OSSEOINTEGRATION – MOLECULAR EVENTS AT THE BONE-IMPLANT INTERFACE- A REVIEW .

Dr. Sagrika Shukla, Dr. Ashi Chug, Dr. Lanka Mahesh, Dr. Sanjay Jadwani.

Abstract

Osseointegration is probably the most studied and most investigated area in implantology. A thorough and complete understanding of what happens at the bone-implant interface is important for the implantologist and the manufacturer, thereby enabling the implant manufacturer a product which conforms to the standards and giving better clinical predictability to the clinician. This article serves to be a comprehensive review of literature on the various aspects of Osseointegration today, considering the scope and the depth at which investigators are involved in research on the subject.

Key words – Osseointegration, bone-implant interface.

Introduction :

Collins in 1954 and Southam & Selwyn in 1970, disagreed and disapproved with the idea of bone to implant contact without formation of a fibrous layer, since decades there had been a thought of development of fibrous layer around implant, diminishing its integrity with bone[1,2]. Prof. Per-Ingvar Brånemark and his colleagues in 1950s and 1960s, while examining microcirculation of bone and wound healing through means of vital microscopy[1] accidentally discovered the process of Osseointegration, and a new dimension in the field of implantology had been reached which gave better treatment options to patients improving function and overall health. What became significant with that innocuous sagacity was the adherence of bone with titanium metal, without the formation of fibrous layer, which could not be removed without fracture[3].

The term Osseointegration was first used by Prof I-P Branemark[5], since then it has been used to describe the procedure of bone attachment with titanium. Though lately, the Glossary of Prosthetic Terms

(Sixth Edition) lists the terms Osseointegration and osteointegration but recommends the use of the term osseous integration[4]. However, in this article term Osseointegration will be used.

Osseointegration was originally defined as, a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant by Branemark in 1985[6]. Albrektsson[7] in 1981 defined it as, a direct on light microscopical level, contact between living bone and implant. Steinemann[8] in 1986 defined it as, A bony attachment with resistance to shear and tensile forces. Branemark[9] in 1990, then gave a modified definition of his own – "A continuing structural and functional coexistence, possibly in a symbolic manner, between differentiated, adequately remodeling, biologic tissues and strictly defined and controlled synthetic components providing lasting specific clinical functions without initiating rejection mechanism."

Bone

Osseointegration is an ongoing procedure representing process of formation and adaptation to function and repair, which takes place due to Osteoblastic and Osteoclastic activity of bone, also known as coupling[10-13].

Osteoblasts are of mesenchymal origin and differentiate under the influence of local growth factors such as fibroblastic growth factor (FGFs), bone morphogentic proteins (BMPs), and Wnt proteins, and also require transcription of Runx2 and Osterix transcription factors[14]. Osteoblasts also govern the activity of osteoclasts by secreting Osteoprotegrin (OPG), which is a decoy RANK, which inhibits osteoclastic bone resorption[15].

Osteoclasts are bone resorbing cells and function in conjuction with Osteoblasts.

Osteocytes are the new cells which get trapped inside the new bone matrix. They communicate with other bone cells through numerous cellular membrane protrusions that lie in tunnels, known as Canaliculi. The function of these networked cells is partly unknown, nevertheless they are thought to participate in bone resorption[16] and sense mechanical load on bone[17].

Bone lining cells cover majority of quiescent boney surfaces, however their function is still partially unknown and their origin is under debate[10].

Events at Bone-Implant Interface.

As soon as the implant is placed in the prepared site, within nanoseconds there is formation of water molecule layer around it, which is greatly influenced by implant surface[18]. This layer facilitates protein and other molecules to adsorb on the implant surface[19,20]. In the 2nd stage, within 30 seconds to hours after implantation, the implant surface is covered by a layer of extracellular matrix proteins. Its conformation, orientation and composition, depends upon the surface type. These proteins first come from blood and tissue fluids at the wound site and later from the cellular activity in the periprosthetic region[21]. In the 3rd stage, interaction of cells with implant surface via adsorbed protein layer takes place, initiating cellular adhesion, migration and differentiation, which occurs from few hours to several days[22]. This stage is enormously and tightly regulated by ECM proteins, cell surface-bound and cytoskeletal proteins, chemical characteristics, implant topographies and chemical ions released by the surface[23].

