

## **Current Status of Nanotechnology Methods Applied For Dental Implants**

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**ABSTRACT :** *The structural and functional contact of implant surface with the surrounding bone is an important and crucial aspect to determine the long-term success of the device. Current trends have achieved a drastic enhancement in osseointegration at the bone-implant interface after modifying the surface topography of implant surface particularly at the nanoscale level. This review discusses an overview of the most common manufacture techniques and the related cells-surface interactions. It also describes the available data on nanoscale modifications mentioning their risks and benefits. Nanotechnology has opened new opportunities for tissue engineers and biologists to interact and understand relevant biological processes and cell specific functions. Nanoscale modification of titanium endosseous implant surfaces can alter cell behavior and their responses that may significantly benefit dental implant therapy.*

**KEYWORDS:** *Nanotopography; Dental implant; Stem cells; Surface treatment; Osseointegration; Differentiation.*

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### **I. INTRODUCTION**

Dental implants are commonly practiced as an adjunctive therapy for restoring missing teeth. One of the major challenges in implantology is to achieve and maintain the osseointegration, as well as the epithelial attachment of the gingiva with implants. The idea of osseointegration was first emerged in the late 1970 and 1980.<sup>1,2</sup> Osseointegration actually refers to a structural and functional fusion of the implant surface with the surrounding bone. An intimate contact of the gingival tissue with the neck of implant may prevent bacteria colonization leading to peri-implantitis while direct bone bonding may ensure a bio-mechanical anchoring of the implant. Primary stability is the first step of the osseointegration and is related to the mechanical anchorage, design of implants, and bone structure.<sup>3</sup> At a microscopic level, the screw design, the thread shape, and the pitch distance are fundamental to offer stability to dental implants. Abuhussen et al<sup>4</sup> postulated that dental implants should be designed to maximize favorable stresses and to minimize unfavorable stresses along the bone-implant interface. The use of a smaller pitch, deeper threads and longer and larger implants may be of help in increasing the surface area of contact with the surrounding bone.<sup>5-7</sup>

Several studies have been attempted to assess the modification in the bone-implant interactions brought by various surface modifications. Variola et al<sup>8</sup> stressed microscale features, which they believed to create a micro-environment that can modulate recruitment and function of cells. Some researchers<sup>9-11</sup> proved that the roughness of the surface can influence osseointegration by means of cell attraction, improving cell adhesion. However, other researchers<sup>12-14</sup> showed the role of the microscopic features of the implant surface on bone formation at the implant site and believed to be indirectly involved in the osseointegration process. The control

of surface modifications at the protein and cell levels i.e. in the nanometer range, poses a challenge for researchers and dental implants manufacturers.

Nanotechnology has been defined as “the creation of functional materials, devices and systems through control of matter on the nanometer length scale (1-100 nm), and exploitation of novel phenomena and properties (physical, chemical, and biological) at that length scale” (National Aeronautics and space Administration).<sup>12</sup> The term ‘nanotechnology’ was first defined by Norio Taniguchi of the Tokyo Science University in a 1974 paper as follows: ‘Nanotechnology’ mainly consists of the processing of, separation, consolidation and deformation of materials by one atom or one molecule.<sup>15</sup> Nanotechnology involves nano-sized materials which have a size range between 1 and 100 nm ( $10^{-9}$ m). Materials are also classified based on their form and structure as nanostructures, nanocrystals, nanocoatings, nanoparticles, and nanofibers.<sup>16</sup>

Nanotechnologies can create surfaces with controlled topography, and chemistry which would help understanding biological interactions and developing novel implant surfaces with predictable tissue-integrative properties.<sup>9, 17</sup> The application of nanotechnology to biomedical surfaces is explained by the ability of cells to interact with nanometric features, which is mainly mediated by integrins, binding to the arginine-glycine-aspartate sequences of peptides. Cell adhesion to the extra-cellular matrix (ECM) leads to clustering of integrins into focal adhesion complexes (FA), and activates intracellular signaling cascades.<sup>18</sup> Nanofeatures are crucial to modulate stem cells behavior.<sup>19</sup> Osteoblasts are able to “encode” the 3-dimensional characteristics of the surface like lines, pores or dots and modulate their growth according to the suggested structural features.<sup>5, 11, 12</sup> Hence, the surface pattern in particular has been demonstrated to play a key role.

