

## Basis of Bone Metabolism around Dental Implants during Osseointegration and Peri-Implant Bone Loss

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## Basis of Bone Metabolism around Dental Implants during Osseointegration and Peri-Implant Bone Loss

### Abstract

**Background:** Despite the growing number of publications in the field of implant dentistry, there are limited studies to date investigating the biology and metabolism of bone healing around dental implants and their implications in peri-implant marginal bone loss.

**Purpose:** The aim of this review article is to provide a thorough understanding of the biological events taking place during osseointegration and the subsequent early and late phases of bone remodeling around dental implants. An update on the coupling mechanism occurring during bone resorption-bone remodeling is provided, focused on the relevance of the osteocytes, bone lining cells and immune cells during bone maintenance.

**Material and methods:** An electronic and manual literature search was conducted by three independent reviewers in several databases, including MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and Cochrane Oral Health Group Trials Register databases for articles up to September 2016 with no language restriction.

**Results:** Local bone metabolism is subject to signals from systemic calcium-phosphate homeostasis and bone remodeling. Three areas of interest were reviewed due to recent reported compromises in bone healing including the putative effects of 1) cholesterol, 2) hyperlipidemia and 3) low vitamin D intake. Moreover, the prominent influence of osteocytes and immune cells is discussed as being key regulators during dental implant osseointegration and maintenance. These cells are of crucial importance in the presence of biofilm accumulation and their associated byproducts that leads to hard and soft tissue breakdown; the so called peri-implantitis.

**Conclusion:** Factors that could negatively impact osteoclastogenesis or osteal macrophage activation should be monitored in future research including implant placement/torque protocols, bone characteristics, as well as meticulous maintenance programs to favor osseointegration and future long-term stability and success of dental implants.

## Introduction

Primary or mechanical stability in implant dentistry is regarded as a prerequisite for successful osseointegration. The alveolar bone architecture of the implant drilling site dictates the success of anchored endosseous implants. Immediately and up to several months afterwards, a series of cellular and molecular events take place where host tissues biologically integrate the alloplastic material into the native bony structure. While cortical bone has the function of withstanding torsional loading and provides higher initial stability, cancellous bone is richer in vascular canals and thus, vasculature to supply mesenchymal progenitor cells. In this sense, the complex and dynamic process of osseointegration may occur via contact osteogenesis, where the implant surface is populated by bone cells after fixation to form *de novo* bone, or via distance osteogenesis, where bone formation is preceded by the osteoclastogenesis of the existing tissue.<sup>1</sup>

Nowadays, peri-implant disease does not represent an uncommon condition where, with no hesitation, plaque and its byproducts in a susceptible host are the primary etiology, as it has been demonstrated its cause-effect relationship.<sup>2</sup> Moreover, certain risk factors/indicators such as smoking or history of periodontal disease have been strongly linked to the prevalence of peri-implantitis.<sup>3, 4</sup> Nonetheless, other factors such as material biocompatibility, implant placement and material degradation / titanium particle release have been regarded as other potential factors associated with peri-implant bone loss as a prominent matter of discussion of its implication on osseointegration breakdown even in the lack of irritants.<sup>5</sup>

Into the bargain, early peri-implant marginal bone loss was controversial due to limited knowledge on how hard tissue remodels as a consequence of the biological width adaptation. This has resulted in the development of novel modifications to the implant-abutment connections as well as an evolution towards hydrophilic and bioactive implant surfaces for early osseointegration. Nevertheless, a tight link between the osteogenic and osteoclastogenic pathways modulated by complement factor-3 signaling seems to play a further role on osteolysis led by monocytes/macrophages later discussed in this article. Moreover, the inflammatory response may be exacerbated by tissue trauma such as overheating or compression necrosis (i.e., high insertion torque). These might aggravate the peri-implant bone loss even in an aseptic environment, and worsen the implant prognosis due to the increased exposure of developing a later anaerobic infection, namely peri-implantitis. Therefore, the aim of this narrative review is to provide an updated understanding of the biological events that take place during implant osseointegration and subsequent early and late bone remodeling around implants.

### 1. Biology of the bone remodeling

### 1.1 *Role of osteocytes and bone lining cells in the remodeling process*

Osteocytes are the pivotal cells in the regulation of bone mass and structure along with osteoblasts and osteoclasts.<sup>6</sup> Osteoblasts are derived from mesenchymal stem cells and synthesize new bone matrix.<sup>7</sup> Osteoclasts are terminally differentiated multinucleated cells from the monocyte-macrophage lineage and beyond their role in bone resorption, these cells are also a source of cytokines that play an important role in bone homeostasis.<sup>8</sup>

Osteocytes are terminally differentiated osteoblasts with a primary function to support bone structure and mechanosensation.<sup>7</sup> They act as regulators of bone remodeling by modulating osteoclast and osteoblast activities.<sup>8</sup> These stellate-shaped cells are located within lacunae surrounded by mineralized bone matrix and present with connections through cytoplasmic prolongations with surface bone lining cells and also with bone marrow.<sup>9, 8</sup>

Bone lining cells (BLC) are cells involved in bone formation much like pre-osteoblasts, osteoblasts and osteocytes.<sup>10</sup> They are characterized by a flat-shaped architecture along bony surfaces<sup>8</sup> (Figure 1) and may be considered as latent osteoblasts.<sup>11</sup> In human cancellous bone, around 65% of osteoblasts undergo apoptosis with approximately 30% differentiating into osteocytes,<sup>12</sup> and the reduced remnants becoming BLC and chondroid-like cells.<sup>10, 12</sup> BLCs maintain their proliferative capability and often differentiate into other osteogenic cells.<sup>13, 14</sup> Various studies have shown that some factors can induce their proliferation prior to bone formation,<sup>15</sup> while mature osteoblasts are unable to divide.<sup>10</sup> Osteoblasts may also undergo a quiescent stage when there is no bone resorption or remodeling,<sup>13</sup> but the function of BLCs might be more complex than a simple latent state,<sup>16</sup> including catabolic and anabolic bone processes<sup>15</sup> and rapid bone formation under osteogenic signaling.<sup>16</sup>

### 1.2 *Bone remodeling process*

As bone remodeling is a complex process previously discussed in various other excellent review articles<sup>1, 17</sup> and exceeds the limits of this review, we focus specifically on the pivotal implications of osteocytes and BLCs during this process (Figure 2 A). External factors such as mechanical loading, irradiation, parathyroid hormone (PTH), fibroblast growth factor-2 (FGF2), sclerostin inhibition or inflammation may lead BLCs to exit the quiescent stage into an active function phase by reforming cuboidal appearance and their secretory capability.<sup>8, 15, 18</sup> The presence of BLCs observed histologically indicates a strong sign of osteogenic potential<sup>13</sup> and often regarded as a major source of osteoblasts and proliferating pre-osteoblasts in the adult population.<sup>15</sup> This prominent role in new bone formation was previously highlighted<sup>12, 16</sup> when rapid bone formation after mechanical loading without previous bone resorption was observed.