ECM contains information that is interpreted by cells via adhesion structures and influences cell shape, cytoskeletal organization, cell motility and polarity, gene expression, proliferation and survival. It also contains type I collagen, proteoglycans and noncollagenous proteins[24-26].

In actual terms ECM is the mode through which transfer of information takes place via a no of proteins, to name some like, collagen I, fibronectin, thrombospondin, osteonectin, osteopontin, osteoadherin, bone sialopeoteion (BSP), most of them function as cell attachment mediators, some signaling and cell-cell and cell-protein interactions[27]. Certain Plasma proteins like α_2HS-glycoprotein[21]. Also, there is absence of serum proteins like albumin, indicating selective accumulation/deposition of molecules at the interface[21]. Molecules containing Arg-Gly-Asp or RGD sequence are believed to play role in cell adhesion and binding of minerals[21]. This RGD sequence is present in a

number of ECM proteins like fibrin, collagen, fibronectin, vitronectin, osteopontin and bone sailoprotein[28].

Cell attachment is a complex procedure and takes place with the help of Integrins, Focal Adhesion and Filopodia. Integrins are transmembrane cell surface receptors which mediate physical contact of cell to the outside matrix for propagation of signaling from outside-to-inside and vice versa[29,30]. They contain α- and β- subunits, with each cell expressing different mixture of Integrins[31]. Focal adhesion are integrin based molecular compositions of cells participating in adhesion dependent signaling[32,33] and link ECM to the actomyosin sytoskeleton of a cell[34]. These structures are motile, can assemble/disassemble, disperse and recycle according to the cell's need[34,35]. Filopodia are Actin rich cell extensions through which cell adherence takes place on rough surface[36]. Filopodia scan substrate's surface structures and stabilize the cell according to signals received from micro or submicrometre-structured pores which act as a favorable environment during the path-finding phase[36]. Optimum anchorage takes place by specific points along the Filopodia as well as their tips. The tips broaden and branch out to become localized adhesive structures, known as footpads[37]. Cell spreading is mediated by cell membrane extensions at footpads, or by protrusion of a cytoplasmic sheet, i.e. a lamella or a Lamellipodium between adherent Filopodia[37,38,39]. On the other hand cells adhere with smooth surface through focal adhesion. Filopodia while scanning the smooth surface get negative signals and retract back to the cell body, resulting in well-developed stress fibers which exert tension across the cell body making more flattened cells with reduced cellular attachment to their surrounding substrate[18,36].

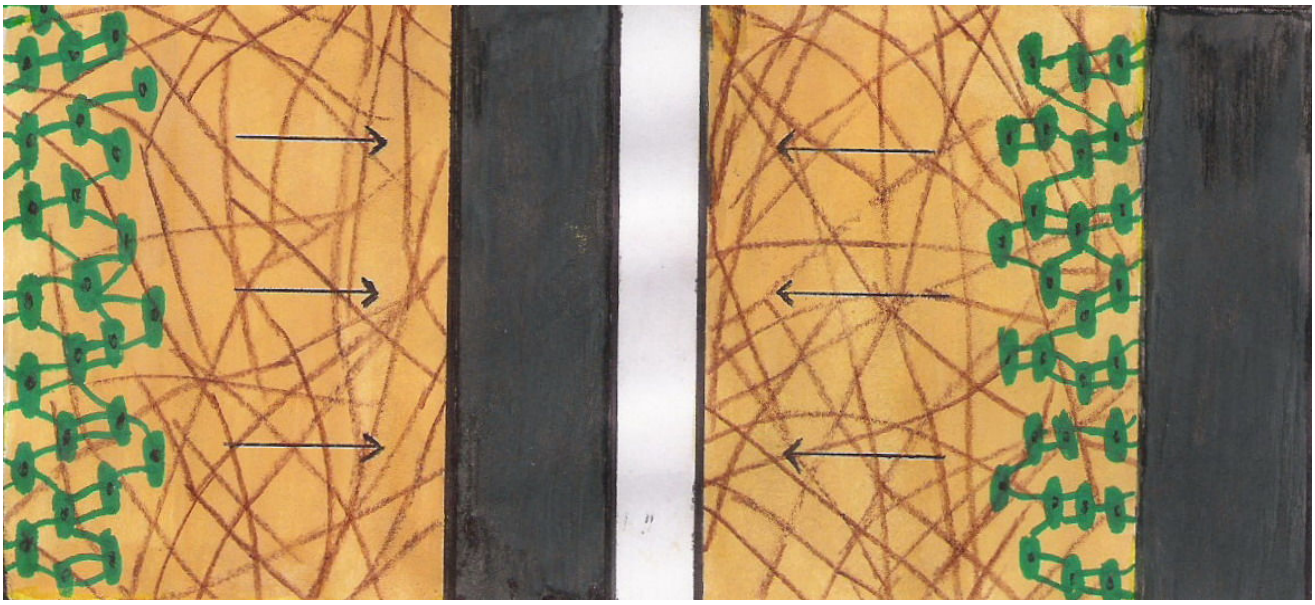
At day 1, on the day of implant placement, there is adsorption of water molecules and platelet which secrete growth factors, signaling and enabling Osteoblasts to adhere at the implant surface via fibronectin mediated focal adhesion[41]. The first cells to migrate at the implant surface are multipotent mesenchymal cells and not committed Osteoblasts[42], and the ability of these cells to differentiate into functional Osteoblasts depends upon local oxygen tension[43], availability of nutrients and local regulatory growth factors, which in turn depends upon vascularity of implant site and host physiology[27]. Migration of these cells also depends upon diminishing oxygen concentration gradient towards the center of the