Several new coating technologies have also been developed for applying hydroxyapatite and related calcium phosphates (CaP), onto the surface of implants. It has been demonstrated that CaP coatings provided titanium implants with an osteoconductive surface.<sup>20, 21</sup> After implantation; CaP coatings undergo dissolution in the peri-implant region which increases ionic strength and saturation of blood. This process leads to the precipitation of biological apatite nanocrystals onto the implant surface, which in turn incorporates proteins and promotes the adhesion of osteoprogenitor cells that would produce the extra-cellular matrix of bone tissue. It has been also shown that osteoclast cells are able to degrade the CaP coatings through enzymes and created pits on the coated surface.<sup>21</sup> The presence of CaP coatings on titanium promotes an early osseointegration of implants with a direct bone bonding as compared to non-coated surfaces. The challenge is to create CaP coatings that would dissolve at a similar rate than bone deposition to achieve a direct bone contact on implant surfaces.

This paper reviews the most common manufacture techniques and the different steps of the interactions between biological fluids, cells, tissues, and surfaces of implants. Recent nanoscale surface modifications and CaP coating technologies of dental implants are also discussed.

## II. NANOFEATURED SURFACE MODIFICATIONS

Surface modifications has been shown to enhance the bone-to-implant contact and improve their clinical performance.<sup>9,22</sup> Numerous techniques are used to create nanofeatures on endosseous implants surfaces, which are as follows,

1. Self-assembly of monolayers (SAMs)
  - A. Anodic Oxidation
  - B. Acid oxidation or Peroxidation
  - C. Alkali treatment (NaOH)
3. Physical Modifications
  - D. Compaction of nanoparticles
  - E. Ion beam deposition
  - F. Plasma Spray
  - G. Grit Blasting
4. Nanoparticle deposition
  - H. Sol-gel (colloidal particle adsorption)
  - I. Discrete crystalline deposition (DCD)
  - J. Lithography and contact printing technique
5. Combination of chemical and physical modifications

### 1. MOLECULAR SELF-ASSEMBLY or SELF-ASSEMBLED MONOLAYERS (SAMs):

Self-assembled monolayers (SAMs) are created by the spontaneous chemisorption and vertical close-packed positioning of molecules onto some specific substrata, where the end-chain group(s) at the interface is exposed.<sup>23</sup> Germanier et al<sup>24</sup> in their histomorphometric study in miniature pigs have demonstrated the role of such functional end-group with an example of using cell adhesive peptide domains appended to SAMs composed of polyethylene glycol (PEG) and applied to the Ti implant surfaces.

## 2. ANODIC OXIDATION:

Anodic oxidation or anodization is one of the most commonly used methods to obtain nanostructured oxides on Ti-based implants.<sup>25-29</sup> Even a nanoscale oxide with diameters of less than 100 nm can be created. The titanium metal acts as the anode, and an inert platinum sheet serves as the cathode. Both these are connected by copper wires and linked to a positive and negative port of a 30 V/ 3 A power supply, respectively. During anodization, both anode and cathode are submerged into diluted hydrogen fluoride (either at 0.5 wt % or 1.5 wt %) in a Teflon beaker. During the process, a strong acid dissolves the oxide layer creating a pattern that follows the consecutive lines of the galvanic current. By the voltage regulation and density, it is possible to control the diameters of nanostructures and the gap between them. This is a relatively simple and economical method of surface modification. Anodic oxidation can create surfaces which have been considered as platforms for drug delivery.

