

REVIEW ARTICLE



A brief overview of cellular and molecular mechanisms of osseointegration

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Received 04 April 2015;

Accepted 06 May 2015

doi: 10.15713/ins.ijcdmr.70

How to cite the article:

Seyed Hadi Hosseini, Mozghan Kazemian, Sajedeh Ghorbanzadeh, "A brief overview of cellular and molecular mechanisms of osseointegration," Int J Contemp Dent Med Rev, vol.2015, Article ID: 010415, 2015. doi: 10.15713/ins.ijcdmr.70

Abstract

Osteointegration is one of the most studied issues and is considered as one of the most evaluated cases in implantology. It is important for implantologists to have an in-depth understanding of what exist in the bone-implant interface. It is a treatment plan that is compatible with standards and provides a better clinical forecast. The present study is a comprehensive review from all that have been conducted regarding various aspects of osteointegration.

Keywords: Bone-implant interface, dental implant, integrin, osseointegration

Introduction

Collins (1954), Southam and Selwyn (1970) refuted the claim that bone-implant connection does not contain any fibrous layer formation and within several decades of development, the layer has existed around the implant and its integration has decreased with the bone.^[1,2] Professor Brånemark *et al.* during 1950s-1960s, while working on microcirculation of bone and lesion treatment via microscope, discovered the osteointegration process accidentally and brought new lights in implantology through better medical choices for patients that increased their performance quality and general health. What seemed significant in this unexpected finding was bone adhesion to titanium with no fibrous layer formation, which was inseparable without fraction.^[3]

It was Professor Brånemark who used the term osteointegration for the first time and since then; the term is used to explain the process of bone-titanium connection. Although in the past, introduced other terms such as "osteointegration" and "osteointegration," it also recommends the term "osseous integration."^[4] In this article, however, we used the term "osteointegration."

Originally, osteointegration was defined by Brånemark in 1985 as a structural and functional connection between the

desired living bone and the surface of an implant that carries graft. In 1981, Albrektsson interpreted this term as a direction in the light microscopic level that establishes a connection between the living bone and the implant.^[5] And in 1986, Steinemann described it as a bone connection with resistance to shear and tensile strength.^[6] In 1990, Brånemark proposed a modified definition - "A feasibly stable structural and functional symbiosis in a symbolic method among distinguished biological tissues with remodeling and strictly controlled synthetic compositions and it is obvious that the last special clinical function is provided before the recoiling mechanism begins."

The Bone

Osteointegration is a developing process that indicates the formation procedure, role, and restoration adoption that, due to osteoplastic and bone osteoplastic activity, is known as a coupling agent as well.^[7-10]

Osteoblasts have mesenchymal origins that are under the influence of topical growth factors such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), and Wnt proteins, which require transcription from Runx2 and

Astrix transcription factors.^[11] Moreover, osteoblasts direct the osteoclasts activity by the secretion of osteoprotegerin, which is a RANK trap that inhibits the reabsorption of osteoblast bone.^[12,13]

Osteoclasts are bone absorbing cells and work in connection with osteoblasts, while osteocytes are new cells that are being trapped inside the new bone matrix. They make a connection with other bone cells through protruding the cell membrane in tunnels, which is called canaliculi. The role of these network cells is relatively unknown and however, they play a significant part in bone absorption^[14] and the sense of mechanical loading.^[15]

Although epithelial bone cells mostly cover the static bone surface, yet their function is somehow unknown, and there are doubts regarding their region.^[16]

Bone-implant interface events

As soon as the implant is placed in the prepared space, within a limited time as long as nanoseconds, the layer of water molecules form in its surrounding, which is considerably under the influence of implant surface.^[17] This layer facilitates protein and other molecule absorptions within the implant surface.^[18,19] In the second stage, within 30 s to several hours after the implant, the surface will be coated with a layer comprised of intercellular matrix proteins. Its structure, inclination, and composition depend on the surface type. These proteins initially come from blood and interstitial fluid in wound location and then derive from cell activity in the area around the prosthesis.^[20] In the third stage, cells interaction with the implant surface occurs via a protein layer, which is initiated by cell adhesion, migration, and differentiation that lasts for several hours or days.^[21] This phase is finely adjusted with extracellular matrix (ECM) proteins, cell surface binding and cytoskeleton proteins, chemical characteristics, binding topography, and chemical ion release.^[22]