Early peak of bone formation after three days was only possible if BLCs underwent reactivation and required their secretory capacities.<sup>12, 16</sup>

Moreover, BLCs exert a prominent function during bone resorption<sup>19</sup> demonstrated by their ability to express key osteoclastogenesis markers including macrophage colony -stimulating factor receptor (M-CSFR) and receptor activator of nuclear factor kappa-B ligand (RANKL).<sup>15</sup> BLCs have been shown to be in close contact with osteoclasts and may also modulate bone remodeling.<sup>19</sup> These cells have been shown to digest the protrusive nonmineralized collagen fibers mediated by matrix metalloproteinases (MMPs) and clean the bone surface in order to facilitate osteoclast attachment to their surface and subsequent resorption process. Furthermore, the activation of BLCs after osteocyte apoptosis leads to the formation of the so-called bone remodeling compartment<sup>17</sup> where osteoclasts resorb bone without damaging the surrounding environment. Later, the osteoprogenitor cells colonize these remaining surfaces left by osteoclastic cells.

After the modulation of bone resorption, BLCs play another important role in the early stages of bone formation by entering the resorption lacunae to remove collagen fibers and debris left by osteoclasts (Figure 2B). Subsequently to this cleaning function, BLCs secrete a layer of fibrillar collagen allowing osteoblasts to attach and deposit new osteoid.<sup>19</sup>

### 1.3 Loading and bone resorption

Osteocytes and BLCs are part of a functional syncytium which regulates communication through gap junctions during their mechanoreceptive function.<sup>6</sup> Based on finite element analysis/models, it has previously been shown that slight loading increases bone formation and inhibits resorption.<sup>20, 21</sup> During this function, BLCs are remodeling activators<sup>22</sup> and promote bone resorption unless an inhibitory signal from osteocytes is present.<sup>23</sup> Bone formation by osteoblasts is downregulated by this inhibitory signal, proportional to the mechanical loading sensed by osteocytes.<sup>23, 24</sup> Thus, an increase in bone remodeling may be observed when the strength of the inhibitory signal is low, triggered by a small generation signal or transmission failure. Bone disuse state without mechanical loading is an example of low strain-generated signal and the consequence is the activation of BLCs and bone loss. Transmission failure can be observed in cyclic loading, microdamage (microcracks) or diffuse bone damage.<sup>23</sup> Presence of microcracks or diffuse damage may impair the intracellular and/or extracellular flow of signals between osteocytes and BLCs and also may increase the presence of cytokines or Ca<sup>++</sup> ions resulting in signal reduction.<sup>23</sup>

Moreover, excessive loading and microdamage have been related to higher osteocyte apoptosis.<sup>24,36</sup> Interestingly, computer simulations stated that the inhibitory signal from osteocytes are significantly lower when cell apoptosis is present.<sup>25</sup> Indeed, the greatest reduction in signal is when osteocyte apoptosis occurs nearest to the bone surface.<sup>25</sup> Bone surface is mechanically more sensitive than the inner portions of the bone<sup>25,26</sup> and therefore apoptosis location is more relevant than the total amount of cellular programmed death.<sup>27</sup> In addition, osteocyte apoptosis is followed by an increase release of RANKL in bone leading to osteoclastogenesis and bone resorption.<sup>28,29</sup>

Under functional loading conditions, the molecular signals from osteocytes leads to a coupling of bone formation and bone resorption, being first increased in higher levels of loading and later in lower levels of strain.<sup>16</sup> A study utilizing a simulation model by finite elements<sup>21</sup> has demonstrated that strain-induced signals from osteocytes guide the osteoclast resorption direction,<sup>6</sup> meanwhile unloading leads to random resorption.<sup>21</sup> Among other factors, this osteoclast guidance is related to the differences in canalicular flow and the levels of nitric oxide (NO) in the osteocytes.<sup>6,30</sup>

#### *1.4 Regulation of osteocytes*

Osteocytes maintain an important role during bone formation and resorption and are the major source of RANKL in bone,<sup>29</sup> required for osteoclast differentiation and function.<sup>31</sup> Osteocytes also function through Wnt signaling pathways and regulate osteoblast proliferation, differentiation and survival.<sup>29,32</sup> Wnt has also been suggested to be involved in the induction of bone formation even in cases where fibrous encapsulation predominates.<sup>33</sup>

Some pathologies may also influence RANKL expression of osteocytes. For example, the presence of estrogens promotes osteocyte viability and reduces cortical bone resorption, but lower levels has also been shown to promote osteocyte apoptosis and raise the levels of RANKL.<sup>34</sup> Inflammatory mediators such as interleukin -I (IL-1), IL-6 and tumor necrosis-factor (TNF) alpha also increase the levels of RANKL and induce osteocyte death.<sup>35</sup> Finally, PTH basal levels seem to maintain bone remodeling by raising RANKL and lowering osteoprotegerin (OPG) release by osteocytes.<sup>29</sup>

### 1.5 Bone remodeling around dental implants

After dental implants are anchored, a sequence of immune-inflammatory responses followed by angiogenesis and eventually osteogenesis take place to achieve osseointegration. This is influenced by the implant surface characteristics owing to the ability for protein adsorption based on implant surface topography and hydrophilicity. Accordingly, thrombin and fibrinogen adhere to the implant surface. Later, neutrophils populate the implant recipient site before the monocytes and macrophages infiltrate the area. These events fulfill a key role on the early homeostasis as they release the cytokines and growth factors that stimulate collagen matrix deposition around the titanium oxide layer leading to newly-formed woven bone (usually occurs 5 days later). In a matter of 8 to 12 weeks, lamellar bone initiates the biological stability, namely osseointegration.<sup>1</sup>

As it occurs with the natural dentition, implants are subjected to soft and hard tissue remodeling after restoration delivery. Biologic width in humans around dental implants has recently been shown to be ~3.5mm.<sup>36</sup> This physiological bone remodeling mechanism to a foreign body is led by RANKL, which promotes macrophage activation into osteoclasts. When early implant marginal bone loss exposes the implant microtexture, contamination by bacterial and its byproducts is facilitated and thus, the infiltration of large proportions of CD68- and myeloperoxidase (MPO)-positive cells are capable of breaking down the peri-implant structures.<sup>37</sup>

It has been suggested that the microgap in two-piece implants might be associated with the up regulation of the inflammatory cell infiltrate leading to crestal bone loss.<sup>38, 39</sup> The abutment connection on the endosseous portion of the implant leaves a gap in a range of 10- to 50-micrometers.<sup>38</sup> A pumping effect of the fluid contained in the implant cavities might shift inwards to the peri-implant compartment due to the cyclical loading of the implant/abutment interface<sup>38, 40</sup> and facilitate the colonization of the gap by putative pathogens. These organic fluids with bacteria products and endotoxins could upregulate the expression of pro-inflammatory cytokines in the peri-implant tissues and stimulate the chemotaxis of active osteoclasts.<sup>38</sup> Over the time, leakage associated to micromovements leads to steady inflammatory reaction,<sup>41</sup> bone loss around the implant neck and later, in the presence of biofilm, to peri-implantitis.<sup>42</sup> It seems that internal implant connections provide better sealing than the external ones.<sup>42, 43</sup> Tesmer et al. reported a higher number of colonies former unit (CFU) of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in samples of trilobe connection vs. Morse cone connection in an *in vitro* study.<sup>44</sup> Moreover, conical seal systems have been related to less crestal bone loss.<sup>43</sup> Nonetheless, it remains to be elucidated the association of the gap size or microbial leakage at the implant-abutment connection with the crestal bone loss.<sup>45</sup> In addition, it



remains controversial the influence of the position of the implant-abutment connection on crestal bone loss.<sup>45</sup> Even though, a greater density of neutrophils have been reported in subcrestal interfaces vs. supracrestal location.<sup>38, 39</sup> Further improvements on the implant-abutment sealing together with encouraging to use the original components might minimize the crestal bone loss associated to microgap inflammation.