By regulating the voltage time, nanofeature properties could be controlled. It has been reported that the diameters of the nanotubes could be modulated to a range from 20 to 150 nm by modifying voltage conditions.<sup>30</sup> It has been found that TiO<sub>2</sub> nanotube arrays were more uniform on electro-polished titanium than on machined one.<sup>31</sup> Alkaline phosphatase (ALP) is a marker of osteogenic differentiation. TiO<sub>2</sub> nanotubes with a diameter of 100 nm improved the production of ALP activity by osteoblastic cells as compared to 30-70 nm diameter nanotube surfaces.<sup>32</sup> This increased ALP activity demonstrate enhanced bone tissue integrative properties. Von Wilmsky et al<sup>33</sup> concluded that implant surface with interface features of 30 nm TiO<sub>2</sub> nanotubes positively influence bone-implant contact and peri-implant bone formation.

## 3. ACID OXIDATION OR PEROXIDATION:

The combination of strong acids can be effectively used to create nanopits of 20-100 nm diameters on titanium surface.<sup>34</sup> The titanium surface is etched with a solution of strong acids, e.g., H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> or HCL/ H<sub>2</sub>O<sub>2</sub> or HF/ H<sub>2</sub>O<sub>2</sub>, at a constant temperature and for a specific duration and then stopped by adding distilled water. The surface is washed further with ethanol in an ultrasonic bath for 20 minutes and dried.<sup>35</sup> The treatment with H<sub>2</sub>O<sub>2</sub>/HCL has been shown to create novel nanostructures of amorphous titanium oxide on the implant surface.<sup>36</sup> It was shown that HCL/H<sub>2</sub>O<sub>2</sub> treatment increased the adsorption of RGD peptides onto the implant surface.<sup>37</sup> Treatment with HF/ H<sub>2</sub>O<sub>2</sub> also creates nanostructures on TiO<sub>2</sub> grit blasted surfaces.<sup>38</sup> Several studies and investigations support the observation that HF acid treatment with TiO<sub>2</sub> grit blasted Ti implants has shown rapid bone accrual at the implant surface.<sup>39-44</sup>

Isa and colleagues<sup>39</sup> have documented that fluoride-modified Ti-surface appeared to optimize the upregulation of cbfa-1, a transcription factor that is essential for the maturation and differentiation of mesenchymal stem cells into osteoblasts. Guo and colleagues<sup>41</sup> compared TiO-blasted surfaces vs TiO-blasted followed by HF acid treatment and reported that only HF acid treated surface had nano-scaled features and support the osteogenic adherent cell response compared to TiO-blast adherent cells. Berglundh and co-workers<sup>43</sup> found that fluoride-modified implant surface enhances and promotes osseointegration in the early phase of healing following implant installation in six mongrel dogs. The parameters like temperature, duration, and solutes can be adjusted to modify the number and depth of nanostructures, which further modulate cell function. The processing of titanium screw-shaped implants with H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> creates a nanopattern which has been demonstrated *in vivo* to be linked with an enhanced osteogenesis.<sup>34</sup> It has been also observed that oxidative nanopatterning promoted the growth of the stem cells.<sup>45</sup>

## 4. ALKALI TREATMENT:

NaOH treatment is popular among current dental implant researchers, which chemically reacts with the implant surfaces exposing reactive groups and creates nanoscale topography. Zhou et al<sup>46</sup> have demonstrated that NaOH application catalyzes the production of Ti nanostructures outward from the Ti surface. NaOH solution treatment forms a sodium titanate gel layer on the Ti surface, which allows hydroxy-apatite deposition. Similar reaction has also been noted with other metals such as zirconium and aluminium.<sup>47-49</sup> Oh et al<sup>50</sup> have reported an accelerated nano-scale HA-crystal growth on TiO<sub>2</sub>-nanotubes chemically treated with NaOH when tested in a simulated body fluid (SBF).

