

Treatments for enhancing the biocompatibility of titanium implants

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Titanium surface treatment is a crucial process for achieving sufficient osseointegration of an implant into the bone. If the implant does not heal sufficiently, serious complications may occur, e.g. infection, inflammation, aseptic loosening of the implant, or the stress-shielding effect, as a result of which the implant may need to be reoperated. After a titanium graft has been implanted, several interactions are crucial in order to create a strong bone-implant connection. It is essential that cells adhere to the surface of the implant. Surface roughness has a significant influence on cell adhesion, and also on improving and accelerating osseointegration. Other highly important factors are biocompatibility and resistance to bacterial contamination. Bio-inertness of titanium is ensured by the protective film of titanium oxides that forms spontaneously on its surface. This film prevents the penetration of metal compounds, and it is well-adhesive for calcium and phosphate ions, which are necessary for the formation of the mineralized bone structure. Since the presence of the film alone is not sufficient for the biocompatibility of titanium, a suitable surface finish is required to create a firm bone-implant connection. In this review, we explain and compare the most widely-used methods for modulating the surface roughness of titanium implants in order to enhance cell adhesion on the surface of the implant, e.g. plasma spraying, sandblasting, acid etching, laser treatment, sol-gel etc., The methods are divided into three overlapping groups, according to the type of modification.

Key words: titanium treatment, osseointegration, biocompatibility, surface modification

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INTRODUCTION

Bone is the second most commonly transplanted tissue^{1,2}. For present-day treatment of degenerative diseases, such as arthritis and traumatic bone damage, the replacement of bone tissue by an implant is an option when conservative treatment has already failed. Bone tissue is characterized by excellent regenerative and remodelling capabilities. There are several methods for treating bone diseases and injuries. In the case of small-scale tissue damage, the bone is self-regenerating. For larger-scale injuries, it is necessary to use optimal bone replacement therapy³. However, many traumatic and also non-traumatic bone injuries require treatment with bone substitutes or with grafts, depending on the extent of the defect and the loss of bone volume^{1,2}.

One approach for the treatment of traumatic bone damage is to transplant a bone graft, which may be of autologous, allogeneic or xenogeneic origin⁴. This method is necessary to maintain the patient's quality of life, and it is mainly used for treating disorders accompanied by a bone volume loss, e.g. due to non-union as a result of bone fractures, removal of bone neoplasm, osteomyelitis, osteonecrosis, cyst formation, etc⁵. An *os ilium* bone graft has been considered as the "gold standard", but the use of bone tissue from an allogeneic donor is ten times more common than the use of an autologous graft⁶. These clas-

sical operations are often associated with graft problems, donor morbidity, low graft availability, and, in the case of allogeneic grafts, with the risk of disease transmission and an undesirable immunological response of the organism^{5,6}.

Synthetic grafts and implants, made of a variety of metallic, ceramic and polymer-based materials, are currently successfully used, but they also have limitations that lead to implant failure and to the need to reoperate^{5,7}. Biomaterials used for bone implantation should meet high requirements, such as long-term material durability, biocompatibility, corrosion and wear resistance, and biomechanical compatibility⁸. Implants for replacing missing or damaged bones, or for interconnecting bone fragments, must not only be mechanically resistant, but must also quickly integrate with the host organism and must perform their functions as soon as possible and for as long as possible².

A biomaterial is defined as any organic or inorganic material used in medical devices interacting with biological systems in order to treat, enhance or replace any tissue, organ or function of the human body. Several materials are used for implantation into the human body, namely various types of metals (non-corrosive steel, cobalt alloys, titanium alloys), ceramics (alumina, zirconium, calcium phosphate), and natural or synthetic polymers⁹.

After a biomaterial has been implanted into the patient's body, there are mutual interactions of the two sys-

tems¹⁰. This reaction is dependent on the physicochemical properties of the material, which can induce various cellular responses. Thus these properties affect the adhesion of the cells to the implant¹¹. Sometime after implantation, undesirable problems may occur, such as infection, inflammation, insufficient healing or aseptic loosening of the implant, or the stress-shielding effect, where the bone degrades at the point of contact with the implant¹². In addition, the material may release toxic substances (aluminium, vanadium, etc.) into the surrounding tissue, leading to damage to the tissue¹³.

A biomaterial can be evaluated by the following biological properties, which express the cell response to the implant material: osteoconduction, osteoinduction, osteogenesis, and osseointegration. Osteoconduction refers to the ability of the implant to bind osteoblasts and progenitor cells within its three-dimensional structure, which is accompanied by cell migration and cell growth¹⁴. Osteoinductive materials stimulate the differentiation of primitive, undifferentiated cells towards osteoblasts, leading to osteogenesis¹⁵, which is defined as the generation of a new bone tissue from cells of the host tissue or of an autologous graft¹⁶. The term osseointegration refers to the ability of an implant to anchor into the surrounding bone tissue without forming an interlayer of fibrous tissue¹⁴.

Proper incorporation of a titanium implant into the bone tissue consists of the following steps: I) formation of a hematoma, inflammation, release of bone-inducing factors, migration of cells to the site of the injury, II) anchoring the implant into the adjacent bone, III) vascular neogenesis, IV) migration, proliferation and phenotypic maturation of osteoblasts, tissue ossification, and V) bone modelling and remodelling, establishing a strong connection between the bone and the implant¹⁷.

