Dentistry
ISSN: 2161-1122

Elkhidir and Cheng, Dentistry 2017, 7:6
DOI: 10.4172/2161-1122.1000435

Research Open Access

Achieving Superosseointegration: The Photofunctionalization Effect

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Recieved Date: May 02, 2017; **Accepted Date:** May 09, 2017; **Published Date:** May 16, 2017

Abstract

Osseointegration is the backbone of successful implant stability. Biological ageing of titanium implants decreases its bioactivity leading to less bone to implant contact. Ultraviolet photofunctionalization reverses the ageing process, increases the bone-implant contact to almost 100% in what is known as "Superosseointegration", and therefore increasing the strength and the primary stability of implants while decreasing the healing time. Photofunctionalization was shown to improve the prognosis, decreased morbidity and to have a significant impact on clinical practice. The aim of this review is to explain the events on the molecular level, the clinical implications of photofunctionalization and to highlight some of the other applications associated with this new technology.

Keywords: Osseointegration; Photofunctionalization; Biological ageing

Osseointegration

OI is the "direct structural and functional connection between ordered living bone and the surface of the load - covering implant at the histological level [1-3]. When this bone opposition occurs, without any soft tissue intervention between the bone and the implant surface, it translates clinically into a long-term rigid, stable fixation of implants into the surrounding bone [2]. Osseointegration is a prerequisite for primary implant stability and is by far the most important factor to consider before establishing any further treatment [3]. Knowing the biological events and the healing process occurring after placing an implant is therefore significant for further understanding of the different factors acting on this bone-implant interface.

Mechanism of Osseointegration

The process of bone formation involves attachment, settlement, proliferation, and maturation of osteoblasts followed by mineralisation around the secreted proteins [4]. Similar to primary bone healing, there is a cascade of biological events occurring around the implant fixture. The wound healing process starts within a few seconds of blood contact on the implant surface. Thrombocytes initiate the coagulation cascade, and then inflammatory cytokines along with other growth factors and vasoactive amines are released leading to the migration and proliferation of phagocytic cells. Neutrophils peak at the first three days following surgery to remove the dead cells and residues of bacterial extracellular matrix. Macrophages follow to help in the degradation process and express more cytokines, inducing further recruitment of osteogenic and endothelial progenitors [5]. Angiogenesis within the fibrin matrix occurs simultaneously with removal of coagulum. The formed fibrin matrix acts as a scaffold for colonisation of the migrating and differentiation of osteogenic cells such as osteoblasts and the recruited mesenchymal stem cells (MSCs) arriving as early as the first day. Upon arrival of these osteoprogenitor cells, matrix formation and mineralisation initiates by contact osteogenesis [2,3]. Following osteoid deposition on the implant surface, immature woven bone starts remodelling at the second week and becomes replaced by mature lamellar bone within three months [5] as shown in Figure 1.

Titanium

In the recent decades, Implant biomaterials have been widely investigated, aiming at finding and developing the most biocompatible implants biomaterials which can achieve high levels of OI and implant stability. Despite exhibiting better biocompatible nature and less foreign body reaction compared to other conventional materials [3,6] pure titanium and titanium alloys were found to also have good mechanical reliability, high corrosion resistance, as well as exhibiting low modulus of elasticity and considerable fatigue strength [7,8]. These excellent mechanical and superior physiochemical properties makes titanium much more favourable than other alloys like stainless steel or chrome-cobalt in regards to biocompatibility and clinical choice.

Tissue response is largely dependent on the nature of the implant surface. Unlike bioactive surfaces which participates actively in OI, bioinert surfaces does not play a role in this process [9]. Titanium and titanium alloys are considered bioinert according to their surface oxides, so the OI on their surfaces occur due to a lack of negative tissue response rather than occurring due to a positive one [3]. Titanium implant surfaces are osteoconductive and allows bone growth on its surface by distant osteogenesis [3]. Surface modification approaches have been focused on making it more osteoinductive in order to stimulate and recruit osteogenic cells and to exhibit contact osteogenesis [10].

Biological Aging of Titanium

The term biological ageing refers to the time-related degradation of the physiochemical properties of the implant surface [11-16]. It is well documented that following a sufficient healing period after placing titanium implants, the osseointegration level is usually less than ideal and the BIC does not reach 100% [12,13].