wound, which happens due to local ischemia and necrosis because of cessation of circulation and lack of oxygen supply for the osteocytes due to broken cappilaries[40]. Neutrophils are the most numerous cells peaking at 24-48 h, but later macrophages rapidly become predominant. Both these cells are involved in clot and necrotic tissue formation.

On day 3, cells around implant activate osteoblast related transcription factors Runx2 and Op[44]. By day 4, Necrotic bone created during surgery gets resorbed, and a well defined interface zone is formed[45]. By day 5, there is evidence of new bone formation and presence of Alkaline Phosphatase activity is seen, indicating onset of mineralization and evidence of matrix remodeling[44]. At the end of 1 week, Osseous matrix adherence on implant surface can be easily distinguished, ECM gets anchored in the cavities on the surface[44] and Bone to implant contact ratio becomes 35.8 ± 7.2%[46]. By day 16, implant surface is well covered and extensively integrated in a mixture of mineralized tissue, osteoid and dense matrix[45].

By day 28, at the end of 4 weeks, there is Intimate bone contact over the whole length of the implant surface and also at the neck, Collagen fibers and Osteoblasts make bulk of tissue layer adjacent to implant, Collagen fibers orient themselves parallel to the implant surface, cells, ECM proteins and mineralized bone tissue appear in direct contact with implant and bone to implant contact ratio becomes 46.3 ± 17.7%[46]. According to Davies (2003)[40] and Puleo-Nanci (1999)[21], bone formation occurring in 2 directions, from implant surface towards bone and from bone towards implant surface also known as contact osteogenesis and distance osteogenesis (fig 1&2). In contact osteogenesis bone forms at a 30% faster rate[21]. In this, the implant surface has to be colonized with bone cells before bone matrix formation can begin, the same mechanism that takes place during remodeling procedure, also known as de novo bone formation[40]. In distance osteogenesis, new bone is not forming at the implant surface, but implant gets surrounded by bone. This procedure is expected to occur in cortical bone healing[40].

Initially woven bone is formed, which has osteoids in its matrix. At the end of 12 weeks, newly developed bone integrated at implant surface with intimate contact of mature lamellar bone with titanium surface[45].



Distance osteogenesis. contact osteogenesis.

CONCLUSION:

Osseointegration is a very complex procedure, there are still many micro and macro molecular aspects of bone-implant interface that need to be understood and elucidated. But with the experiments and studies done by many authors so far, we can state that healing patterns in cortical and trabecular bone are different. Cortical healing relies on osteonal remodeling, while, trabecular healing on the phenomena of osteoconduction and de novo bone formation[40].