On the molecular level, immediately after implantation, water molecules begin to adsorb to the implant surface, forming a mono-molecular or bi-molecular layer. The arrangement of the water molecules depends on the atomic structure of the implant surface. Hydrated ions such as Cl⁻, Na⁺ or Ca²⁺ are attached to the surface of the water layer. Further, depending on the physicochemical, biochemical and topographical properties of the implant surface, blood proteins and other tissue proteins are adsorbed, desorbed and again re-adsorbed onto the layer. The cellular response can be positively influenced by the application of an inorganic calcium phosphate layer¹⁸. Other biomolecular factors, e.g. growth factors, induce a cellular response by activating specific signalling pathways. Various surface irregularities, e.g. protrusions, cavities, depressions at the macro-, micro- or even nanoscale level, also influence the interaction of the material with its biological environment. As a result, a film of proteins with various geometrical conformations is formed on the surface of the implant, and this further affects the cell adhesion to the implant and other cellular responses. Specific bioactive sites in these proteins, e.g. oligopeptidic sequences such as Arg-Gly-Asp (RGD), are recognized by integrins, i.e. cell adhesion transmembrane receptors, which mediate the association of proteins adsorbed to the implant with the cell cytoskeleton¹⁹.

Taken together, the biocompatibility of the implant is dependent on the ability of osteoblasts to adhere to the implant surface¹⁵. The ability of osteoblasts to adhere and spread influences their further ability to proliferate and differentiate. This process is essential to create a mechanically strong bone-to-implant linkage without a fibrous interstitial layer, i.e. without a layer containing fibroblasts and extracellular matrix.

The most widely-used bone implant materials are titanium alloys, especially for their inertness towards tissues and body fluids, their high tensile strength, high corrosion resistance, and biocompatibility²⁰. Titanium bio-inertness is mainly ensured by its high reactivity and oxygen affinity, which causes the formation of a protective film (TiO₂, Ti₂O₃ or TiO) on the surface of the metal, which is constantly renewed and is thermodynamically stable²¹. This film prevents the penetration of metal compounds from the implant material into the osteoblast culture or into the bone tissue *in vivo*, although not entirely, due to a continuous slow passive diffusion of the compounds through the oxide layer. At the same time, this film is well-adhesive for the calcium and phosphate ions necessary for the formation of the mineralized bone structure. However, this oxide layer is not ideally mechanically resistant and is not sufficiently bioactive to support direct binding of the implant and the bone. The use of such an implant then leads to the formation of fibrous tissue between the implant and the bone, which causes insufficient osseointegration and can lead to the implant being released even a long time after implantation^{22,23}.

Titanium and its alloys are the most widely-used materials for orthopaedic joint replacements, and also for the screws, splints and bone repair studs that are used for fixing internal bone fractures. The frequent use of titanium is due not only to the nature of the material itself, but also to the wide range of possible structural and chemical surface treatments by which this material can be modified to increase its usability in orthopaedics, and also in stomatology. The organism must not react to the titanium alloy as a foreign material; otherwise, a fibrous interlayer is formed which separates the implant from the bone, resulting in micro-movements and non-healing of the implant²⁴. Due to its mechanical properties, pure titanium without admixtures is not suitable. However, there are safe materials, such as niobium, zirconium and tantalum, that have been used as admixtures to produce titanium alloys. This has resulted in high-strength, low-weight alloys with excellent corrosion resistance and biocompatibility⁸. Due to the relatively low cost and the good workability of the material, Ti6Al4V is the most widely-used alloy²⁵.

The first applications of Ti-based implants included the use of polished metal implants. Subsequently, emphasis was placed on modifying the surface of the implant to accelerate osseointegration. For example, a polished titanium implant does not provide sufficiently fast osseointegration for some dental implants, so a very rough surface was developed, resulting in faster osseointegration²⁶. In order to improve the interaction of an implant with the surrounding tissue, its surface is treated by modifying its topography or by applying various surface layers.

The clinical success of these implants is determined by their interaction with biological fluids and tissues, and by conditions that would promote early osseointegration²⁷. By appropriately modifying the surface, a functional bone-implant interface can be adjusted and increasing osseointegration of the implant is achieved. This reduces the healing time of the implant, and allows early loading of the implant²². Another reason for treating the surface is to eliminate the formation of a soft fibrous tissue interlayer between the implant and the bone⁴. Currently, several types of metallic material coatings are used in practical applications. Their advantages are a relatively high rate of healing and high bone strength after osseointegration, as well as long-term stability in the host bone^{2,4}.

AN OVERVIEW OF TITANIUM IMPLANT SURFACE MODIFICATIONS

The speed and the quality of osseointegration are related to the surface properties of implants, e.g. surface texture, topography, wettability, roughness, chemical composition and electrical tension of the surface. One way to increase the integration of osteoblasts with a titanium surface is by treating titanium and its alloys at the level of surface topography and surface morphology. Surface treatments are generally divided into additive methods and subtractive methods. Additive methods can be subdivided into two groups. In the first case, the material is only applied on the surface, without chemical bonding. This group includes plasma spraying, hydroxyapatite coatings, aluminium coatings, and calcium phosphate (CaP) coatings. The second option is to impregnate the substance into the implant material when chemical bonds are formed. This group includes impregnating the TiO₂ oxide layer on the implant surface of the implant with calcium phosphate crystals or incorporating fluoride ions into the titanium surface. Subtractive methods remove material particles and plastically deform the surface of the material, changing its roughness^{28,29}.