As aforementioned, peri-implant implant bone undergoes remodeling after surgery trauma and due to the biological width establishment, but also it keeps in active bone remodeling during years as stimulated by the masticatory loading after the post-implantation healing.<sup>46-48</sup> In a short-term follow-up study in dogs, Gyoon-Kim et al. reported that newly formed bone had lower ability to resist elastic, plastic and viscous deformation but higher viscoelastic capacity to absorb deformation energy than the old resorbed bone. This fact might explain why bone is able to bear the impact of masticatory loading transmitted from the implant in absence of the periodontal ligament.<sup>46</sup> Baldassarri et al. demonstrated that bone only reaches maturity after 5 years of loading and an increase of elastic modulus and hardness have been observed during that time in human retrieved implants.<sup>47</sup> Interestingly, a reduction in osteocyte density in samples after long period of loading have been reported,<sup>48</sup> and a possible explanation was the limited number of cells needed to maintain bone homeostasis after bone is matured, well aligned and biomechanically competent.<sup>48</sup> In this sense, initial healing process takes up to the first year of loading and imply the remodeling of initial woven bone and a high number of osteocytes. The second stages comprises up to the fifth year when bone matures after another active remodeling period and also a higher presence of osteocytes. Last, the third stage seems to imply a reduction in osteocytes numbers and bone remodeling.<sup>48</sup>

## **2. Excess of implant torque on bone healing**

### ***2.1. Bone biology under implant insertion***

Adequate implant insertion torque (IT) values (25-45 *Ncm*) have been suggested to prevent micro-movement that could lead to fibrous encapsulation. On the flip side, high insertion torque has also been associated with an increase in critical pressure triggering microfractures and bone necrosis (Figure 3). It has been shown in animal models that high IT elicits a complex microdamage being a strong stimulator for initiating targeted bone remodeling. Moreover, it was evidenced that cortical bone resorption occurs on the surface of differently oriented Haversian and Volkmann canals.<sup>49</sup> This was in agreement with a radiographic, histomorphometrical and histological investigation that clearly identified that implants with a high IT (>50 *Ncm*) are

subject to greater peri-implant bone loss in the early stages of healing compared to those more passively placed.<sup>50</sup> Additionally, a recent multiscale analysis revealed that under drilling to achieve high IT, a double layer of dead and dying osteocytes was observed when compared to low IT.<sup>51</sup> Moreover, it was shown that osteocyte lacunar density in human cortical bone is associated with micro-cracks accumulation and porosity increase with age.<sup>48 52</sup> This finding highlights the importance of minimizing microfractures as a consequence of high IT to predictably preserve the peri-implant bone level. Thereby, it seems highly coherent to state that the lack of primary stability may potentially jeopardize osseointegration, high IT might not favor the preservation of the peri-implant tissue level.

Finite elements studies have shown that loading increases bone formation and inhibits the resorption and that bone disuse promote bone loss,<sup>20 21</sup> so a contradiction between recommending low IT levels and may be observed. However, increased IT may lead to osteocyte apoptosis and consequently may promote higher levels of RANKL and VEGF secretion to the surrounding environment to remove apoptotic cells.<sup>29 53</sup> Higher levels of RANKL has previously been reported 100-200 microns away from microcracks and lower levels of OPG were observed up to 200 microns away from microcracks.<sup>53</sup> Verborgt et al. reported that viable osteocytes next to microdamaged areas promoted cell apoptosis by expressing higher levels of Bax gene and that the highest levels of the anti-apoptotic protein Bcl-2 was reached 1-2 mm away from microcracks.<sup>54</sup> Moreover, osteoclasts not only remodel disused bone, but also damaged bone like microcracks.<sup>55</sup> In this area two stimuli promote osteoclast activation.<sup>21</sup> First of all, dying osteocytes release chemotactil signals to attract osteoclasts and raise RANKL levels. Secondly, osteocyte-dead areas do not emit the osteoclast inhibitory signals. These signals might be transmitted preferently following the direction where the bone was deposited,<sup>21</sup> triggering greater peri-implant bone loss in the crestal area.

Alveolar bone density further influences primary stability. An early publication in the field of implantology classified the maxillary ridges in four major types.<sup>56</sup> Accordingly, denser bone is located in the anterior mandibular region, whereas more porous trabecular bone is detected in the posterior maxillary area. Recent findings seem to point to the influence of bone atrophy on bone density.<sup>57</sup> Cortical bone has a higher elastic modulus<sup>58</sup> and compressive strength when compared to cancellous bone.<sup>59</sup> Nonetheless, it is worth noting that the restrained vascularity of compact bone, implying minimal to no migration of differentiating osteogenic cells, may result in peri-implant bone loss in the event of trauma. In the same way, Kristensen et

al. reported the three main routes of osteoblast recruitment during remodeling: approximately 20% of the osteoblastic cells come from reactivation of BLC on quiescent surfaces, 50% are canopy cells from the mesenchymal bone marrow and the last third are vascular-associated osteoprogenitors like pericytes that reach the bone remodeling area by the canopies.<sup>60</sup> Limited blood supply and absence of bone marrow might limit the amount of osteoblast cells in the bone remodeling area, and in some cases the area may not reach the critical osteoblastic cell density needed for bone formation to occur,<sup>60</sup> showing an arrested reversal area where only bone resorption can take place.<sup>61</sup>

Simons et al. studied the association of the proportion of cancellous/cortical bone on marginal bone loss.<sup>62</sup> The authors identified that higher cancellous proportion (>50-60%), and early bone loss was significantly minimized (~0.6-0.7mm) when compared to implant recipient sites (<30%) of cancellous content (~1.5mm). Therefore, high IT should be omitted, in particular in the presence of a thick cortical layer. In order to avoid microfractures as a consequence of high IT, tapping should be advocated.

In summary, cortical bone presents with several disadvantages when compared to trabecular bone. The limited bone supply may impair osteoprogenitors presence and the critical osteoblastic cell density required for bone repair might not be reached.<sup>60</sup> Secondly, the crestal bone is exposed to the highest strain levels<sup>63</sup> and correlates with the most mechanically sensitive area in bone.<sup>25, 26</sup> This highest strain levels are located on the area of first contact when two different materials are in contact and one is loaded.<sup>64</sup> Moreover, osteocyte apoptosis at the surface promotes a more potent resorptive signal than found in deeper bone tissues.<sup>25, 26</sup> Thirdly, according to histological reports, the highest density of osteocyte canaliculi are observed at the bone surface perpendicular to the loading force,<sup>6</sup> therefore a greater amount of damage in this zone might be occurring. For such reasons, implant placement protocols (including IT) are crucial parameters necessitating adequate control according to their bone characteristics.