## 5. COMPACTION OF NANOPARTICLES:

This is one of the approaches of physical methods which involve compaction of nanoparticles of TiO<sub>2</sub> vs micron-level particles to yield surfaces with nanoscale grain boundaries.<sup>51</sup> The main advantage of compaction of nanoparticles is that it conserves the chemistry of the surface among different topographies.

**6. ION BEAM DEPOSITION:**

Coelho and Suzuki<sup>52</sup> reported an ion beam deposition (e.g. hydroxyapatite) as an alternative method of depositing nanoscale material on to the implant surface. They have shown an in-vitro thin-film process as an alternative method for surface incorporation of bioceramics on dental endosseous implants, while doing an experimental study in dogs.

**7. PLASMA SPRAY:**

The plasma spray can create engineered-surface nanostructures of less than 100 nm diameters. Initially, a vacuum is used to remove all contaminants, and then kinetic energy guides the charged metallic ions or plasma to the implant surface. Various materials (Ag, Au, Ti, etc.) can be coated onto a wide range of underlying structures (metals, polymers, and ceramics).<sup>53</sup> Reising et al<sup>53</sup> also found a greater calcium deposition on the nano Ti-coated surfaces when compared to uncoated surfaces. The most popular coating method is plasma spraying of HA<sup>54-59</sup> But; this method only controls the initial coating thickness and composition. The elevated temperature required in this processing causes partial thermal decomposition of HA, which further forms highly soluble amorphous CaP (22-62%)<sup>60-64</sup> or  $\alpha$ -TCP,  $\beta$ -TCP, tetra-CaP, and calcium oxide<sup>65-67</sup>. This leads to the formation of unacceptable and heterogeneous coatings. Such coatings may create problems like unreliable adhesion,<sup>68-75</sup> continued dissolution of the coating,<sup>76</sup> partial crystallization of the coating,<sup>77-79</sup> and poor biological stability of the coating<sup>80, 81</sup>. This may cause the catastrophic failure of the implant at the coating-substrate interface<sup>76</sup> or the significant degradation in the fatigue resistance and endurance strength of the implant alloy<sup>82</sup>.

**8. GRIT BLASTING:**

This technique creates a porous layer on the implant surface which is achieved through the collision with microscopic particles. The thickness of the porous layer can be modified by the regulation of granular size of the particles. The rough surface, thus created has been demonstrated to stimulate osteoblastic gene expression, and to enhance bone-implant fixation.<sup>39, 83</sup>

Variola et al<sup>34</sup> demonstrated the creation of 50-200 nm porous layer on titanium implant surface by using the combination of blasting and hydrogen fluoride treatment. They found that the majority of implants yielded good osseointegration and stability at one year after surgery. Alumina is one of the most commonly used materials for particle blasting. Aparicio et al<sup>84</sup> showed that alumina particle detachment during the healing process and then absorption by the surrounding tissue could compromise osseointegration. Other researchers also have shown that grit blasting residue may interfere with the osseointegration of the titanium dental implants.<sup>85-87</sup>

Grit blasting with Biphasic Calcium Phosphate (BCP) ceramic particles has shown a high degree of surface roughness and particle free surfaces after acid etching of titanium implants. It has been shown that BCP grit-blasted surfaces stimulated an early osteoblast differentiation and bone apposition as compared to mirror-polished or alumina grit-blasted titanium.<sup>88, 89</sup> TiO<sub>2</sub>-grit blasting materials has shown interesting results in an experimental research.<sup>90</sup> Ivanoff et al<sup>90</sup> has shown a significant enhancement of bone-to-implant contact with TiO<sub>2</sub>-blasted implants than with machined surfaces. This result was confirmed by Rasmusson et al<sup>91</sup> after performing an experimental study in the dog mandible.