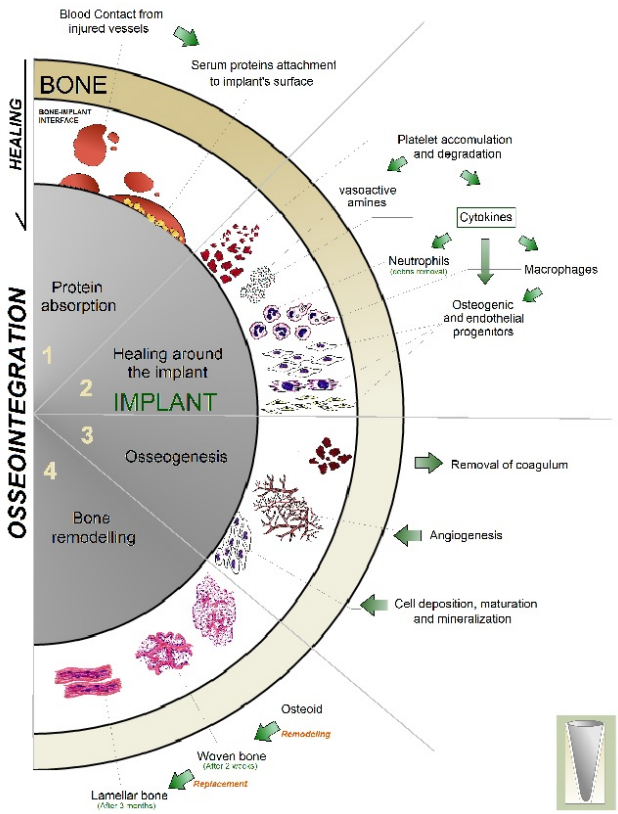

Figure 1: Schematic illustration of the process of osseointegration around the implant surface. 1) Serum proteins form a layer on the implant surface, 2) Clot formation and inflammatory process begin. Neutrophils remove debris and dead cells from the Extracellular matrix, cytokines released from platelets and further by macrophages are responsible for recruitment of osteogenic and endothelial progenitors (Osteoblasts, hMSCs, fibroblasts). 3) Angiogenesis starts simultaneously with the removal of the blood clot, and bone starts forming at the implant surface. 4) Osteoid becomes woven bone within 2 weeks and gets replaced by lamellar bone within 3 months.

This phenomenon is largely attributed to the unavoidable deposition of carbon from the atmosphere forming a layer of hydrocarbon on the titanium surface leading to significant loss of the hydrophilicity and surface charge from the implant surface. This causes impairment of the physiochemical and biological capabilities, resulting in a substantial decrease in osteoconductivity, poor osteogenic recruitment and proliferation as well as reduced absorption of serum proteins [17-19].

Extracellular matrix (ECM) serum proteins play a major role in accelerating osteoinduction and bone integration [20-22]. However, the affinity of these negatively charged serum proteins to the implant becomes severely affected by the gradual time-dependent change of the surface from positive to negative on the aged titanium surfaces [22-26]. As a result, the amount of bone covering implants and the strength of initial fixation is reduced to less than half compared to those seen in new surface [18].

Photofunctionalization

Light wavelengths visible to the human eyes ranges from 400 to 700 nm. Ultraviolet (UV) light on the other hand can be either UVA (320-400 nm), UVB (290-320 nm) or UVC (10-290 nm). The range used in biological investigations is 200-400 nm [27]. UVA light can remove hydrocarbons through inducing TiO2 photocatalysis [28]. UVC irradiation is considered superior in reducing the surface carbon levels, improving the hydrophilicity and enhancing the protein adsorption and cell function [29,30].

Ultraviolet (UV) PhF is the surface modification changes on titanium surfaces following UV treatment, including the alteration of physicochemical properties and the enhancement in biologic capability. It is a unique and simple mechanism which remarkably increases the biologic capacity of titanium implants and enhances osseointegration to nearly 100% BIC (Superosseointegration) compared to less than 55% for untreated implants [11,12,31,32].