## 2.2. Implant outcomes under high and low implant torque

While the belief behind achieving high primary stability has been the goal for many clinicians based on the belief that osseointegration would be better warranted, current clinical research seems to indicate that high implant torque might be pernicious for the peri-implant bone level. Certainly, for immediate implant placement with/-out immediate loading, solid primary stability is necessary (>32 Ncm).<sup>65, 66</sup> Nonetheless, in delayed implant placement, understanding

the resorption process of the bundle bone and the establishment of the bone macro-architecture and bone density might dictate the drilling sequence and IT. As such, when implant placement is applied under high IT ( $\geq 50$  Ncm), it has been shown to be more prone to marginal bone loss and recession, notably in the presence of a thin buccal bone.<sup>67</sup> Alike, when compared to even higher IT threshold ( $>70$  Ncm), it was evidenced that marginal bone loss was substantially higher.<sup>68</sup> Strikingly, an association was found statistically correlated when including all the values (up to 176 Ncm). This finding further reflects the role that bone structure plays and its influence on the fate of the peri-implant bone level.

For the aforementioned reasons, novel approaches for implant placement are being investigated. One example is the use of simplified drilling methods that have not seemed to jeopardize the process of osseointegration.<sup>67, 68</sup> For example, wider implants installed under higher IT have shown adequate secondary stability and high bone-to-implant contact (BIC); although it was demonstrated that a certain healing delaying was found due to the necrosis of the existing bone.<sup>69</sup> Alike, findings from another group also indicated that even submerged implants inserted at 0 Ncm torque displayed similar outcomes compared to those inserted at 30 Ncm or 70 Ncm at 4 months.<sup>70</sup> In partial agreement, Campos et al. found that although the BIC was not affected, adequate drilling to achieve passive implantation outperformed over-/under-drilling by means of the bone area fraction occupied.<sup>71</sup> Hence, clinical outcomes echo the uncertain impact high IT might have on peri-implant bone loss compared to low IT. Future research are currently investigating alternative strategies including the application of osseodensification protocols,<sup>72</sup> lasers<sup>73 74</sup> or ultrasound tools<sup>75 76 77 78</sup> to enhance osseointegration.

### 3. Trauma from occlusion

Although peri-implantitis and overload in conjunction with the host characteristics may be the major etiological agents causing late failures,<sup>79</sup> the influence of trauma from occlusion on peri-implant disease has not been yet elucidated.<sup>80</sup> In a systematic review based on animal studies, Chambrone et al. reported that occlusal overload may lead to bone loss in the presence of dental plaque and to a higher bone density if plaque control is performed.<sup>81</sup> Heitz-Mayfield et al. reported in a dog study that overload did not impact on healthy implants, with no differences in BIC.<sup>82</sup> It is important to highlight also the series of animal studies from Miyata et al., who reported that inadequate oral hygiene and trauma from occlusion resulted in peri-implant tissue breakdown in monkeys,<sup>83, 84</sup> even in the lack of plaque-induced inflammation.<sup>83</sup> Along these lines, a systematic review<sup>85</sup> reported that supra-occlusal contacts on non-inflamed peri-implant bone tissues did not cause bone catabolism, whereas supra-occlusal contacts combined with

inflammation significantly increased the plaque-induced bone loss. Accordingly, it was concluded that the effect of implant overloading on bone/implant loss in clinically well-integrated implants is poorly reported and there is not enough evidence to support a cause-and-effect relationship.<sup>85</sup> Moreover, a clinical and radiographic study in *Macaca Fascicularis* confirmed that overloading might trigger the loss of osseointegration 4.5-15.5 months after the overload was commenced in the vast majority of implants examined. Interestingly, it was further shown that, although excessive marginal bone loss was observed (1.8mm), no implant was lost when plaque was disrupted but in the lack of occlusal overloading.<sup>86</sup>

In summary, cautious conclusions should be done on this topic due to a limited and risk-biased literature and also due to the fact that most of the knowledge in this field is derived from animal experimental studies.

#### 4. Factors affecting bone metabolism

##### 4.1 Cholesterol and fatty acids

In the last three decades, the consumption of high-fat and high-cholesterol-diets have increased<sup>87</sup> and as a consequence, the morbidity and mortality of obesity-related diseases such as cardiovascular disease and hyper-inflamed conditions have also increased.<sup>88, 89</sup> Obesity has also been related with an enhanced hazard of periodontal disease in man.<sup>90, 91</sup>

Although obesity and high levels of cholesterol production have been linked for years, the relation between obesity and serum levels is low.<sup>92, 93, 94</sup> Similarly, the relationship between bone and body fat is complex and not totally understood to now.<sup>95</sup> Bone marrow fat (BMF) is the accumulation of fat cells inside the bone marrow tissue.<sup>96</sup> An inverse correlation between bone mass and BMF has been reported.<sup>95-98</sup> Higher adipogenesis in BM may result in lower osteoblastogenesis and these adipocytes can secrete saturated fatty acids which may impair osteoblast viability by inducing apoptosis and autophagy.<sup>96, 97</sup> Adipocytes can also release pro-inflammatory and osteoclastogenic cytokines (e.g., TNF $\alpha$  and IL-6), adipokines and express RANKL.<sup>95, 96, 98-100</sup>

In other words, fatty acids<sup>97</sup> and high levels of cholesterol<sup>101</sup> may disturb the bone formation/bone resorption equilibrium by down-regulating the Wnt signaling pathway.<sup>102</sup> This is probably due to the effects of higher levels of TNF $\alpha$  and sclerostin.<sup>103</sup> Wnt pathway balances the mesenchymal stem cells differentiation by inhibiting of adipogenesis and promoting osteoblast proliferation, maturation and differentiation.<sup>97</sup> Animal studies have shown more bone resorption,

less bone formation and bone mass and higher levels of bone turnover markers after rich-cholesterol diets.<sup>97 101 104 105 106</sup>

In addition, obesity induces a systemic inflammation condition with high levels of circulating cytokines and increased production of monocytes, neutrophils<sup>107, 108</sup> and adipose tissue macrophages.<sup>109, 110</sup> These cytokines and the accumulation of cholesterol in macrophages can alter the ratio of M1/M2 macrophages promoting an M1 pro-inflammatory environment thereby increasing the numbers of monocytes/macrophages in circulation.<sup>109, 111</sup>

The influence of obesity and increased levels of cholesterol and triglycerides have been extensively described in the medical field but the effect of hyperlipidemia on dental implant osseointegration has not yet been fully elucidated.<sup>112</sup> Significantly more peri-implant bone loss, reduced bone formation and lower strength in the bone-implant interface has previously been reported in mice after a 12 week high-fat diet.<sup>112</sup> On the other hand, Dündar et al. (2016) reported that there was no difference in BIC 12 weeks after implant placement between rabbits following a 3-month high-fat diet versus normal diet.<sup>113</sup> As hyperlipidemia might impair bone quantity and density, negative effects might be speculated on implant osseointegration although no conclusive evidence to date has been found.