Bone formation at the bone injury site takes place due to coupling mechanism, according to frost this mechanism of formation and resorption must exist. Biomechanical environment at the fracture site immensely influences the development of cartilage and bone.

When an implant placement site is prepared, a wound is created, and healing at the bone-implant phase takes place in the same manner as it does at the bone fracture site, thus they both begin with a breach in an intact skeletal site, an immune response, neo-vascularization, and recruitment of skeletal progenitor cells. However in a fracture, some skeletal progenitor cells differentiate into chondrocytes, while others into Osteoblasts, followed by endrochondral ossification. Where as, around an implant all skeletal progenitor cells differentiate into Osteoblasts, followed by intramembranous ossification. Another difference in implant

healing is that the process of Osseointegration is largely influenced by implant surface, chemical composition and implant biomechanics[44].

Thus, as soon as the implant is placed there is aggregation of platelets[40]. These platelets secrete growth factors like Platelet Derived Growth Factor (PDGF-BB), insulin-like growth factors (IGF-1, IGF-2), fibroblastic growth factors (a-FGF, b-FGF), Transforming growth factor beta (TGF β's), bone morphogenic proteins (BMPs)[27] and vasoactive factors serotonin and histamine[40], (fig 3). These growth factors further differentiate, proliferate and attach Osteoblasts with titanium surface (fig 4.) and form new bone matrix (fig 5). The transcription factor protein core binding-facto-alpha (Cbfa1) regulates this development[47].

There is enough evidence based literature demonstrating a direct relation between Osseointegration and surface topography. It has been proved that rough surface enhances the process of Osseointegration with better attachment, resulting in Filopodia, also there is four-fold increase in Cbfa1 on rough surface[47]. It has also been postulated that an increase in surface area is not a decisive factor for regulating cell growth at bone-implant interface, but the implant's surface topography plays a vital role and changes cell structure accordingly, as on smooth surface Osteoblasts are oriented in parallel manner, and on rough surface cells have a stellate shape[36]. Thus new bone matrix at the bone-implant interface is formed through osteoconduction, osteoinduction, osteogenesis and osteopromotion[27].

Osteoconduction means directing bone forming activity to a particular site or surface, like, Hydroxyapatite coatings serve as scaffold for cells to attach and grow[27,40]. Osteoinduction involves the recruitment of mesenchymal stem cells to become osteoblasts. Implant surfaces are not osteoinductive. Osteogenesis refers to the stimulation of committed osteoprogenitor cell proliferation and encouragement of osteoblast biosynthetic activity. The 4th term osteopromotion is relatively new and refers to bone formation at local osseous sites using membrane barrier techniques and is in use for clinical promotion of cells only[27].

Apart from the details provided in this article there are many unanswered questions, however there is no other area of study in implantology being investigated as thoroughly as osseointegration and the results of ongoing and future research projects would provide an even better understanding and a more clear picture at what happens at

the bone implant interface thereby providing implantologists adequate information required to provide the patient best possible clinical care after understanding patient's bone physiology and bio-physical need.

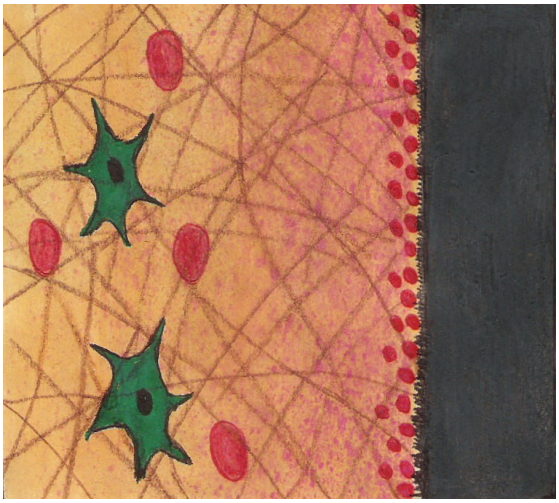


Fig 3.