PhF improves protein affinity to the implant surface, and drastically enhances physiological function as well as the expression of osteogenic cells phenotypes [11,18,19,32,33]. This "upgrade" in the OI capacity at the implant surfaces is caused fundamentally by inducing three property changes on the titanium implant surface:

- PhF regenerates the lost hydrophilicity caused by biological age ing of titanium, it converts titanium surfaces from hydrophobic to "Superhydrophilic" [12-15].
- It optimises the electrostatic status of the surface, reverting it from electronegative back to original electropositive status found on fresh titanium surfaces [18,19].
- It removes the significant amount of hydrocarbon that unavoidably accumulates on the surface by time (Figure 2) [31-33].

Hydrophilicity

Although the wettability of titanium surface is not an indicator of it's bioactivity, recent studies demonstrated that hydrophilicity and electric charge play key roles in the initial attachment of cells to UV-functionalized titanium [29,34]. A newly processed titanium surface is "superhydrophilic". This term is used when the contact angle of water to the surface is less than 5 degrees. Due to ageing of titanium, the implant surface gradually becomes hydrophobic with a contact angle more than 60 degrees after 4 weeks of processing [29]. UV treatment converts the aged surface from hydrophobic to superhydrophilic in conjunction with the removal of hydrocarbon contamination from the titanium's surface. The bioactivity of a UV-treated of 4 week old titanium surfaces becomes even higher than that of a newly processed surfaces [16,34].

Electrostatic status

UV-treated titanium surfaces are electropositive, whereas aged titanium surfaces are electronegative [18]. PhF of titanium oxide (TiO_2) causes excitement of an electron from valence band to conduction band and creates a positive hole on the superficial layer [19]. This results in the conversion of relevant Ti^{4+} sites to Ti^{3+} sites which are favourable for dissociative water adsorption to form basic Ti-OH groups [28,31]. The surface oxygen vacancies eliminate the need for inorganic bridges for cell attachment and adhesion and hence, enabling direct protein–titanium interaction or even a direct cell-titanium interaction. UV treatment, therefore results in more cells

attraction and stronger cell adhesion by converting the titanium surface from bioinert to bioactive [19].

Hydrocarbon removal

In contrast to other techniques aimed at modifying the surface topography of implants, such as oxidising, sandblasting, acid etching [35,36]. UV treatment does not Alter the topography, but it rather removes the surface hydrocarbons which are responsible for biological ageing. The surface of bulk titanium is made of a semiconductor TiO_2. Therefore, UV treatment removes hydrocarbons by induced photocatalytic activity of TiO_2 and a direct decomposition by UV. The removal of hydrocarbon results in the exposure of Ti^{4+} and may facilitate its interaction with biological cells that are electronegatively charged [28,31]. The level of hydrocarbons on the TiO_2 surface is inversely related to the level of protein absorption and osteoblast attachment [18,33].

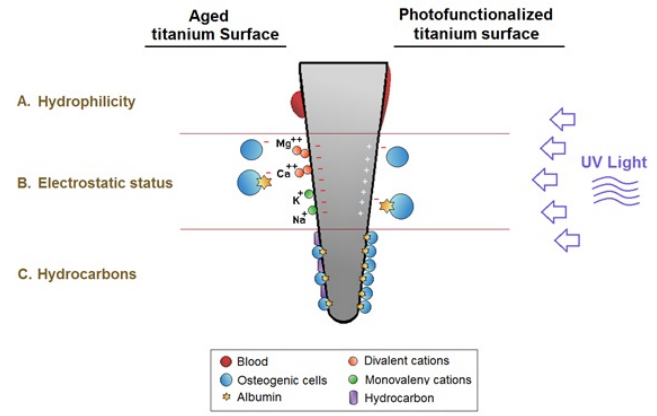

Figure 2: Schematic comparison between the physiochemical properties of the titanium's surface as received "aged" and the titanium's surface following PhF. A) Photofunctionalized surface shows much higher wettability across the implant surface than the non-treated surface. B) The surface charge on the UV treated surface becomes positive, allowing the negatively charged osteoblasts and stem cells to attach alone or through serum proteins. The non-treated surface is negatively charged and the only method of cells to attach is via bridging divalent cations (Mg++, Ca ++). Monovalent cations (Na+ and K+) competitively inhibit cell attachment. C) Photofuncionalization removes the hydrocarbon layer from the surface allowing for more protein absorption, and better attachment and spread of osteoblasts.