Another way to classify surface treatments, based on the type of modification, is to divide them into three groups: mechanical, chemical and physical surface treatments. However, these three groups overlap each other. The basic mechanical treatments include machining, grinding, polishing and blasting. Chemical methods, such as etching with alkali or acids, deposition of surfaces with chemical bonds on an implant, and anodization, are used not only to roughen the surface, but also to modify the chemical composition of the implant material and affect its surface wettability. Physical methods include plasma spraying and thermal spraying, laser surface treatment, spraying and ion deposition. Another option is to create a biologically active surface by depositing another layer on the surface of the implant by physicochemical and biochemical deposition methods.

The primary aim of all these methods is to roughen the originally smooth surface of titanium implants. This is expected to lead to higher cell adhesion to the implant and higher cell metabolic activity, as demonstrated by *in*

vitro experiments. These experiments are followed by *in vivo* experiments, in order to verify the findings obtained *in vitro*, and also to investigate the effects of the material modifications on the whole organism. It was shown that physical surface treatments (such as surface roughness) play a more important role than chemical modifications³⁰. Proper implant modification should result in improved bone-implant attachment and increased mechanical resistance after implantation. Many studies have demonstrated the optimization of dental and orthopaedic implants by modifying their surfaces chemically or topographically, e.g. by blasting, hydroxyapatite deposition by plasma, sandblasting, etching or anodizing³¹. Modifying the surface not only changes the microstructure, but also leads to other changes, especially a higher local density of the electrostatic charge and attractive forces, contributing to the acceleration of bone healing after implantation³². The modifications mentioned here, particularly acid and alkali etching or plasma treatment, can also lead to oxidation of the material surface, i.e. the formation of oxygen-containing chemical functional groups on this surface, which increase the wettability of the material surface, improve the adsorption of cell adhesion-mediating molecules from the ambient environment, and finally enhance the cell adhesion and growth¹³.

All these methods improve the osseointegration of the implant to a certain extent, but they also have limits that have a negative effect on the long-term endurance of the implant in the bone. According to Jemat et al.³³, plasma spray coating is one of the most widely-used treatments, with a proportion of approximately 40%. Other often-used treatments are acid etching, sandblasting and a combination of these treatments (SLA surfaces). Other treatments, such as ion implantation, laser treatment and magnetron sputtering are less used³³. The principle of the basic methods and their use in implantology is described in the following paragraphs.

MECHANICAL METHODS OF SURFACE TREATMENT AND THE IMPACT OF ROUGHNESS ON THE BIOCOMPATIBILITY OF TITANIUM SURFACES

As was mentioned above, the originally smooth implant surface needs to be roughened to increase the osseointegration of titanium implants. Generally, this can be achieved mechanically by abrasion, i.e. by removing material from the surface of the implant to the desired roughness, and in some cases by smoothing, i.e. by polishing the material. Microwells and other surface irregularities increase the size of the cell-binding surface of the material, which contributes to better cell adhesion to the implant surface. In addition, the larger surface of the material leads to improved biomineralization³⁴. Machining, brushing, polishing and blasting are used to take material from the surface and to shape it. A further effect is that the structure of the material is changed, whereby the crystalline structure becomes amorphous, and the surface hardness increases²².

The impact of mechanical surface treatment on implant osseointegration has been investigated many times. In general, a disadvantage of mechanical methods is that they are uncontrollable and inaccurate, and that substances with cytotoxic properties may be used³⁵. However, several factors influence the cellular response, the roughness of the titanium surface being the most important in mechanical machining. The optimal titanium roughness for osseointegration of the implant has not yet been well defined. Generally, the roughness can be divided into three areas according to grain size: macro-, micro- and nanoscopic scale. In addition to the surface roughness, an essential factor is the crystallographic orientation in the titanium material, which influences the geometrical conformation of the cell adhesion-mediating proteins, and thus the cell binding and proliferation. This inherent characteristic of a polycrystalline material can be used to improve its surface bioactivity and biocompatibility, and could therefore offer a way to improve the properties of titanium-based implants³⁶.

The roughness in the macroscopic scale can be defined in ranges from millimetres to hundreds of micrometres. Many studies have shown a positive effect of this modification, especially in the early fixation and the long-term mechanical stability of the implant in bone, in comparison with a smooth surface. This modification also significantly improves the resistance to the torsion forces acting at the bone-implant interface in comparison with other treatments³⁷. In such modified implants, however, the incidence of peri-implantitis is increased, and there is also a risk that ions will be released from the implant³⁸.