#### **4.2 Vitamin D**

Vitamin D is a fat-soluble hormone that regulates calcium phosphate homeostasis and mineral bone metabolism.<sup>114</sup> It is transformed into the active form (1,25-dihydroxy vitamin D3) by hydroxylation, firstly in the liver and then in the kidney.<sup>115</sup> This vitamin can stimulate osteoblast bone matrix production, coupling bone resorption to formation and optimize bone remodeling.<sup>116</sup> It increases calcium absorption in the intestine leading to a reduction in PTH secretion and lower systemic bone resorption<sup>115, 117, 118</sup> with a possible inhibition of osteoclastogenesis.<sup>119</sup> 1,25-dihydroxy vitamin D3 can stimulate bone resorption by binding to osteoblast vitamin D receptors (VDR) and by altering the balance between RANKL and OPG.<sup>120-123</sup>

Vitamin D is a common substance in the prevention and treatment of osteoporosis but research investigating its effects during dental implant osseointegration remains limited.<sup>118</sup> In animals, Kelly et al. (2009) studied the osseointegration process in rats with deficiency in Vitamin D and reported lower BIC values and mechanical bone strength after 2 weeks post-implant placement.<sup>124</sup> Noteworthy however is that implant failure might be confounded by the rising insufficiency of vitamin D prevalence in various patient populations.<sup>124</sup> Zhou et al. (2012) reported an improved titanium screw fixation in ovariectomized rats after 8 weeks of oral

treatment with vitamin D, showing a significant increment of peri-implant bone density, bone-implant contact (1.5 times higher) and peri-implant trabecular microarchitecture.<sup>118</sup> Similar results were reported in mice with chronic kidney disease (CKD), suggesting that vitamin D treatment may be an effective approach for implant placement in patients with CKD.<sup>125</sup> Recently, the effect of topical application of vitamin D (10%)<sup>126</sup> and melatonin (5%)<sup>127</sup> solutions on the surface of immediate implants placed in dogs was evaluated. Both topical applications improved significantly new bone formation around implants and reduced crestal bone loss at 12 weeks following surgery,<sup>127</sup> standing out the positive correlation between vitamin D and early stages of osseointegration. Therefore, these results may suggest that vitamin D has a protective effect on bone healing after implant insertion.<sup>118</sup>

Schulze-Spate et al. (2016), in a randomized, double-blind, placebo-controlled clinical trial in humans, reported no differences in bone formation nor in graft resorption after maxillary sinus augmentation procedure with vitamin D and calcium supplement.<sup>123</sup> Only a difference in the number of bone-resorbing osteoclasts was assessed, finding a higher bone remodeling activity related to higher vitamin D levels.<sup>123</sup> A retrospective study to correlate early implant failure and low serum levels of vitamin D<sup>128</sup> showed a higher incidence of the implant failure rate in these patients but a correlation between both factors could not be determined. Therefore, vitamin D seems to improve bone health and implant healing but further research is needed to obtain an adequate level of evidence.

### **4.3 Hyperglycemia**

The number of adults with diabetes in the world increased from 108 million in 1980 to 422 million in 2014.<sup>129</sup> Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin. Type 2 diabetes (formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises the majority of people with diabetes around the world and is largely the result of excess body weight and physical inactivity. It is characterized by hyperglycemia, insulin resistance and relative insulin deficiency.<sup>130</sup>

Diabetes mellitus has been related to a deficient metabolism of the skeletal tissue due to a suppressed osteoblastic function and lower bone formation potential, independently of the type of bone, the location and mechanical loading.<sup>131</sup> A higher risk of implant failures have been related to uncontrolled diabetes<sup>132</sup> and non-diagnosed diabetes might be a possible reason of failed implants for unknown reasons.<sup>133</sup>

Ajami et al. reported a delayed bone formation and remodeling in hyperglycemic rats. Early bone mineralization might be affected due to a compromised intra-fibrillar collagen mineralization whereas inter-fibrillar and cement line mineralization remained normal.<sup>134</sup> Other mechanism could be the fact that diabetes promotes a hypercoagulative state and a delay in fibrin clot resolution due to an increased thrombin formation, platelet activation and fibrin resistance.<sup>133</sup><sup>135</sup> These facts hinder platelet cytokines and growth factor release and cause a limited pericytes and endothelial migration into the implant surface together with a reduced angiogenesis.<sup>136</sup> Moreover, hyperglycemic conditions are related to a reduction in bone formation markers like osteocalcin and bone-specific alkaline phosphatase and also bone resorption markers like C-terminal telopeptide of collagetype I (CTX).<sup>137</sup> Serum levels of osteoprotegerin (OPG) are increased following an episode of hyperglycemia and it also leads to a lower bone density due to the accumulation of advanced glycation end products (AGEs) that affect the organic bone matrix, reduce osteoblast proliferation and function and increase osteoclast resorption.<sup>137 138, 139</sup>

Moraschini et al. concluded in a systematic review that the rate of implant failure is not higher for diabetic subjects than non-diabetic ones, nor between type 1 and type 2 diabetic subjects.<sup>140</sup> However, non-diabetic patients showed a statistically significant less crestal bone loss than diabetic patients.<sup>140</sup> Further studies are needed to elucidate the impact of hyperglycemia upon dental implants.

#### **4.4 Other factors**

Not only metabolic issues can influence bone remodeling. Some patient-specific factors like medication intake might induce changes in bone cells and bone turnover and lead to bone loss around dental implants.<sup>141</sup> Higher bone turnover seems to expose more implant surface<sup>140</sup> and mandible might be a particularly vulnerable location.<sup>141</sup> Serotonin reuptake inhibitors and proton pump inhibitors have been related to an increase in bone loss and higher implant failure<sup>142</sup>. so an updated and thorough medical records are advocated to avoid complications.

Other patients might present some degree of hypersensitivity to titanium particles<sup>143</sup> or ions released from implant surface.<sup>141</sup> The corrosion of the implant surface or the dioxide layer titanium degradation can release particles that induce inflammatory reactions in the peri-implant tissues.<sup>144</sup> Aseptic loosening is the main reason for implant hip long-term failures.<sup>145</sup> According this model, wear particles are recognized as foreign body substances and phagocytosed by macrophages.<sup>144</sup> Later, M1 cells release inflammatory cytokines that promote osteoclastogenesis and osteolysis of the peri-implant bone.<sup>144</sup>



## 5. Role of macrophages in bone and peri-implant breakdown and regeneration

Macrophages play a prominent and central role in bone homeostasis and bone/biomaterial integration around dental implants.<sup>146</sup> Specifically in bone tissues, a special subset of macrophages, termed osteal macrophages (or OsteoMacs), have recently been hypothesized to play a pivotal role in the fate of implant osseointegration.<sup>146</sup> The general role of OsteoMacs in bone is to act as immune surveillance cells within their microenvironment.<sup>147, 148</sup> Yet when a foreign body biomaterial such as a dental implant is inserted trans-mucosally into the alveolar bone, a rapid accumulation of macrophages is typically found at the implant surface.<sup>149</sup> Chehroudi et al. clearly showed that bone formation on rough titanium dental implant surfaces was routinely preceded by macrophage accumulation (prior to bone deposition).<sup>149</sup> Despite this prominent finding, it is interesting to note that over 90% of research to date has focused on osteoblast and fibroblast behavior to material surfaces with only a small percentage (10%) dedicated to immune cell interactions including monocytes, macrophages, osteoclasts, leukocytes and multinucleated giant cells (MNGCs).<sup>150</sup> This major discrepancy is difficult to understand given the fact that macrophages and immune cells in general dictate how biomaterials will eventually be integrated into host tissues.