ECM carries information that could be decoded by cells and cohesive structures, as well as cell shape, organizing cytoskeleton, mobility and polarity of the cell, gene expression, proliferation, and survival. The process includes collagen Type I, proteoglycans, and nano collagen proteins.^[23-25]

In fact, ECM is a data transmitting method included in a number of proteins, such as collagen I, fibronectin, thrombospondin, osteonectin, osteopontin, osteoadrin, and bone sialoprotein (BSP), as well as specific plasma proteins like α 2HS glycoprotein,^[20] which mostly acts like cell adhesion interfaces and some as messengers with cell to cell/cell to protein interaction.^[26] Moreover, protein serums like albumin are absent which indicates selective agglomeration/sedimentation of molecules in the interface. Molecules that contain Arg-Gly-ASP or RGD sequence contribute to cell adhesion and mineral binding. This RGD sequence is present in a number of ECM proteins such as fibrin, collagen, fibronectin, vitronectin, osteopontin, and BSP.^[27]

Cellular connection is a complicated process and forms by integrin, focal adhesion, and filopodia. Integrins are membrane

transporters of cell surface receptors that mediate between the physical binding of cell to the outside of matrix to broadcast messages from outside in and vice versa.^[28,29] They have α and β subunits with cells, expressing various combinations of integrins.^[30] Canonical cohesion of integrin is based on cell molecule compositions participating in a messaging-based cohesion^[31,32] and binding ECM to the actomyosin of cell cytoskeleton.^[33] These structures are moving and based on the cell, could be bonded, detached, dispersed, and recovered.^[33,34] Filopodia is an actin-rich cell appendage that, along the cell, causes cohesion on rough surfaces.^[35] Surface structures scan the filopodia layer and stabilize the cell in line with the receiving signals from cavities with micro and sub-micrometer structures, acting as an appropriate setting during the route finding phase. A desirable support will shape with certain points along the filopodia as well as the tip of these points. These tips that become wide and branch outward will convert the sticky structures that are known as footpads. Cell expansion interfaces with cell membrane appendages in footpads or with the bulge of a cytoplasmic disk, which means that it is similar to a lamella or lamellipodium between sticky filopodia.^[36-38] On the other hand, cells stick to the flat surface through canonical cohesion. When this surface is scanned, filopodia receives negative signals, which go back to the cell body. The process leads to developed stress fibers that impose the stress along the cell body and by increasing the cells' connection to the surrounding, they will become smoother.^[35]

During the first day of implant placement, it is the water molecules and platelet absorption that secretes the growth factors. Moreover, messaging and provoking osteoblasts to stick in cell level becomes possible through the aid of fibronectin that is interfacing with canonical cohesion.^[39] Pluripotent mesenchymal cells are the first to migrate along the implant level. They do not deliver osteoblasts.^[40] The ability of these cells to distinguish active osteoblasts depends on topical oxygen tension, food availability, and local regulatory growth factors; all of which depend on the angiogenesis of implant position and the physiology of transplant.^[41] Migration of these cells is also contingent to the decrease of oxygen concentration gradient toward the center of wound that is due to local ischemia and necrosis. Local ischemia and necrosis are the result of circulation stop and lack of oxygen for osteoblasts due to the breakage of capillaries. Although neutrophils are the most abundant cells to reach their peaks (maximum) within 24-48 h, yet later on macrophages become dominant. Both of these cells are involved in forming clots and tissue necrosis.