**8. SOL-GEL TRANSFORMATION:**

Ben-Nissan and Choi<sup>92</sup> have discussed sol-gel transformation of bioactive nanocoatings for medical applications. These approaches achieve deposition of nanometer-scale calcium phosphate accretions to the implant surface.<sup>93-97</sup> The resultant atomic-scale interactions thus developed exhibits a strong physical bonding.<sup>92, 98-100</sup> Sol-gel technique may offer a more accurate compositional control and the possibility of fabricating much thinner coatings which establishes biological stability. The processing technique and the nature of the coating might be altered to modulate the coating-substrate strength.

**9. DISCRETE CRYSTALLINE DEPOSITION (DCD):**

This modified approach was reported by Nishimura et al<sup>101</sup> who demonstrated a directed approach to assembly of CaPO<sub>4</sub> nanofeatures on dual acid-etched cpTi implant surfaces. The deposition of discrete nanoparticles (20-40 nm) on an acid-etched Ti surface have shown an increased mechanical interlocking with the surrounding bone and the early healing of bone at the implant surface in a rat model. The major risk for DSD is detachment of coating and toxicity of related debris. In this regard, Gutwein and Webster<sup>102</sup> evaluated the relationship of particle size, cell viability, and proliferation in the presence of nanophase particles compared to conventional alumina and titania micron-particles. They found that nanoparticles of alumina and titania possessed less negative impact in cell viability and proliferation. Mendes et al<sup>103</sup> have shown the effect of DCD of CaPO<sub>4</sub>

nanocrystals on bone-bonding to Ti surfaces. They suggested that the quantum interaction of high electron density at the atomic level can enforce high bond strength between the substrate and nanoscale coating.

#### **10. LITHOGRAPHY AND CONTACT PRINTING TECHNIQUE:**

An optical method typically lithography is used to create nanoscale topography on titanium surface. This approach is reliant on wavelength specific dimensions to achieve the appropriate nanoscale modification.<sup>46</sup> These labor intensive methods require considerable development before its clinical application.

#### **PROTEIN-SURFACE INTERACTIONS:**

Balsundaram and colleagues<sup>104</sup> have shown that alteration in initial protein-surface interaction is a critical and responsible aspect controlling osteoblastic adhesion. Protein-surface interaction is the initial step of the osseointegration process. After implantation, protein adsorption occurs on the implant surfaces, which further mediate subsequent cell adhesion and proliferation. Fath and colleagues<sup>105</sup> have highlighted that cell adhesion to ECM proteins is mediated via integrin receptors, which transmit signals through focal contacts.

Tosatti et al<sup>106</sup> have shown that RGD-containing peptide GCRGYGRGDSPG reduces enhancement of osteoblastic differentiation by poly (L-lysine)-graft-poly (ethylene glycol)-coated Ti-surfaces. They found that integrins bind the RGD motif in cell adhesion proteins. Sinha and Tuan<sup>107</sup> demonstrated the role of fibronectin or vitronectin in mediating cell adhesion of osteoblasts and other cells to synthetic orthopedic implant material surfaces. Nanoscale topographic modification can regulate cell spreading and focal adhesion (FA) dynamics.<sup>108</sup>

Altering the surface energy of a biomaterial substrate is a classical approach to change cell-surface interactions. A change in surface energy dramatically affects the ECM protein adsorption onto surfaces. Several studies of SAMs have shown that hydrophobic groups are more likely to adsorb albumin which is not replaced by ECM proteins. This subsequently blocks the cell adhesion. Hydrophobic surfaces permitted an interchange of adsorbed albumin by ECM proteins.<sup>109</sup> Modification with nanoscale topography drastically alters the protein-surface interactions. An increased adsorption of vitronectin on nanostructured surfaces has been observed when compared to conventional topography.<sup>110, 111</sup> Webster and colleagues<sup>111</sup> found an increased osteoblast adhesion on nanophase ceramics, when compared to other cell types, such as fibroblasts.