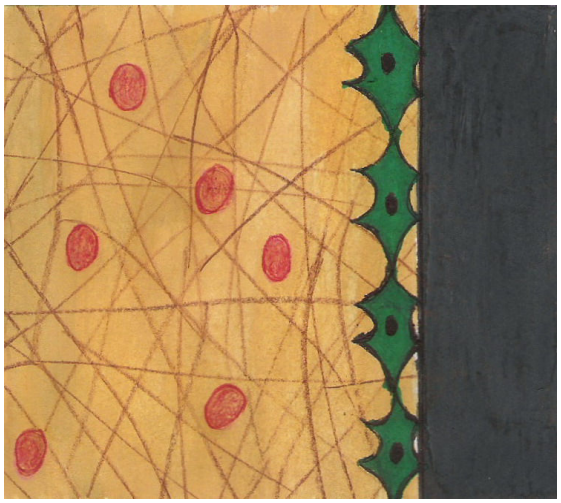


Fig. 4.

Fig. 5.

REFERENCES:

1. Brånemark R, Brånemark P-I, Björn Rydevik, Robert R. Myers, osseointegration in skeletal reconstruction and rehabilitation: a review. J of rehab research & deve. Vol 38 no. 2 2001: 175-181
2. Southam JC, Selwyn R. Structural changes around screws used in the treatment of fractured human mandibles. Br J Oral Surg 1971;8:211–21.
3. Brånemark P-I. Osseointegration and its experimental studies. J Prosthetic Dentistry 1983;50:399–410.
4. The glossary of prosthetic terms. 6th ed. J Prosthet Dent 1994;71:89.
5. Brånemark P-I,Zarb GA,Albrektsson T. Tissue-integrated prostheses: osseointegration in clinical dentistry. Chicago: Quintessence International; 1985.
6. Branemark P-I: Introduction to Osseointegration. In Branemark P-I, Zarb G, Albrektsson T (eds): Tissue Integrated Prosthesis. Chicago, Quint Publishing, 1985, pp 11-76.
7. Albrektsson T, Branemark P-I, Hansson HA, Lindstorm J: Osseointegrated titanium implants. Requirements for ensuring Long lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 52:155,1981.
8. Steinemann SG, Eulenverger J, Maeusli PA, Schroeder A: Adhesion of bone to titanium. Adv biomater 6:409, 1986.
9. Branemark P-I: Precision, Predictability, Gothenburg, Sweden. Institute for Applied Biotechnology, 1990.
10. Muhonen, Virpi, Bone–Biomaterial Interface. The effects of surface modified NiTi shape memory alloy on bone cells and tissue. Acta Univ. Oul. D 974, 2008.
11. Rodan GA, Martin TJ. Role of Osteoblasts in Hormonal Control of Bone Resorption – a hypothesis. Calcify Tissue Int (1981) 33:349-351
12. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J & Boyle WJ (1998) Osteoprotegerin ligand is a cytokine that regulates

osteoclast differentiation and activation. Cell 93: 165-176.
13. Martin TJ, Sims NA (2005) Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med 11: 76-81.
14. Komori T (2008) Regulation of bone development and maintenance by Runx2. Front Biosci 13: 898-903.
15. Baron R (2003) General principles of bone biology. In Primer on the Metabolic Bone Disease and Disorders of Mineral Metabolism, 5 ed. American Society for Bone and Mineral Research,p 1-8.
16. Shimizu H, Sakamoto M, Sakamoto S (1990) Bone resorption by isolated osteoclasts in living versus devitalized bone: differences in mode and extent and the effects of human recombinant tissue inhibitor of metalloproteinases. J Bone Miner Res 5: 411-418.
17. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K (2007) Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab 5: 464-475.
18. Singhatanadgit W. Biologic Responses to New Advanced Surface Modifications of Endosseous Medical Implants. Bone and Tissue Regeneration Insights 2009:2 1–11.
19. Sharad AG, Tomlins PE. Biocompatibility and the efficacy of Medical Implants. Regen Med. 2006;1:789-800.
20. Thevenot P, Hu W, Tang L. Surface Chemistry Influences Implant biocompatibility. Curr Top Med Chem. 2008;8:270-80.
21. DA Puleo, A Nanci. Understanding and controlling the bone-implant interface. Biomaterials 20 (1999) 2311-2321.
22. Wilson CJ, Clegg RE, leavesley dl, pearey mj. Meditation of biomaterial cell interactions by adsorbed proteins: a review. Tissue Eng. 2005;11:1-18.
23. Ratner BD, Bryant SJ. Biomaterials: where we have been and where we are going. Annu Rev Biomed Eng. 2004;6:41-75.
24. Damsky CH & Werb Z. Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. Curr Opin Cell Biol 4:772-781.
25. Globus RK, Doty SB, Lull JC, Holmuhamedov E, Humphries