Studies from basic research have been pivotal to better understand the role of macrophages in bone biology. A series of key studies on OsteoMacs has shown that their removal during bone development is consistently found associated with a reduction in bone modeling, bone remodeling and bone repair.<sup>151-154</sup> Furthermore, in primary osteoblast cultures (containing macrophages), the simple removal of macrophages from these *in vitro* systems leads to a 23-fold decrease in the mineralization potential of bone-cells.<sup>153, 155</sup> Therefore, while basic studies have clearly pointed to their vast and substantial role in bone biology, much less information is available concerning the response of macrophages to implanted biomaterials. It is therefore pivotal to better characterize how immune cells and macrophages behave in relation to dental implant osseointegration and maintenance.

### 5.1. Macrophage polarization: M1-M2 phenotypes

While the objective of this review is not to highlight macrophage biology, it is important to note that they are some of the most plastic cell types found in the human body. They polarize completely from the classical M1 macrophage (involved in tissue pro-inflammation) towards M2 (tissue regeneration) macrophages. They may also fuse into osteoclasts and resorb bone or fuse

into multinucleated giant cells (MNGCs) where their role remains poorly defined.<sup>156, 157</sup> Major differences between M1 and M2 macrophages is that M1 macrophages have their arginine metabolism shifted to nitric oxide (NO) and citrulline, whereas M2 macrophages are shifted towards ornithine and polyamines.<sup>158</sup> M1-macrophages produce NO as a main effector molecule capable of inhibiting cell proliferation,<sup>159</sup> while M2-macrophages generate ornithine increasing cell proliferation and repair through polyamine and collagen synthesis.<sup>160</sup>

During dental implant osseointegration, classical M1-macrophages secrete a wide array of pro-inflammatory cytokines including TNF-alpha, IL-1Beta, IL-6, IL-12, MMP2, MMP9 typically induced by IFN- $\gamma$  + LPS or TNF- $\alpha$  (*in vitro*).<sup>159, 161</sup> In contrast, M2-macrophages are produced in response to IL-4 or IL-13 and also secrete a wide variety of pro-regenerative cytokines including PDGF-BB, TGB1, VEGF, IL-4, IL-10, CCL18 (Table 1). As can be expected, their polarization around implant surfaces is highly relevant for implant integration and long-term stability. Interestingly, Spiller et al. showed that macrophages can completely polarize from M2 wound-healing macrophages towards M1 pro-inflammatory macrophages within as little as 3 days and vice versa.<sup>162</sup> Therefore, their role, especially as it relates to peri-implant infection, is extremely vital for the long-term maintenance of dental implants.

### 5.2 Impact of implant surface topography and chemistry on macrophage behavior

As previously mentioned, one area of research that has been largely omitted is the effect of implant surface material, topography, chemistry and composition on immune cell behavior. While this topic has recently been reviewed,<sup>146</sup> it is important to note that surface roughness in general tends to increase a pro-inflammatory response. It has been shown that roughness [e.g., sandblasted acid etched (SLA)] surfaces tends to increase M1 macrophage polarization,<sup>163-165</sup> whereas a modification to their surface chemistry has been shown to reduce this pro-inflammatory response (modified-SLA surfaces).<sup>164, 165</sup> Despite this, a great deal of information concerning the behavior of monocytes/macrophages as well as their fusion to MNGCs remains unknown. A small percentage of dental implants are lost every year for yet known reasons unassociated with peri-implant infections.<sup>146, 166</sup> This is most likely caused by immune cell biocompatibility interactions not yet fully understood and future research in this field is likely to further advance of understanding of the prominent role of immune cells during early and late stages of implant osseointegration.

### *5.3 Macrophages, Immune Cells and the Foreign Body Reaction*

It has been reported that implant osseointegration is a long-term equilibrium between host immune cells and bone biomaterials.<sup>167-169</sup> The literature showed MNGC accumulation on implant surfaces leads to biomaterial breakdown and possible implant failure/rejection.<sup>167-169</sup> These papers provide a platform whereby implant osseointegration and eventual peri-implant bone loss is likely a direct result of a M1/M2 shift in macrophage polarization. Interestingly, invading periodontal pathogens are known to secrete lipopolysaccharides (LPS), a known and direct molecule influencing pro-inflammatory M1-macrophage polarization.<sup>170</sup> Hence, it is important to examine foreign body reaction, equilibrium between M1 and M2 macrophage and MNGC polarization. Furthermore, these papers stressed heavily on material rejection with MNGCs accumulation on the implant surface. While MNGCs have certainly been implicated in bone biomaterial material rejection,<sup>167-169</sup> it is interesting to note that accumulating evidence has now shown that MNGCs (which are hypothetically derived from the fusion of macrophages) are also capable of polarizing towards M1-MNGCs and M2-MNGCs. In addition, other researchers have shown that MNGCs are capable of expression M2-macrophage markers following macrophage accumulation on their biomaterial surface.<sup>171</sup> Specifically around bone grafting materials, MNGCs have been shown to exist in stable human bone many years following their implantation<sup>172-174</sup> and have been associated with a rapid increase in tissue vascularization.<sup>175-178</sup> It is evident that much further research is needed to better understand their role in bone biomaterial integration and implant osseointegration.

It is therefore a necessity to accurately characterize immune cells such as macrophages and MNGCs and their interaction with dental implants, their osseointegration and their maintenance. It is likely that both cells are prone to reversibly shifting their polarization from M1/M2 macrophage/MGNCs yet little research to date has been performed as it relates to dental implants. Furthermore, many cell types are found in small spaces within the oral cavity around dental implants including osteoblasts, osteoclasts, osteocytes, fibroblasts, endothelial cells, leukocytes etc. Since macrophages (and most likely MNGCs) express high levels of cytokines, it becomes highly relevant to determine how cell-cell communication occurs between macrophages/MNGCs and other cell types (via direct cell contact or paracrine activity) during bone remodeling of implants. This field of study has thus far been left entirely unstudied yet possesses major clinical implications.