Scotchford and co-workers<sup>23</sup> have found higher adsorption of fibronectin on hydrophilic gold SAMs surfaces with greater FA formation; evident in the human osteoblast-like cells adhered to hydrophobic SAM treated surfaces. Lim and colleagues<sup>112</sup> have demonstrated that the protein adsorption, cell adhesion and attachment are directly related to an increased FA kinase activity. Cavalcanti-Adam and colleagues<sup>108</sup> have shown that the cell spreading and FA dynamics are regulated by spacing of integrin ligands. They found that the cells cultured on a 58 nm nanopattern formed normal FA, whereas those plated on a 108 nm nanopattern failed to develop FA. Park and Webster<sup>113</sup> also mentioned the creation of nanoscale surface roughness as the determining factor for protein-surface interactions.

#### **CELL BEHAVIOUR DYNAMICS:**

Nanostructured topography affects cell behavior such as cell adhesion, spreading and motility. Brunette<sup>114</sup> has shown that substratum nanosurface topography influences cell behavior dynamics including its adhesion, spreading and motility by both direct and indirect interactions. Andersson and colleagues<sup>115</sup> have demonstrated the influence of Ti-nanoscale features on the epithelial cell morphology and cytokine production. They compared cell behaviours on Ti-substrates with 15 mm wide and 185 nm deep grooves vs Ti-substrates with 100 nm high, 168 nm diameter hemispherical nanopillars. The cells appeared partially aligned to the grooves and showed a cytokine release similar to that shown by cells on flat surface topography. Cells on hemispherical nanopillars had a smaller area and more membrane projections. Morphological variation correlated with decreased protein secretion. It has been suggested that 70-100 nm features of an implant surface are scaled to function directly with the FA of cells.

Wan et al<sup>116</sup> have shown that osteoprogenitor cell adhesion was enhanced on poly-L-lactide (PLLA) and polystyrene (PS) surface with nanoscale and micron-scale roughness compared to smooth surfaces. OCT-1 osteoblast-like cells grew along the surface with two different nanoscale surfaces (PLLA) and grew inside micron-scale pits of PS. Webster and Ejiófor<sup>51</sup> also reported the similar findings while comparing nano- and micron-scale grain boundary effects on osteoblast cell adhesion and proliferation. Teixeira and co-workers<sup>117</sup> have studied epithelial contact guidance on well-defined micro- and nanostructured substrates. They have demonstrated that when cells bridge nanoscale patterns, integrin binding was limited to substrate-adsorbed proteins on the top of the ridges. Topographic features smaller than FA architecture confines the cell attachment to the top portion of the topographic feature. The depth details of the correlation between nanofeatured topography and cell adhesion are emerging. The current understanding of nanotopography influence on adherent osteoblast behavior needs further scientific research.

Dalby and co-workers<sup>118</sup> investigated osteoprogenitor response to defined topographies with nanoscale depths. They showed that high pit density reduced cell spreading and ordered arrays of nanopits were effective in this regard. Randomly created nanopits led to move cell spreading. Nanostructured surface presents

an opportunity to modulate cell behavior (cell adhesion and spreading). Lim and co-workers<sup>112</sup> studied the human foetal osteoblastic cell responses to polymer-demixed nanotopographic interfaces. They found that the cell adhesion was influenced by nanotopography (PLLA substrate with 3-45 nm nanofeatures) and interdependent on substratum surface characteristics of topography and surface chemistry. However, Cai and colleagues<sup>119</sup> found no major differences in fibronectin adsorption or cell proliferation on 2 vs 20 nm Ti-films. These findings may be because of cell-type specific responses to nanofeatures of a given substrate surface.