During the third day, related osteoblast transcription factors of Runx2 and Op are activated by the cells around the implant. By the 4th day, the created necrotic bone within the surgery is reabsorbed and a certain interface zone is formed. In the 5th day, some evidences are observable from the new bone formation and the presence of alkaline phosphatase activity, which indicates the beginning of mineralization and matrix remodeling.^[42] By the end of first week, the cohesion of bone matrix on the surface of implant could be recognized easily, ECM becomes engaged

in the surface and bone cavities reach to $35.8 \pm 7.2\%$ implant connection ratio. Up to the 16th day, the implant surface becomes fully and abundantly coated with a mixture of mineralized tissues, osteoid, and dense matrix.^[43]

On the 28th day, which is the end of 4th week, the main bone establishes a complete binding along the implant surface and also in the neck, collagen fibers, and osteoblasts create a volume of tissue layer adjacent to the implant; while collagen fibers incline toward themselves becoming parallel to the implant surface and cells, ECM proteins, and mineralized bone tissue appear in direct relationship with the implant and bone to reach the size of $46.3 \pm 17.7\%$ in implant ratio.^[44] According to Davies,^[45] Puleo and Nanci,^[20] ossification occurs in two directions, from implant surface toward the bone and from the bone toward the implant surface, which is known as bone regeneration and distance bone regeneration [Figures 1 and 2]. In the process of bone regeneration within the contact area, the bone gets shaped 30% faster. In this mode, prior to the formation of bone matrix, the implant surface clone with bone cells, and the identical mesenchyme, which was created during the remodeling

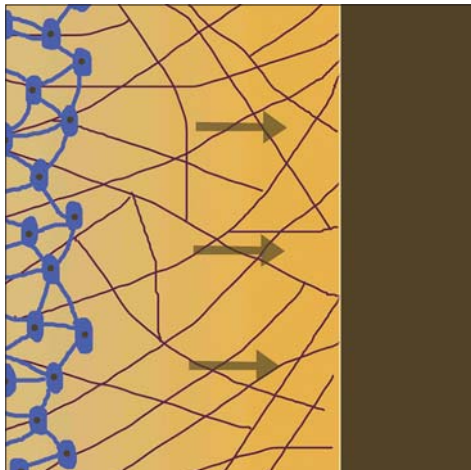


Figure 1: Distance osteogenesis

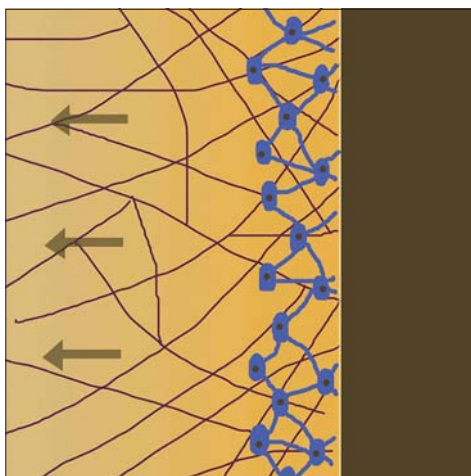


Figure 2: Contact osteogenesis

process is recognized as new bone formation. In distance bone regeneration, the new bone is formed on the surface of implant while the implant is covered by the surrounding bone. It is expected that this procedure occurs in cortical bone healing.^[45] The initial bones are formed woven, which have osteoid in their matrix. At the end of 12th week, the new bone that is formed at the implant surface will be uniformed with a body connection of mature lamellar bone with titanium surface.^[43]

Conclusion

Osteointegration is a fairly complicated process and the aspects of micro and micro molecule bone-implant interface are the matters of controversy. However, through the use of conducted tests and studies of many authors, we can claim that the treatment patterns in cortical and trabecular bones are different. Cortical healing depends on the remodeling of haversian, while trabecular healing is based on osteoconduction phenomenon (bone growth on the surface) and the formation of new bone.

Bone formation in the position of bone lesion takes place due to coupling mechanism and according to frost, the mechanism of formation and reabsorption should be existing. The biomechanical milieu in position failure immediately affects cartilage and bone development.