UV Effect at the Molecular Level

Studies have shown that PhF of titanium surfaces increases protein absorption, enhances osteogenic migration and attachment, as well as osteoblastic proliferation and differentiation [12,19,34-41].

Protein absorption

Biocompatibility of any material is highly dependent on the capacity of protein adsorption on it's surface [18]. Adhesive ECM proteins and transmembrane cellular receptors mediate cell attachment to titanium surfaces [29]. ECM fibronectin interacts with cell membrane integrins to facilitate osteoblastic adhesion and proliferation on the implant surface [12,19]. Vinculin is a cell-binding membrane cytoskeletal protein that binds (via other adhesion complex proteins) to integrins and actin filaments [37], which are cell adhesion membrane filaments that are highly essential in the establishment of cell adhesion and in cytoskeletal development [38,39]. Integrins are transmembranous "bridging" receptors. They bind to ECM proteins such as fibronectin and collagen and initiate cell attachment by ligand-specific RGD peptide interactions [40,41]. Actin is a major constitiuents of stress fibres which largely forms lamellipodia and filopodia extensions. Actin filaments also maintains cellular shape and are responsible for tension resistance [34,39]. The loss of vinculin prevents cell adhesion and spreading, stress fiber formation, and cellular extensions (Figure 3) [42,43].

Albumin on the other hand serves a carrier for molecules and plays a role in the metabolism of vitamin D in osteoblasts [44]. Albumin also regulates cytoplasmic calcium and stimulates osteoblast proliferationn [19,40,45]. Calcium regulation is important for attraction of anionic proteins because titanium surfaces have a net negative charge and must therefore first be bridged by divalent cations to compete with monovalent cations, such as Na+ and K+ which might block the anion sites, making a large part of the titanium surface bioinert for proteins and cells. However, although albumin enhances the fibronectin-integrin interactions [46]. Albumin itself is a competitive inhibitor to cell attachment [47-49], and it gets gradually replaced by fibronectin in reaction to the increased hyrophilicity following UV treatment (Figure 2) [50].

The amount of protein adsorption is inversely correlated with the amount of hydrocarbon on titanium surfaces [33]. Protein absorption drops to less than 50% in aged titanium surfaces. PhF of titanium surfaces causes a substantial increase in protein expression not only compared to aged implant surfaces but to new surfaces as well [12,19,34].

Osteoblast Attachment and Spreading

The initial attachment and spreading of osteoblasts is affected by the surface properties of different implant biomaterials [51,52] and it plays a critical role in OI [29]. Failure of cell attachment and spread on titanium surfaces leaves the cells undifferentiated and suspended in a circular form [53-56]. Adhesion of osteoblasts is regulated by the Rho family GTPases. These are signaling G proteins which play an important role in the dynamics of intracellular actin leading to assembly of stress fibres and in turn, contributes to the osteoblastic mobility and adhesive affinity to the titanium surface [34].

Biological ageing causes the number of attached osteoblasts to be reduced by about 50% and the spread of osteoblasts becomes noticeably delayed [12-15]. UV treatment restores the hydrophilicity and the electrostatic status of the surfaces leading to enhancned formation of focal adhesion complexes and the subsequent GTPase protein gene expression, causing a subsequent increase in the quality and quantity of osteoblastic adhesion and spreading.

Osteoblast Differentiation and Mineralization

Proper OI requires a well-mineralized matrix around the implant surfaces. The recruited osteogenic cells should be completely matured and differentiated in order to produce bone matrix and then mineralize. Alkaline phosphatase (ALP) is a byproduct and an indicator of maturing osteoblastic activity. Elevated levels of this enzyme are suggestive of enhanced osteogenic activity and active bone deposition [13,29].