Researchers<sup>118, 120</sup> reported that fibroblast and MSCs motility varied drastically across a small range of nanostructures. Hansen and co-workers<sup>121</sup> studied the effect of surface nanoscale topography (11-38 nm high islands) on elastic modulus of individual osteoblastic cells (MC3T3-E) as determined by atomic force microscopy (AFM). With AFM, they measured higher cellular modulus values for cells on nanofeatured surfaces compared with cells on flat control surfaces. They found that nanoscale topography influences the actual mechanical properties of the individual cell. These individual cell responses may be due to the resultant integrin-based remodeling of the cytoskeleton or more complex biophysical changes in the cell membrane. The future research on cell spreading or cell motility may be a valuable achievement for biomaterial engineering of implant-bone-mucosa interface.

#### MESENCHYMAL STEM CELLS AND DENTAL IMPLANT SURFACE:

Mesenchymal stem cells (MSCs) are generally defined as stem cells that are able to self-renew and to differentiate into various specialized tissues like fat, bone and cartilage, neural cells.<sup>122</sup> These cells are derived from somatic tissue which can be differentiated into mesenchymal lineages such as bone, cartilage, fat, and skin. MSCs are conventionally defined as adherent, non-hematopoietic cells expressing markers such as CD13, CD29, CD44, CD54, CD73, CD90, CD105, and CD166, and being negative for CD14, CD34, and CD45.<sup>123, 124</sup> MSCs were originally identified in the bone marrow<sup>125</sup>, but have been also extracted from tissues like adipose<sup>126, 127</sup>, heart<sup>128</sup>, dental pulp<sup>129</sup>, peripheral blood<sup>130</sup>, and cord blood<sup>131</sup>. These cell can differentiate into adipocytes<sup>132</sup>, chondrocytes<sup>126</sup>, osteoblasts<sup>133</sup>, neurons<sup>134, 135</sup>, muscles<sup>135, 136</sup>, and hepatocytes<sup>137</sup> *in vitro* after treatment with induction agents.

The main functions of MSCs are tissue development, homeostasis and repair of damaged tissue. MSCs represents an innovative tool in regenerative medicine and odontoiatric field stem cell biology is fulfilling tools for the development of biomedical devices for bone or tooth restoration.<sup>138</sup>

The integration of implant with the surrounding bone and gingival tissue depends on healthy interaction between old tissue and implant surface. The real challenge is the capability of the implant surface to guide and direct colonization of cells and their differentiation. Tissue regeneration is a well organized and sequential process which follows cell migration, adhesion, proliferation, and differentiation. Researchers showed that some factors present in tissues and secreted during the inflammatory phase are able to attract MSCs to the injured site.<sup>139, 140</sup> It has been shown that MSCs migration and proliferation were stimulated *in vitro* by many growth factors including PDGF<sup>141, 142</sup>, EGF<sup>142, 143</sup>, VEGF<sup>144</sup>, TGF- $\beta$ <sup>141, 145</sup>, and BMP-2 and BM P-4<sup>141, 144</sup>. These growth factors are released in the surrounding injured sites by cells involved in healing process. Also, plasma clot serves as a meshwork to fibrin molecules and releases system for bioactive factors that attract and differentiate MSCs into specific lineages (including growth factors).<sup>146-148</sup>

Rock et al<sup>149</sup> has shown the contribution of platelets in the production of cryoprecipitates for use in a fibrin glue. Thus, they demonstrated the role of the platelet factors to stimulate the proliferation of MSCs. The plasma clot in contact with the surface of implant represents a 3-dimensional micro-porous structure that allows diffusion of regulatory system.<sup>150, 151</sup> Recruited MSCs at the injured site; adhere on the local ECM (extracellular matrix) and on the implant surface, which initiates an extensive proliferation to regenerate new tissue. Also, surface modifications of implants in the nanometer range enhanced the biological responses.