It is also interesting to note that more recent research from the field of cardiovascular disease has shown that the calcification of arteries is a direct result of macrophages and MNGC polarization towards M2-macrophage/M2-MNGCs in the intima layers of arterial walls where they express high levels of IFN-gamma.<sup>179, 180</sup> It has been shown that macrophages polarize towards M2 phenotypes and begin to form ectopic bone in areas where bone should otherwise not be formed.<sup>146</sup> Hence, it is interesting to point out that in this scenario, immune cells (such as macrophages) are dictating new bone formation. Therefore, growing evidence from many fields has now shown that macrophages playing a vast and substantial role in bone modeling and homeostasis.

### Conclusions

The present review highlights some of the recent advancements in the area of bone remodeling around dental implants in both health and disease conditions. While peri-implant bone remodeling has received much attention, it remains important to better understand how loading and implant bed preparation affects bone lining cells and osteocyte viability and signaling at early stages of healing. Furthermore, the effects of systemic levels of cholesterol, fatty acids and vitamin D are discussed as potential responsible factors for early implant loss and long-term implant stability. We also stress out the prominent role of immune cells (e.g., OsteoMacs and multinucleated giant cells) and their impact during dental implant osseointegration and maintenance.

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### *Figures and legends.*

Table 1. Cytokines released by M1 and M2 macrophages.

Figure 1. Histological section of maxillary sinus lateral wall with cortical bone surface covered by bone lining cells (Immunofluorescence for Tubulin and DAPI, 20x).

Figure 2. Bone remodeling diagram. A; Cells involved in the process (modified from Weilbaecher, K. N., Guise, T. A., & McCauley, L. K. (2011). Cancer to bone: a fatal attraction. *Nature Reviews Cancer*, 11(6), 411-425. B; Bone remodeling after excessive implant torque. 1) Excessive torque promotes bone damage including the osteocyte network. 2) Osteoblasts and osteoclasts are recruited from the blood, the marrow or from BLCs to populate the bone remodeling compartment. 3) Osteoclasts remove the damaged bone. 4) Bone lining cells clean the debris after osteoclast resorption. 5) BLCs secrete fibrillar collagen. 6) This collagen layer allows osteoblasts to attach. 7) Osteoblasts deposit osteoid to fill the compartment. 8) Osteoblasts trapped into the osteoid become osteocytes or bone lining cells where most undergo apoptosis (modified from Seeman E. Bone modeling and remodeling. *Crit Rev Eukaryot Gene Expr* 2009; **19**: 219-233).

Figure 3. Bone microcracks as a consequence of excessive implant torque (modified from Cha JY, Pereira MD, Smith AA, Houschyar KS, Yin X, Mouraret S, Brunski JB, Helms JA. Multiscale analyses of the bone-implant interface. *J Dent Res* 2015; **94**: 482-490.

Upper images (A, B, C) : Schematic of the osteotomy relative to the implant's external diameter. Image A shows an oversized preparation with the presence of a gap (\*) between the implant and bone. This gap is filled with fibrous tissue. Image B shows a osteotomy where the implant reached low torque and a smaller gap (\*) is observed. Image C shows an undersized osteotomy that induces a high torque without gap bone-implant.

Image D: Illustration of compressive strain fields around an implant placed with low torque. Only 15-20 microns of the thread engaged in bone and promoted a small region of moderate strain.

Image E: Illustration of increased compressive strain fields around an implant placed under

increased torque. The threads engaged deeply in bone and created a larger region of high strain around the threads and the implant body. Image F: Illustration of photoelastic stress around an high-torque implant.

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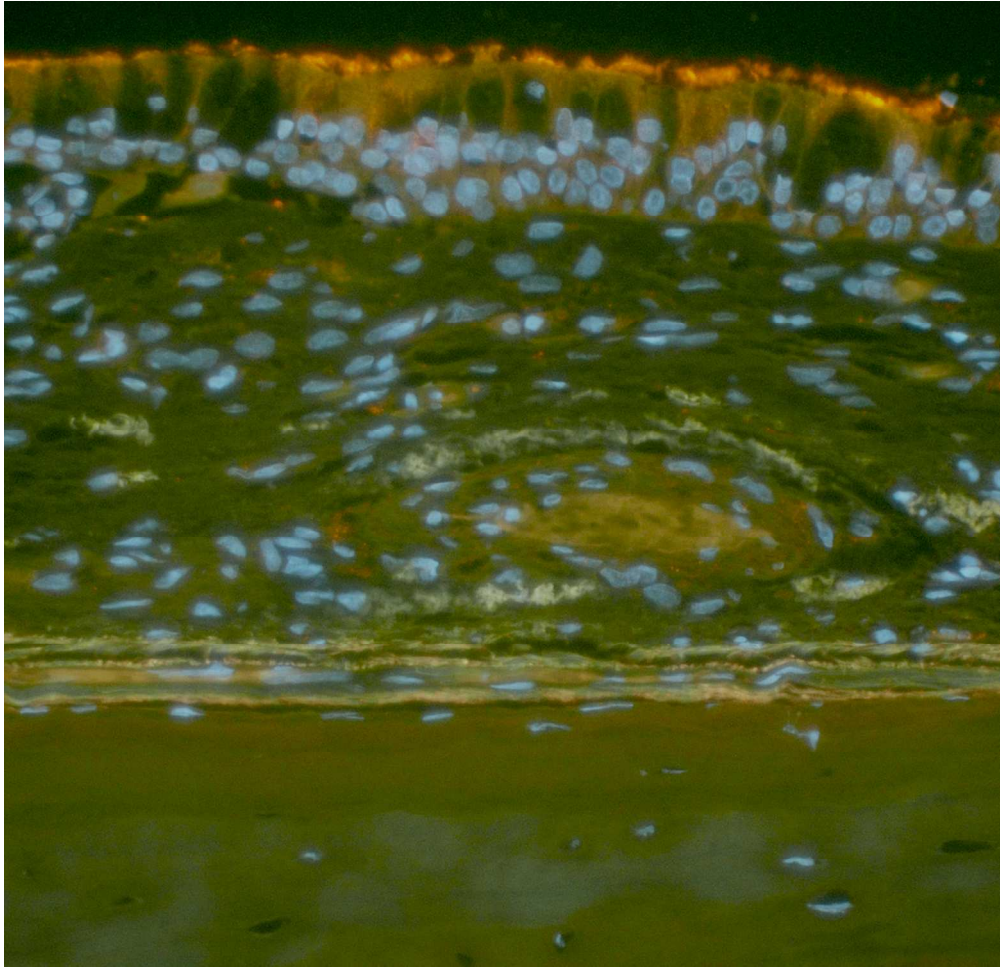


Figure 1. Histological section of maxillary sinus lateral wall with cortical bone surface covered by bone lining cells (Immunofluorescence for Tubulin and DAPI, 20x).