Implants with slightly rough surfaces in the micrometre scale achieve better properties. Microstructures with a grain size of approximately 1-10 μm minimize the risks mentioned above while maximizing the effect of attaching the implant to the bone and osseointegration³⁹. A surface with hemispherical holes 4 μm in diameter and 1.5 μm in depth was found to be optimal⁴⁰. However, even micro-scale mechanical treatment of titanium implants does not mimic the surface properties and the mechanical properties of bone, so that it can lead to implant failure due to insufficient osseointegration, bone loss or implant loosening²². It should also be taken into account that the cells, including osteoblasts, usually spread over tens of micrometers, and therefore the micrometer-scale roughness can hamper cell adhesion, spreading and subsequent proliferation, especially at higher densities of micrometer-scale irregularities on the material surface. The sharp spike-like morphology of the cells can also hamper cell adhesion, spreading and subsequent proliferation¹³.

Roughness in the micrometer scale can be achieved by mechanical machining, where various types of creases, wrinkles and dimples are formed on the surface. Another approach is to roughen the titanium surface by blasting it with hard ceramic particles. This is called sandblasting. Surface roughness is anisotropic, and the degree of roughness depends on the particle size⁴¹.

The most similar surfaces to the bone structure are on nanostructured materials, i.e. materials with irregularities less than 100 nm in size (nanomaterials). Modifications

that produce nanoscale surface roughness improve the adhesion of osteoblasts to the implant surface, as they contain sharp convex edges or spikes of nanorough titanium surfaces, where the magnitude of the negative surface charge density is highest³⁴. Different roughness of nanoparticles induces different adhesion energy, leading to high adhesion of osteoblasts and thus osseointegration, as has been confirmed by many investigations. In addition, it is believed that on nanostructured surfaces, cell adhesion-mediating proteins such as fibronectin and vitronectin are adsorbed in almost physiological conformation. This makes specific sites in these proteins, e.g. RGD-containing oligopeptides, accessible to integrin adhesion receptors on cells. Moreover, the nanostructured surfaces promote preferential adsorption of vitronectin, which is explained by its relatively small and linear molecule. Vitronectin is then preferentially recognized by osteoblasts rather than by other cell types, because osteoblasts bind specifically the Lys-Arg-Ser-Arg (KRSR) sequence in the vitronectin molecule. This suggests that nanostructured surfaces could lower the risk of fibrous encapsulation of a bone implant and could enhance its osseointegration¹³. However, it is currently complicated to fabricate a surface of reproducible nanoscale roughness. In addition, the optimum nanotopography has not yet been established for the adsorption of proteins leading to osseointegration⁴², although some studies, e.g. studies performed on poly(lactide-co-glycolide) (PLGA) enriched with nanophase titania⁴³, and on nanocrystalline diamond films⁴⁴ suggest that the most appropriate nanoscale surface roughness for osseointegration is root mean square (RMS) roughness close to ca. 75 nm, which is the surface roughness of the natural bone.

Sandblasting and abrasion of surfaces

The most commonly used mechanical treatment for obtaining implant roughness is sandblasting with hard ceramic particles of irregular shape. The particles are driven by compressed air and, due to their high speed, they form dimples on the surface⁴⁵. Various surface roughnesses can be produced, depending on the size of the particles. The ideal sandblasting material must be biocompatible and chemically stable, and must not hamper the osseointegration of the implant, e.g. by residues on the implant surface after modification, which may unfavourably affect the osseointegration of the implant. Alumina (Al_2O_3), titanium dioxide, calcium phosphate or bioactive glass is usually used as the sandblasting material^{27,45}. Improved osseointegration of sandblasted surfaces was confirmed by *in vivo* studies, where titanium dioxide and alumina utilization increased the biomechanical fixation of the implant in comparison with a smooth surface titanium implant³⁷.

The main limitation of alumina is the adherence of particles to the surface of the implant, and these particles cannot be eliminated by subsequent ultrasonic or acid purification or sterilization^{27,45}. The particles have a negative effect on the osseointegration of the implant and release residual material into the surrounding tissues. Furthermore, the corrosion resistance of titanium is impaired in the physiological environment⁴⁶.

In experimental studies, the use of titanium dioxide as a sandblasting material leads to better bone-implant attachment than machined surfaces⁴⁷. A closer and stronger bone-implant contact was achieved. In clinical trials, better bone implant properties have been demonstrated ten years after implantation. However, it has been proved that while the mechanical fixation to the bone is improved after the use of ceramic particle blasting, the biological fixation remains constant⁴⁸.

Calcium phosphate particles have been reported to be highly biocompatible, and mixtures with these particles (hydroxyapatite, beta-tricalcium phosphate and other substances) are therefore considered to be the ideal sandblasting material. Any residues on the surface of the implant after treatment are not a problem, as the phosphates are resorbable and osteoconductive. Experimental studies have shown a higher percentage of bone-implant contacts in this type of surface modification than for machined surfaces⁴⁹.

Other surface modification methods include surface mechanical attrition treatment (SMAT), which involves impacting the surfaces by milling balls made from alumina. Many types of research have been carried out on SMAT (ref.^{50,51}), showing that it improves the tensile strength and the fatigue resistance of metals⁵². After this treatment had been applied, pure titanium implants exhibited high strength. A nanocrystalline coating with increased strength, microhardness and corrosion resistance, which induced the cellular response, was also formed on the material surface⁵³.