MJ & Damsky CH. Fibronectin is a survival factor for differentiated osteoblasts. J Cell Sci 111: 1385-1393.
26. Moursi AM, Damsky CH, Lull J, Zimmerman D, Doty SB, Aota S, Globus & RK. Fibronectin regulates calvarial osteoblast differentiation. J Cell Sci 109: 1369-1380.
27. Cooper LF. Biologic determinants of bone formation for osseointegration: Clues for Future clinical improvements. J Prosthet Dent 1998;80:439-49.
28. Schwartz Z, Lohmann CH, Oefinger J et al. Implant surface characteristics modulate differentiation behavior of cells in the osteoblastic lineage. Adv Dent Res 1999;13:38-48.
29. Hynes RO (1987) Integrins: a family of cell surface receptors. Cell 48: 549-554.
30. Takagi J, Petre BM, Walz T & Springer TA (2002) Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. Cell 110: 599-11.
31. Clover J, Dodds RA & Gowen M (1992) Integrin subunit expression by human osteoblasts and osteoclasts in situ and in culture. J Cell Sci 103: 267-271.
32. Abercrombie M, Heaysman JE & Pegrum SM (1971) The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. Exp Cell Res 67: 359-367.
33. Avnur Z & Geiger B. The removal of extracellular fibronectin from areas of cell-substrate contact. Cell 25: 121-132.
34. Sastry SK & Burridge K (2000) Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. Exp Cell Res 261: 25-36.
35. Smilenov LB, Mikhailov A, Pelham RJ, Marcantonio EE & Gundersen GG (1999) Focal adhesion motility revealed in stationary fibroblasts. Science 286: 1172-1174.
36. Zhu X, Chen J, Scheideler L et al. Cellular reactions of Osteoblasts to micron- and submicron-scale porous structures of titanium surfaces. Cells Tissues Organs 2004;178:13-22.
37. Rosen, J.J., L.A. Culp (1977) Morphology and cellular origins of substrate-attached material from mouse fibroblasts. Exp Cell Res 107:139–149.

38. Albrecht-Buehler, G. (1976) Filopodia of spreading 3T3 cells: do they have a substrate-exploring function? J Cell Biol 69: 275–286.
39. Adams, J.C. (2001) Cell-matrix contact structures. Cell Mol Life Sci 58: 371–392.
40. Davies JE. Understanding peri-implant endosseous healing. J of Dnt Edu 67;8:931-949.
41. Ulrich Joos, Andre Büchter, Hans-Peter Wiesmann, Ulrich Meyer. Strain driven fast osseointegration of implants. Head & Face Medicine 2005, 1:6.
42. Schwartz Z, Martin JY, Dean DD et al (1996). Effects of titanium surface roughness on chondrocyte proliferation, matrix production and differentiation depends on the state of cell maturation. J Biomed Mater Res 30:145-155.
43. Rajpurohit R, Koch CJ, Tao Z et al (1996). Adaptation of chondrocytes to low oxygen tension: relationship between hypoxia and cellular metabolism. J Cell Physiol 168:424-432.
44. Colnat C et al. Molecular Analysis of Healing at a Bone-Implant Interface. J Dent Res 86(9):862-867, 2007.
45. Rita Depprich, Holger Zipprich, Michelle Ommerborn et al. Osseointegration of zirconia implants: an SEM observation of the bone-implant interface. Head & Face Medicine 2008, 4:25.
46. Andre Büchter, Ulrich Joos, Hans-Peter Wiesmann et al. Biological and biomechanical evaluation of interface reaction at conical screw-type implants. Head & Face Medicine2006, 2:5.
47. Schneider GB, Perinpanayagam H, Clegg M et al. implant surface roughness affects osteoblast gene expression. J Dent Res 82(5):372-376, 2003.