131x126mm (300 x 300 DPI)

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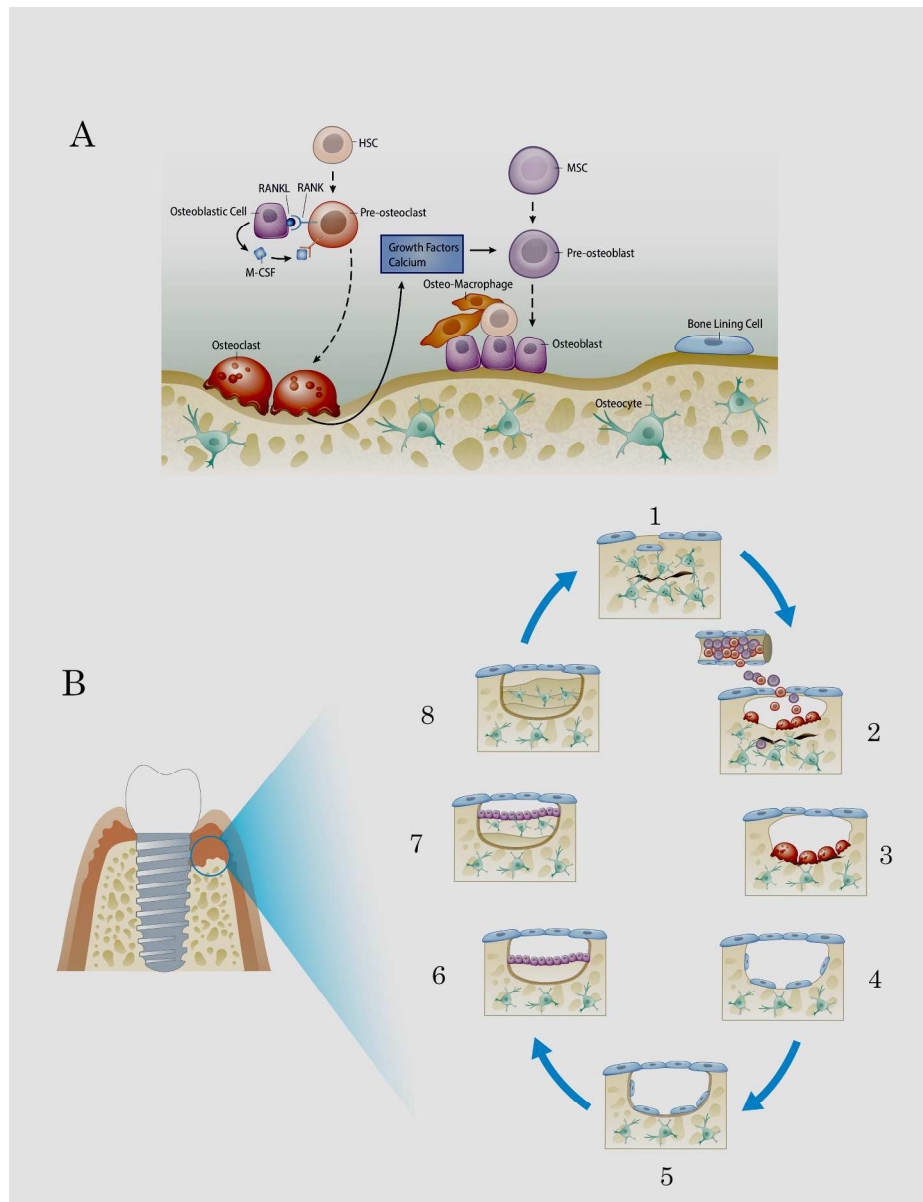


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215x279mm (300 x 300 DPI)

Accepted Article

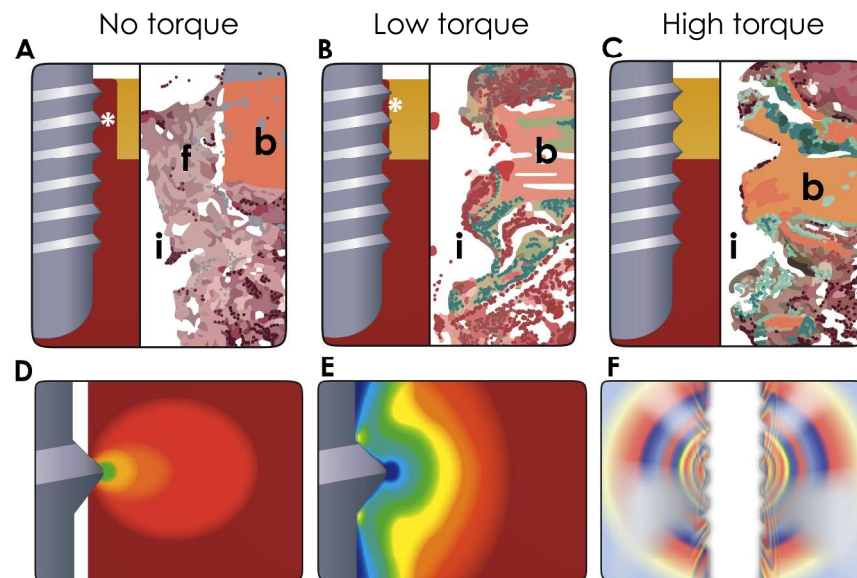


Figure 3. Bone microcracks as a consequence of excessive implant torque (modified from Cha JY, Pereira MD, Smith AA, Houschyar KS, Yin X, Mouraret S, Brunski JB, Helms JA. Multiscale analyses of the bone-implant interface. *J Dent Res* 2015; 94: 482-490.

Upper images (A, B, C) : Schematic of the osteotomy relative to the implant's external diameter. Image A shows an oversized preparation with the presence of a gap (\*) between the implant and bone. This gap is filled with fibrous tissue. Image B shows a osteotomy where the implant reached low torque and a smaller gap (\*) is observed. Image C shows an undersized osteotomy that induces a high torque without gap bone-implant.

Image D: Illustration of compressive strain fields around an implant placed with low torque. Only 15-20 microns of the thread engaged in bone and promoted a small region of moderate strain. Image E: Illustration of increased compressive strain fields around an implant placed under increased torque. The threads engaged deeply in bone and created a larger region of high strain around the threads and the implant body. Image F: Illustration of photoelastic stress around an high-torque implant.

220x154mm (300 x 300 DPI)

A

**Table 1:** Markers of macrophages of M1 and M2 phenotypes, adapted from <sup>136</sup>. Those highlighted in yellow are most common markers utilized to investigate M1 and M2 macrophage polarization.

M1	M2
<b>B 7 (CD80)</b>	<b>M130 (CD163)</b>
<b>B7.2 (CD86)</b>	<b>CD206 (MRC1, mannose receptor)</b>
<b>CCR7 (MCP-3)</b>	<b>FcεRII (CD23)</b>
<b>CCL22 (MDC1)</b>	<b>CD36</b>
<b>CD64</b>	
CXCL10 (IP-10)	IL-1 Ra
SOCS1	
TLR-2	Nucleotide receptors (GPR86, GPR105,
TLR-4	P2Y8, P2Y11, P2Y12)
FcγRIII (CD16)	C-type lectin-like receptor dectin-1
FcγRII (CD32)	DC-SIGN (CD209)
LAM-1 (CD62)	DCIR (CLECSF6)
IL-1 R1	CLACSF13
IL-7R (CD127)	FIZZI, ST2 (mouse)
IL-2R (α chain)	Phagocyte receptors (SR-A, M60)
IL-15R (α chain)	CXCR4, fusin (CD184)
IL-17R (CTLA8) (CDw217)	TRAIL

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