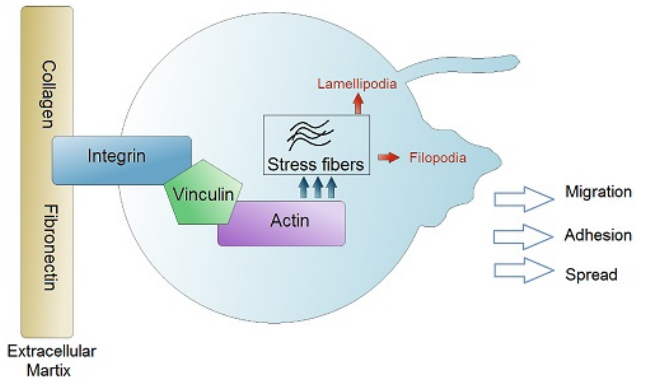

Figure 3: ECM collagen and fibronectin interacts with transmembranous integrins. Vinculin binds integrins to actin filaments, which are major constituents of cellular cytoskeleton and stress fibres. Lamellipodia and filopodia assess in cellular migration, adhesion and spreading.

Despite the drastic reduction of the total ALP activity on aged titanium surfaces by more than 50% [13,57] the cell-based ALP activity and osteoblastic gene expressions are not affected significantly. This indicates that the decreased levels of ALP activity and cell differentiation is actually due to the overall inhibition of osteoblastic attachment and proliferation [34] rather than biological ageing process itself. The increased rates of protein absorption and osteoblasic adhesion and proliferation on photofunctionalized titanium surfaces are therefore the reason behind the increased number of differentiated cells on the implant surface, as well as the increased matrix deposition and mineralisation.

Antibacterial effect of PhF

Preventing colonisation of bacteria on the implant surface is critical. However, bacterial contamination of implant surfaces during surgery is unavoidable. There are billions of bacteria in the oral cavity and over 600 different species [58,59]. Furthermore, a portion of the implant is positioned transmucosally during the healing period and is exposed to bacteria which is very similar to those found in periodontal disease [60,61]. These bacteria can utilise salivary and blood proteins to enhance their attachment on the implant surface [62-64]. Elimination of pathogenic microorganisms from implant surfaces is essential to avoid implant failure. Ultraviolet disinfection has been proven to effectively eradicate numerous bacterial species [65-70].

The bacterial charged cell surface interacts with the charged molecules or ions on the surface of implants. UV treatment of TiO_2 reduces the population of oral bacteria attached to the surface by roughly more than 3 folds during the first 6 hours following implant placement. This is the critical period prior to the formation of the blood clot which acts as a barrier to bacterial access, and also when the freshly implanted fixture is still highly susceptible to bacterial colonisation. Enhanced UV-mediated osteogenic adhesion to the implant surface causes osteoblasts to compete with microorganisms for space and competitively inhibit the bacterial capacity to proliferate and attach to the implant surface [69]. UV treatment reduces the biomass and decreases the area covered by the bacterial biofilm and hence, reduces the risk of implant infection and failure [71].

Clinical significance of PhF

Time-related biological degradation of titanium surfaces has an adverse effect on the osseointegration capacity and subsequently on the whole healing process. The amount of BIC gets reduced significantly due to biological ageing of titanium. PhF of dental implants prior to their placement reverses the ageing process and results in direct bone formation on the implant surface "Contact Osteogenesis" [72]. UV treatment of titanium surfaces increases the BIC from 55% to a near maximum level of 98.2% [11,12,31,73-75] and also leads to a 3-fold increase in the strength of bone-implant integration. This highly enhances the primary stability, even in implants placed without cortical bone support. The initial stability is crucial in avoiding micromovements wich may adversely affect the osseointegration process [72].

Implant stability quotient (ISQ) is a reliable and a valid method for measuring the primary stability [75-78]. The Push-in value is the breakage strength of osseointegration while the implant is being pushed. Both ISQ and Push-in values were higher in UV treated surfaces than not only aged titanium, but to new "as-received" titanium surfaces [73]. Implants show better load distribution and the mechanical stress in the peri-implant marginal bone is reduced [11,12, 22,31,29]. Furthermore, the osseointegration process occurs 4 times faster and the average healing time required before functional loading reduces by one half compared to the non-photofunctionalized implants [11,31,74].

PhF allows for a quicker loading protocol and a decreased overall treatment time. It also permits the use of shorter and small-diameter implants without compromising the success rate [74]. This opens new treatment possibilities for the use of these smaller implants in more complex cases with higher load or space requirements.