CHEMICAL SURFACE TREATMENT

An increase in the osseointegration of titanium, which is otherwise inert to the cells, can be achieved by modifying the implant with a chemical coating. Titanium surface chemical treatments are based on the reaction of titanium with other chemicals, most commonly with acids, bases, hydrogen peroxide, and other passivating reagents⁵⁴. One of the most widely-used methods is apatite-based inorganic coatings; other methods include plasma spraying, electrodeposition, acidic and alkaline modifications, chemical vapour deposition, etc.

In general, chemical treatments result in increased titanium biocompatibility, conductivity, and bioactivity. They further modulate the surface roughness to optimum values, resulting in better cell adhesion. In this context, a wide range of novel biocompatible inorganic and organic coatings have been developed. Inorganic coatings include nanocrystalline diamond particles and films^{44,55} and zeolite films, e.g. silicalite-1 films (for a review, see⁵⁶). Organic coatings include particularly coatings based on ECM proteins, e.g. collagen⁵⁷, fibronectin⁵⁸, vitronectin⁵⁹, elastin-like proteins⁶⁰ and peptides, such as RGD- or KRSR-containing adhesion oligopeptides⁶¹, and antimicrobial LL-37 peptides, which also stimulated the migration and osteogenic differentiation of bone marrow mesenchymal stem cells⁶². However, these coatings, although promising, still remain at the experimental level.

Acid etching

Strong acids such as H_2SO_4 , HNO_3 , HF, and combinations of these acids, are used not only for roughening the titanium surface but also for cleaning it. The titanium surface etched by an acid is covered with microdimples 0.5 - 2 μm in diameter, which induce cell adhesion to the surface, and thus support subsequent osseointegration and bone formation, as has been proved by several studies⁶³. Various degrees of surface roughness can be achieved by selecting the appropriate type of acid and other parameters (concentration, temperature, etching time)⁶⁴. Using this method, however, it is not possible to create a specific, well-defined surface topography. Sulfuric acid is commonly used to modify titanium surfaces for biological applications, and gradually higher concentrations of this acid have induced an increase in surface roughness⁶⁵. Hydrofluoric acid is used for etching ceramic substrates in order to enlarge their binding surface for various reagents, rather than for treating titanium alloys⁶⁶. A mixture of two acids can also be used, or two acids can be applied sequentially. This process led to the formation of a specific surface topography that promoted the adsorption of fibrin, a provisional ECM protein, which accelerated cell adhesion to the implant and osteoconduction of the implant, especially when the acids were heated⁶⁷.

Larger amounts of bone-to-implant contacts, better osseointegration and bond strength, and less bone resorption at the bone-implant interface were found in acid-etched implants than on mechanically machined surfaces⁶⁸. Reactions of titanium with fluoride ions have a positive effect on the adhered cells, because of the production of soluble titanium fluoride (TiF_4), which promotes osseointegration⁶⁹.

As was mentioned above, acid etching can increase the surface wettability of titanium implants. The underlying mechanism is, at least in part, due to oxidation of the material surface⁷⁰. However, acid etching may also damage the protective oxide layer on the titanium surface. In this way, it can affect the cell proliferation by chemical components released from the implant⁷¹. Chemical surface treatment also has an adverse effect on the fatigue resistance of the material, since micro-cracks are formed on the surface of the implant, causing embrittlement of the entire surface⁷². The resulting reduced ductility of the material can lead to fractures of bone and dental implants⁷³.

SLA surfaces

SLA surfaces are obtained by applying acid to the mechanically-treated surface. A combination of sandblasting or blasting with titanium dioxide or with aluminium oxide particles and etching with a hot acid solution (H_2SO_4 , HCl or a combination thereof) is used, leading to the formation of a macroscopically rough dimpled surface. The surface is relatively coarse after blasting or sanding (the pits are in the macrometric scale), and it is irregular. However, after acid etching, the surface is more uniform, with small pits with an average diameter of 1-2 μm ⁶⁵.

The specific method for producing SLA surfaces combines the advantages of macro- and micrometric rough surfaces. The resulting surface achieves high osseointegra-

tion, and biologically active molecules, such as fluoride ions, can be bound to the oxide layer by hydrofluoric acid etching⁷⁴. Promising behaviour of SLA surfaces *in vitro* has also been verified *in vivo*, where improved bone-implant contact, improved stability of the implant in the bone in an early phase (6 weeks after surgery), and faster healing time have been observed⁷⁵. SLA treated surfaces using two-component etching (acids and bases) supported the adhesion of fibroblasts as well as bacterial strains⁷⁶. In addition, etching with two types of acids leads to better osseointegration and improved bioactivity of the implant⁷⁷.

On titanium surfaces, TiO₂ is formed at the interface of the material and the ambient air. This oxide layer can be hydroxylated when exposed to water. Changes in the charge distribution can lead to ion interactions on the surface and around the material, leading to TiO₂ contamination and further loss of hydrophilic material properties⁷⁸.