Under the influence of certain specific factors, MSCs differentiates into osteoblasts in contact with the surrounding bone, while they differentiate into fibroblasts in the gingival tissue region. Sometimes, implant surface is encapsulated by fibrous tissue due to proliferation and differentiation of MSCs into fibroblasts. This fibrous tissue protects biological bonding between implant and juxtaposed bone, which further causes a failure of the implant.<sup>152</sup> Adhesion of fibroblastic cells has been shown to be lower on nanostructured surface compared to machined surfaces.<sup>153</sup> Cohen et al<sup>154</sup> has shown the decreased fibroblast and increased osteoblast functions on ionic plasma deposited nanostructured Ti-coatings. Moreover, Miller et al<sup>155</sup> compared the adhesion of fibroblastic cells and vascular cells to nanostructured poly (lactic co glycolic acid) films, and confirmed the lower fibroblast adhesion to nanoscale structures. Various surface treatments like machining, grit blasting, Ti/HA plasma spray, chemical etching, and anodization can be applied to modify the implant surface. Studies have demonstrated that nanorough Ti<sup>156</sup> and nanotube-like structured Ti can enhance osteoblast adhesion and differentiation compared to their nanosmooth control<sup>157</sup>. Implant surfaces featured with micro-and -nano-pores have demonstrated to enhance greatly growth behavior, matrix production, and gene expression of human osteoblasts and ultimately the osseointegration.<sup>158, 159</sup> Modulation of surface properties, thus control the steps of adhesion, proliferation, and differentiation of MSCs conditioning the tissue integration.

Branemark et al<sup>161</sup> in 1983 described the osseointegration as a direct structural and functional bone to implant contact under functional load. Osseointegration at the tissue-implant interface is influenced by the chemistry, topography, and wettability of implant surfaces. In order to enhance osseointegration, numerous surface treatments at the nanometer scale have been performed on implants and experimented in various animals. Kubo et al<sup>161</sup> observed a substantial increase by 3.1 times in bone-titanium interfacial strength by Ti nanotube (300 nm) at 2 weeks of implantation in femur rats. Ogawa et al<sup>162</sup> found an increased surface area upto 40% and a greater strength of osseointegration for the nanostructured Ti compared to an acid-etched surface, when tested in femur of rats. Some researchers have correlated the initial sequential events in bone formation with the long-term tissue response to these materials in human.<sup>163, 164</sup>

CaP and Hydroxyapatite coatings on Titanium implant surfaces greatly enhance osseointegration. During the healing process, calcium and phosphate ions are released into peri-implant micro-environment, which saturate the localized body fluids precipitating a biological apatite which further acts as a substrate for bone formation. Many researchers have demonstrated the significance of CaP-coated Ti implants for enhancing the osseointegration.<sup>165, 166</sup> Le Guehennec et al<sup>167</sup> have reported the histomorphometric analysis of the osseointegration of four different implant surfaces in the femoral epiphyses of rabbits, after 2 and 8 weeks of healing. They have shown that biomimetic coating method may enhance the osseointegration with the Ti-implant surfaces. For this, the Cap coating should dissolve or degrade by osteoclastic cells at a similar rate than bone apposition. CaP coatings are prepared by biomimetic methods at physiological temperature and pH from simulated body fluids. Liu et al<sup>168</sup> have shown the possibility of the incorporation of growth factors during the precipitation of CaP coatings on Ti-implants. Moreover, they have shown that BMP-2 liberated from biomimetic implant coatings induces and sustains direct ossification in an ectopic rat model.

### III. CONCLUSIONS

Nanofeatured modification by different methods can alter the chemistry and surface topography of the implant surface. Many studies have reported that nanometer-controlled surfaces have a great effect on adsorption of proteins, blood clot formation, and cell behaviors which occur after implantation of dental implants. These early events have an effective role on the migration, adhesion, proliferation, and differentiation of MSCs. Nanostructured surfaces may control the differentiation pathways into specific lineages which further direct the nature of peri-implant tissues. Nanoscale modifications of Ti-endosseous implant surface enhance osseointegration. The outcomes of such biomaterials at nanoscale level may be defined by long-term clinical evaluation.

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