Implant's "Stability dip" is a term used for the weakest stability point throughout the healing period which occurs typically 3 weeks following implant placement due to the osteoblast and osteoclast turnover and bone remodelling. Due to the significant improvement in the OI process, UV treatment of Titanium surfaces eliminates the stability dip, leading to a better and more predictable treatment outcome [79].

Other applications

Other technologies can be incorporated successfully with UV PhF. Nanotechnology is an attractive science with promising applications in the dental field [80]. There is an increasing trend to develop implant surface topographies on a nanoscale level in order to increase it's osteoconductivity [81,82] and enhance the osseointegration process [83-86]. Nanomodification can be done via various approaches such as ion beam deposition, nanoparticle compaction, acid etching, anodising, peroxidation, or chemical conjugation of biomolecules [81,87,88]. Most approaches aim to mimic tissue components by applying nanotopographies such as nanonodules (Figure 4) and nanotubes to the implant surface. Similar to PhF, these "biomimetic" surface features alter the surface interactions with biomolecules and improve the cell adhesion properties [81,89-91]. Hence, it remarkably enhances osteoblastic behaviour and responses, cell attachment, proliferation, alkaline phosphatase activity, and improves protein absorption [67,83,92-95]. Leading to rapid bone healing. Clinically, biomimetic surfaces significantly increases the BIC, strengthens implant fixation up to a 70%, and reduces soft tissue intervention [91]. Combining UV photofunctionalization to these nanoscale

topographies has been shown to provide a synergistic advantage [83,96]. For instance, fluoride treatment or microarc oxidation (MAO) of implant surfaces alone have shown to improve cellular response and bone formation around the implant [97,98], but adding UV light to the equasion further enhances cellular bioactivity and human mesenchymal stem cells (hMSCs) attachement to the implant surface leading to even stronger and a more accelerated osseointegration [82,93,99,100].