Some studies suggest that due to the hydrophobic properties of SLA surfaces and an insufficient nanostructure, these surfaces prevent osteoblast adhesion and differentiation and initial implant fixation⁷⁹. The modSLA method was developed around 2004 to maintain surface hydrophilicity. The production method is similar to the method for classical SLA surfaces. However, but immediately after formation, the modSLA surfaces are rinsed and are further stored in an isotonic sodium chloride solution under nitrogen to ensure surface hydrophilicity⁷⁸. Unlike SLA surfaces, the modSLA surfaces did not have a negative effect on proliferation and differentiation of osteoblast-like cells⁸⁰.

Titanium anodization

Another way to modify the surface of titanium and its alloys is to create nanotubular layers of titanium oxides by anodic oxidation. Nanotubular films are bound to the titanium surface by the chemical-thermal method of anodization. There are changes in the microstructure of the titanium oxide crystals⁸¹. The resulting morphology of the tubular oxide layer can be achieved by adjusting appropriate parameters, i.e. the anodizing voltage, the current density, and also the concentration, the composition, the pH and the temperature of the electrolyte. The resulting oxide layer is more than 1000 nm in thickness. Anodization uses a high current density of 200 A/m² or a potential of up to 100 V. The use of concentrated acids causes the oxide layers to dissolve and re-solidify along the flow lines, creating micropores⁸². Anodized surfaces produce a greater bone response than machined surfaces⁸³.

Anodization is a relatively advantageous and efficient titanium treatment technique. Its advantage in comparison with mechanical methods and etching lies in the controllability of the resulting titanium topography. The resulting coating is very well adherent, mechanically resistant and highly bioactive⁸⁴. A variable nanostructure of the titanium coating can be achieved by applying various currents, but the optimum topography has not yet been determined⁸⁵. *In vitro* and *in vivo* tests point to enhanced bone cell functions and good osseointegration, leading to a reduced risk of implant failure⁸¹.

Surface bioactivity can be enhanced by incorporating organic ions from the electrolyte into the surface structure (Ca, P, Mg). This leads to an increased bone response to the artificial material, resulting in better osseointegration, osteoconduction and bone-implant attachment⁸⁶. Incorporating Ag into a PLGA coating on nanotubular surfaces can prevent bacterial contamination of the anodized surfaces⁸¹.

PHYSICAL TREATMENTS OF TITANIUM SURFACES

Physical surface treatment involves applying thermal, kinetic or electrical energy without chemical bonding of the coating to the material surface.

Laser treatment

Applying a laser to a titanium surface may have various effects with various origins. When a high-power laser is applied to the titanium surface, melting and evaporation of a certain amount of material from the surface is induced. This changes the surface topography, and microstructures of micro- or nanoscale level are created. The melting and re-solidification of the material also changes its wettability, affecting the protein adsorption, the subsequent cell adhesion, and thus the biocompatibility of the modified surface. In addition to treating the material mechanically, which changes its morphology and roughness, it is also possible to induce changes in the chemical composition of the material surface by glazing. Depending on the parameters of the laser treatment and of the gaseous atmosphere (O₂, N₂, air, inert gases), various coatings on titanium can be produced chemically and structurally⁸⁷.

The basic method is surface texturization with the use of a laser, where the laser beam melts the material, and the removed material is then blown off the surface by a gas jet, most often with the use of argon. Various surface properties, differing in roughness and morphology, can be achieved by varying the parameters of the laser (power, movement speed, duty cycle, frequency). The purpose of the gushing gas is to remove the material, to cool the workplace in order to avoid heat damage, and to prevent surface oxidation and/or surface nitriding in the case of argon⁸⁸. Controlled formation of microstructures, particularly microgrooves, leads to cell orientation in the direction of the grooves, resulting in more effective bone regeneration on the implant surface⁸⁹. However, according to another study, laser treatment leads increased roughness and wettability, but the biocompatibility of these surfaces is worse than that of machined surfaces⁹⁰.

Lithographic methods are also used to change the surface morphology, using a mask on the implant surface in order to select areas to be removed or retained. The desired surface morphology is created by the form of the mask. The material is then removed by laser thermal decomposition⁹¹.

Finally, a laser is used to coat the surface of titanium with materials supporting the osseointegration and the

biocompatibility of the material. Calcium phosphate is the most commonly applied substance in implantology. The high-power laser melts the precursor powder deposited on the implant, which is then firmly adhered to the titanium material⁹². Laser-formed coatings improve the hardness, the corrosion resistance, the wettability and the roughness of the material, while supporting the cleanliness of the material and non-breakage of the oxide layer, which significantly improves the osseointegration of the entire implant⁹³. Pulsed-laser technology can also be applied for depositing Cr-doped or Ti-doped diamond-like carbon films on titanium implants. These films also have promising osseointegration properties⁹⁴.

No residues of chemicals that could have a negative effect on the adhesion and viability of cells remain on the material surface after laser treatment. *In vivo* studies have confirmed the positive effects of laser treatment, especially strong bone binding to the implant material and long-term resistance of the material to torsion strain. However, a disadvantage of all methods mentioned above is their inability to modify complicated surfaces with closed curves. More complex shapes are difficult to adjust for lithographic methods. A general problem with laser treatment is that the material is heated, and this can lead to microcracks and other undesirable microstructures caused by excessive heat⁹⁵.

