as well as changing its bioinert nature.

Wennerberg and Albrektsson, (2000) have shown that a 1-2μm roughness ideally promotes osseointegration. A hydrophilic surface has also shown to favor osseointegration (Buser, et al., 2004).

According to Yan Guo, Matinlinna and Tang, (2012) cellular activity and proliferation can be improved by a negatively charged surface. Studies have shown that cellular viability is decreased when contaminants such as Ti or Al particles can be found on the surface, due to the cytotoxic events they induce. A thin oxide layer can also disfavor cellular viability, since it protects the titanium implant from corrosion (Renvert,Persson, 2009; Cruz et al., 2011).

Since Brannemark used machining to increase the surface roughness of Ti implants, various surface treatments have been introduced, opting to improve osseointegration and biocompatibility of Ti implants. Namely in this study were explored the following surface treatments: grit blasting, acid etching, a combination of the previous two, plasma spraying, hydroxiapatite and titanium coatings and anodic oxidization. Other studies were not explored because the literature was not sufficient to take conclusions of their benefits.

Grit blasting increases roughness parameters of the titanium implant surface, improving osseointegration. As for Oshida, (2010) particles projected during blasting can contaminate the surface by releasing cytotoxic ions, impairing bone formation and inducing inflammation of the surrounding tissue, thus, the probability of implant failure increases.

According to Le Guehennec et al., (2007) acid etching has proven to increase the roughness of Ti implants, promoting early osseointegration and healing of the periimplant area. Each acid treatment produces a distinct surface topography with different roughness parameters. The acid molecules can be found in the surface of titanium implants sometimes in low concentrations, though, this doesn't affect the biocompatibility and cell viability.

Conforto et al., (2004) explains how acid etching can be used after a grit blasting process in order to eliminate the blasted particles, improve surface roughness parameters of Ti implants as well as improve their mechanical performance. Complete elimination of the particles though, is not possible. Rinsing an SLA implant into a nitrogen atmosphere has shown to have increased hydrophilicity (Rupp et al., 2006).

According to Freires de Queiroz et al., (2014) plasma spraying can improve hydrophilicity but also thinner the oxide layer of the Ti. While hydrophilicity increases osseointegration, a thin oxide layer can lead to corrosion and subsequently accumulation of ROS in the surrounding tissues. Generally, plasma spraying has shown to have positive effects on osseointegration and to improve the biocompatibility of the implant.

HA coatings were developed in order to increase the biocompatibility of Ti. While HA interacts with the surrounding tissues in a much better way than Ti, the interface Ti-HA has shown to fail because of delamination of the coating. This occurs due to the mechanical stress and thermal mismatch between Ti and hydroxyapatite (Yang, 2011). In order to improve the mechanical connection of these two materials, a Ti bond can be introduced between the titanium substrate and the HA coat (Yang and Yang, 2013).

According to Oh et al., (2006) anodization provides a porous surface that osteoblasts can proliferate and grow within its pores. The interconnection of cells in this surface is at a very high level, improving by that mechanical interlocking of the bone-implant interface.

According to Yang et al., (2004) anodization turns Ti bioactive, achieving early osseointegration, making immediate loading of an implant possible.

Implant manufacturing brands don’t make available a full description of the treatment procedure their implants are subjected to, so it is not possible to understand all the factors that are responsible for the *in vivo* and *in vitro* results that are published from the authors.

Due to the fact that chemical and physical parameters of an implant surface can influence one another, there is no full understanding of how each one of them separately can influence cellular behavior.

III. Conclusion

This review explored various titanium surface treatments and how they influenced the mechanical, chemical and physical properties of it as well as how these changes influenced its osseointegration and biocompatibility. Through the literature there were indicated the surface characteristics of a titanium dental implant that would ideally influence its osseintegration. The “ideal” surface is one with an average roughness of 1-2μm. An ideal surface should be charged in order to attract calcium ions and proteins that will initiate the matrix formation. A negative charge has been shown to be more desirable. A titanium surface with high free surface energy enhances hydrophilicity by increasing its wettability and promotes adhesion of blood components. An increased wettability enhances the biocompatibility of an implant, promoting interactions between the implant and the biological environment. It is of great importance that no contaminants are presented in a titanium implant surface, as they can induce cytotoxicity in the living tissues. A thick oxide layer is important as well, as it protects the implant from corrosion and subsequently mechanical wear that can lead to leakage of titanium ions and particles which can in turn induce cytotoxicity and genotoxicity to the living tissues.

The biocompatibility of the implant can be improved by introducing a hydroxyapatite coat. The problem with this coat though, is that it fails mechanically because of the thermical and mechanical mismatch between the coat and the bulk material. A bonding coat of Cp-Ti has been shown to improve the mechanical performance of this type of surface. A biomimetic surface that can attach to the implant and succeed mechanically is a very promising direction that could be the future of dental implants.

More studies are necessary to be made that isolate each chemical and physical factor so the understanding of each one can be completely clarified.

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