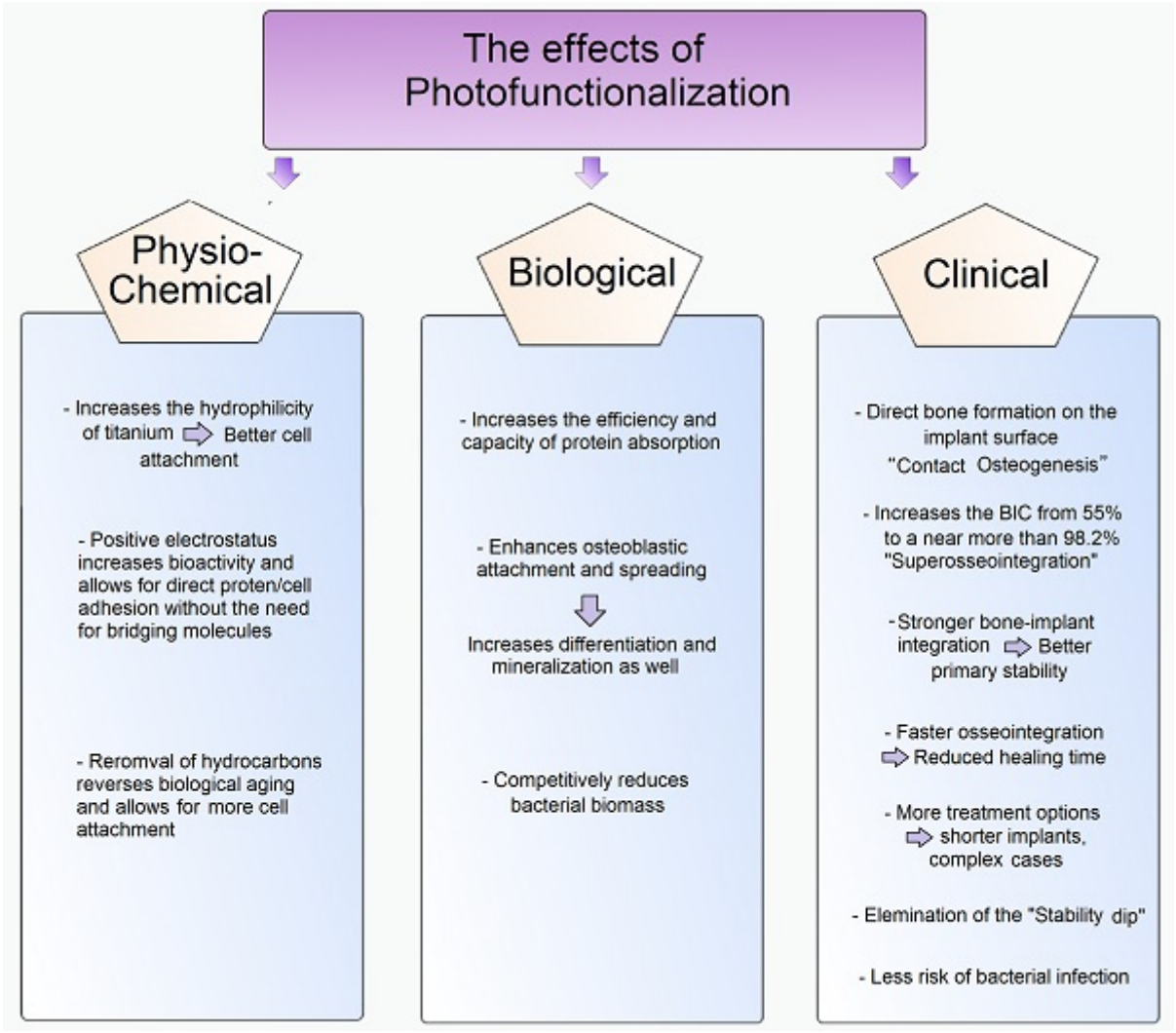

Figure 4: PhF effect on titanium and tissue, and clinical significance.

UV PhF can be utelised in various dental fields. Similarly to the dental implant fixtures, orthodontic miniscrews can also benefit from PhF. The overall success rate of orthodontic miniscrews is around 72% for 6-mm and 8-mm long miniscrews 90% [101,102]. Wider and longer screws provide better anchorage but usually the space for their placement is usually limited, and larger screws are more difficult to remove. Photofunctionalized mini screws were found to provide better anchorage and they displace much less under lateral tipping forces [101]. Photofunctionalization was also found to be effective in increasing the osteoconductivity of titanium mesh and enhancing bone regeneration around it in augmentation procedures [103]. Moreover, UV treatment can also be used to increase the cellular activity and protein adhesion in more advanced tissue engineering techniques such as cell sheet technology [104], which is used to deliver cells in single-sheet form reinforced by a micro-thick titanium framework [105].

Some non-titanium materials were found to be similarly affected by the UV-mediated reversal of biological ageing. UV treatment was associated with a decreased amount of surface carbon in aged chromium–cobalt alloy [29] as well as in ziconium [12], which is a promising alternative material for dental implants because of their mechanical stability, biocompatibility, low plaque attachment, good esthetics and soft tissue compatibility [106-108]. However, low temperature degradation (LTD) is considered a major limitation of this material [109,110], and the oxide layer found on native zirconia makes it bioinert. UV treatment reduces amount of surface carbon, converts zirconium from hydrophobic to hydrophilic and significantly improves the bioactivity of zirconia [111,112].

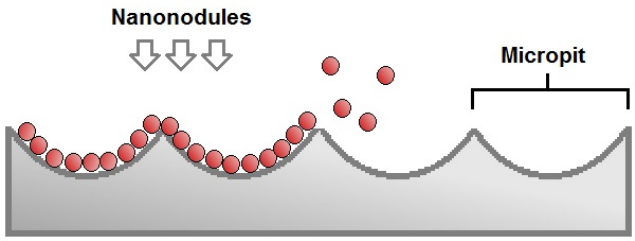

Figure 5: Adding nanoparticles increases the surface area for cellular attachment and enhances cellular bioactivity.

Conclusions

The concept of Superosseointegration is a major breakthrough towards achieving better implant stability and better implant treatment outcomes. PhF increases the implant's primary stability significantly leading to a higher implant success rates and a decreased average healing time. It also allows for more treatment options as shorter and thinner implants can be utilized more frequently without any compromise to the treatment outcome. Further research should be applied for incorporating ultraviolet surface treatment to other dental materials. The clinical ease, low cost of application and significant impact on treatment should also encourage the manufacturers to develop PhF devices affordable for most clinicians.

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