Other laser applications

Powder metal sintering using Liquid Engineered Net Shaping (LENS), and Selective Laser Sintering (SLS) techniques utilizing high-power lasers, are used to create 3D structures of various kinds on titanium implants. As in the case of traditional 3D printers, the input material is melted and then a new structure is formed by a layer-by-layer technique. By adjusting the parameters of the laser, various porosity and material roughness can be achieved⁹⁶. A particular advantage of these methods is that implants with complex shapes and dimensions and with various pore parameters can be created. In addition, implants produced classically (e.g. by CNC milling) are more fragile than implants produced by sintering, and they also have lower fatigue strength⁹⁷.

Plasma spraying and hydroxyapatite-coated surfaces

The most widely-used method for surface treatment of titanium and its alloys is plasma spraying, which creates a morphologically defined porous surface of the implant with an excellent bone response⁹⁸. The coating is formed by heating the particles and injecting them onto a titanium implant, where they condense and form a uniform layer 5 - 400 μm in thickness (at least 40 μm for a uniform coating). The resulting coating is highly porous (e.g. pores $5.7 \pm 0.2 \mu\text{m}$ in diameter using ZrO_2) (ref.⁹⁹). The roughness can be increased by subsequent adjustments, e.g. by acid etching. A suitably high roughness of the implant facilitates its osseointegration with the bone¹⁰⁰. Hydroxyapatite is the most widely-used substance deposited by plasma spraying, as evidenced by the following sources^{79,99,101}.

Calcium phosphates, most often represented by hydroxyapatite, are among the most widespread coatings of titanium implants. There are several methods for depositing hydroxyapatite; the most widely-used is plasma spraying, which is also used commercially²⁷. Many research projects have shown that this modification leads to increased corrosion resistance of the implant, better attachment to the bone, increased mineralization of the tissue in the area surrounding the implant, increased mechanical resistance and biochemical binding of the implant in bone, improved cell proliferation around the implant, and increased osseointegration⁷⁹. The use of hydroxyapatite-coated implants also improves healing - the healing time is significantly shorter than for smooth implants³⁷. After implantation, calcium phosphate is released into the surrounding tissue, causing precipitation of biological apatite on the material surface. The calcium-rich matrix then serves as a suitable substrate for the attachment and growth of osteogenic cells¹⁰². It has been confirmed in long-term clinical experiments that this implant surface achieves a higher degree of biocompatibility and of osseointegration¹⁰³.

Plasma spray coating produces higher surface roughness than other treatments, such as acid etching and blasting¹⁰⁴. According to a clinical study performed on patients with dental implants, bone loss in the first year after implantation was found in the group of patients with plasma-sprayed titanium implants, and also in the group with SLA implants¹⁰⁵.

However, some studies have shown that the use of hydroxyapatite is harmful over a longer period of use¹⁷. Plasma-deposited hydroxyapatite is associated with clinical problems caused by delamination, i.e. separation of the hydroxyapatite layer from the surface of the titanium implant¹⁰⁶. The binding force of hydroxyapatite to titanium decreases over time, because the hydroxyapatite degrades, and even dissolves into the environment around the implant¹⁰⁴. Some studies have shown the wear of the implant, which has been proved by finding metal particles from endosseous implants in bone, liver, spleen, macrophages and para-aortic lymph nodes. There are concerns that these particles released from worn implants may become a source of cancer¹⁰⁰. The rough hydroxyapatite surface also contributes to the adhesion and growth of bacteria on the implant surface, resulting in subsequent inflammation. This occurs particularly in dental implants, due to their size and the complexity of their shape¹⁰⁷. However, research has generally confirmed that, despite these complications, the long-term success rate of hydroxyapatite-coated implants is similar to the success rate for other modified implants¹⁰⁸.

Sol-gel method

Transparent thin oxide layers can be obtained by the sol-gel (solution-gelation) method, where a hydrolytic or polymerization reaction of a colloidal solution (sol) to a solid phase (gel) occurs. Not only the composition of the colloidal solution itself, but also the properties of the gel can be further modulated by doping with other sub-

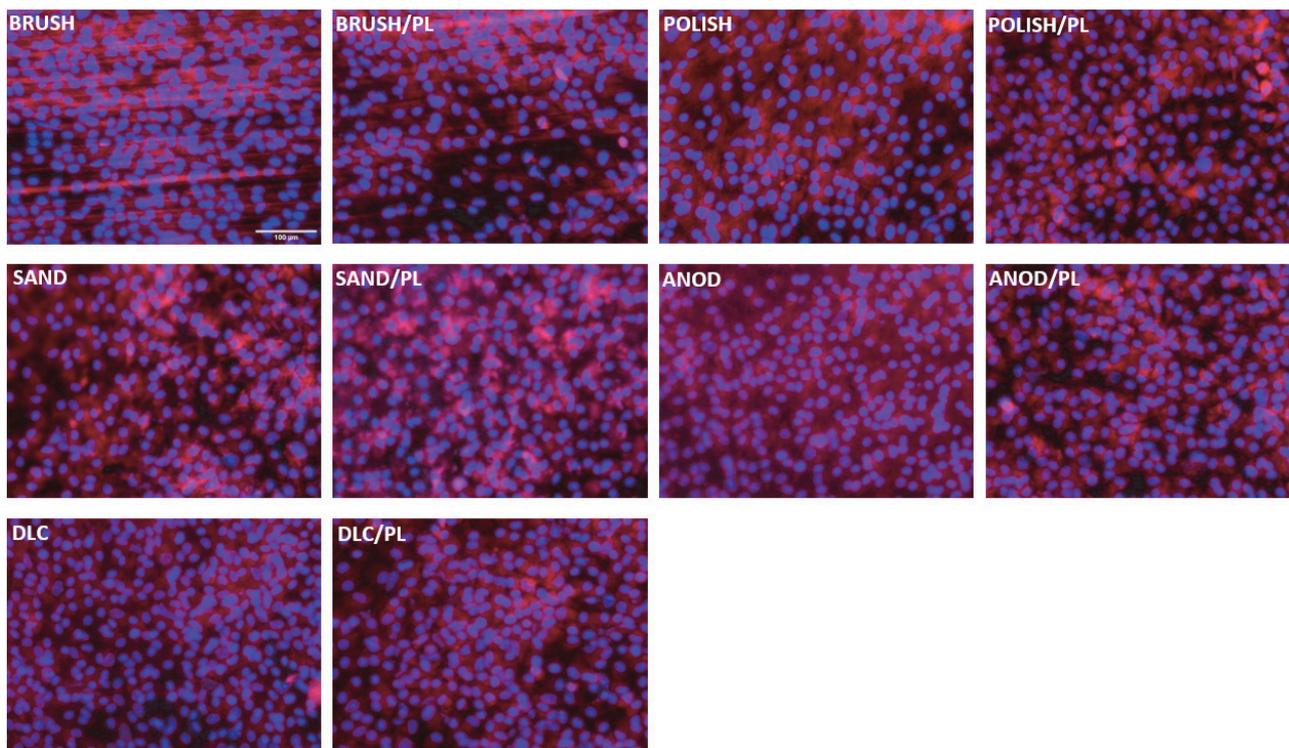


Fig. 1. Porcine adipose tissue-derived stem cells cultured for 7 days on titanium samples with various treatments (BRUSH - brushed, POLISH - polished, SAND - sandblasted, ANOD - anodized, DLC - diamond like carbon-coated), when /PL were oxygen plasma-treated. Cell nuclei were stained with DAPI, and F-actin was stained with TRITC-conjugated phalloidin. Leica DMI8 fluorescence microscope, obj. N-PLAN 10x, scale bar 100 μm .

stances or by annealing at various temperatures. Various production parameters form a gel in the form of monoliths, coatings, foams or fibres with a defined microstructure¹⁰⁹.

The production of sol-gel materials is relatively simple, not requiring conditions such as vacuum, high temperature, etc., and the final costs are relatively low. The favourable properties of the layers lead to their high biocompatibility, and with the use of this method even large implants of complex shapes can be coated. Agents regulating the gel degradation and various drugs for their controlled release can be incorporated into the chemical structure of the gel in order to increase the acceptance of the implant by the patient's body¹¹⁰.

Fig. 1. shows examples of Ti6Al4V samples, modified by various mechanical, chemical and physical methods and seeded with adipose tissue-derived stem cells (ASCs), which are suitable candidates for osteogenic cell differentiation¹¹¹. Specifically, these modifications included brushing, polishing, sandblasting, anodization and coating with diamond-like carbon (DLC). All these modifications were used without or with subsequent oxygen plasma treatment. All modified samples provided good support for the growth of ASCs and for the formation of confluent cell layers on day 7 after seeding. Oxygen plasma treatment had only a minor effect on further improvement of the cell growth, although in our earlier study, performed on nanocrystalline diamond films, this treatment significantly improved the growth and osteogenic differentiation of human osteoblast-like Saos-2 cells¹¹².

CONCLUSION

The aim of this study was to compare surface treatment methods for titanium implants, their complexity of production, the risk of adverse effects due to production, and especially their influence on surrounding cells and tissues. The results of the treatments are highly comparable, and it is therefore not possible to determine a best surface that is universal for all applications. Surfaces with micrometer-scale size irregularities have generally been considered to be more suitable for cell adhesion than smooth titanium surfaces. However, nanostructured surfaces have recently emerged as the most suitable surfaces in terms of their resemblance with the nanoarchitecture of the natural bone matrix. Some surfaces have been evaluated as quite negative during long-term experiments. However, the results of existing studies are inconsistent, and further studies are necessary. The most widely-used methods include hydroxyapatite coating, acid etching, sand blasting and SLA surfaces, which have proved that they are suitable for the treatment of titanium implants, and that their production is less demanding than for other methods. Other methods are not widely used.

Search strategy and selection criteria

This review has focused on methods that are widely used in clinical practice for increasing the osseointegration of titanium implants. Technologies that are now only in development at the experimental level have generally not been included, although some interesting approaches,

particularly those tested by our group, have been briefly mentioned. In addition, methods using surface modification with biological materials are beyond the scope of this review, and have been reviewed only briefly. The methods have been analysed with a view to further investigations of the differentiation of stem cells on titanium implants to increase their biocompatibility. The papers cited here were searched through the keywords “titanium surface treatment” and “titanium osseointegration”. Only articles published in international peer-reviewed journals and written in English have been included in this review.

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