Once the position of implant placement is prepared, a lesion is made and the phase of bone-implant healing is performed by a method similar to healing in the case of a broken bone, since both operations start with penetration in an intact skeletal position, an immune response, a new angiogenesis, and use skeletal progenitor cells. Nevertheless, some skeletal progenitor cells will differentiate into chondrocytes in cases of bone failure, while the others will change into osteoblasts that are followed by ossification inside the cartilage. Around an implant, all skeletal progenitor cells that change into osteoblast are followed with intra-membranous ossification. The other contradiction in implant healing is that the osteointegration process is extremely under the influence of implant surface, chemical composition, and implant biomechanics.^[46,47]

Hence, as soon as the implant is located, platelet density takes place.^[45] These platelets secrete the growth factors, such as platelet-derived growth factor-BB, insulin-like growth factor (IGF, IGF-2), FGFs (a-FGF, b-FGF), transitional growth factor beta, BMPs, vasoactive factors of serotonin and histamine [Figure 3]. These factors are more differentiated, proliferated, and bound to osteoblasts with titanium level [Figure 4] and form a new matrix [Figure 5]. The development is managed by the transcription factor protein core binding-factor-alpha.^[48]

There are ample evidences to prove the direct relationship between osteointegration and superficial topography. It is verified that the rough surface made osteointegration binding better, resulting in filopodia, as well as a four layer increase in *cbfa1*.^[48] We also surmise that, an increase in the surface is not a definitive factor for regulating cell growth in the bone-implant interface. Consequently, implant surface topography

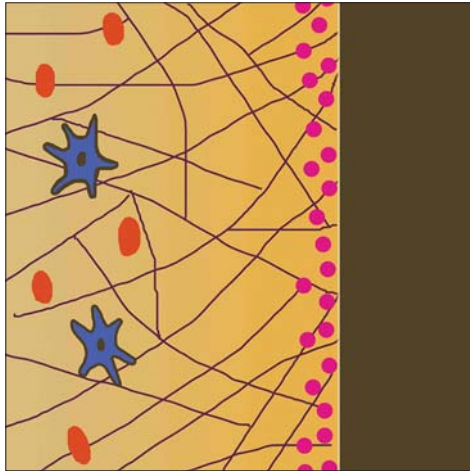


Figure 3: Secretion of growth factors from platelets

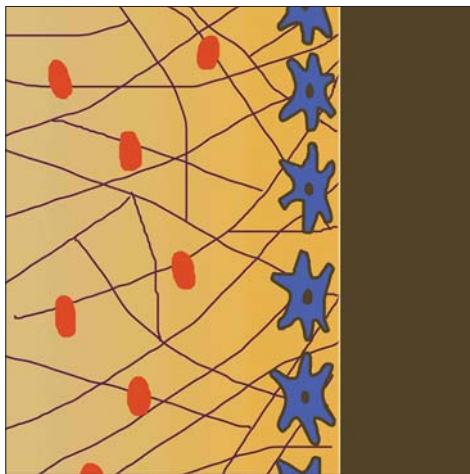


Figure 4: Differentiation and proliferation of growth factors and attachment of osteoblasts with titanium surface

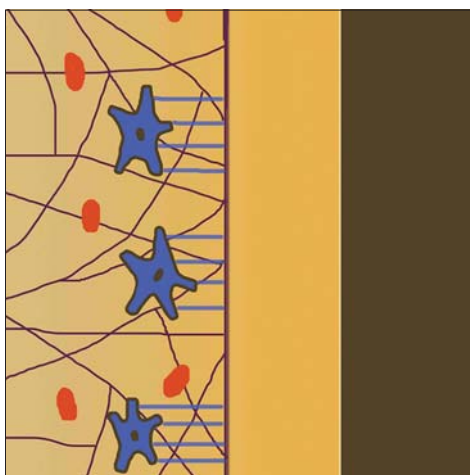


Figure 5: Formation of new bone matrix

plays a significant role and changes the cell structure, as if the smooth surface osteoblasts inclined parallel and are on the

satellite-like rough surface cells.^[35] Therefore, the bone matrix in the bone-implant interface is formed during the bone growth on the surface [osteoconduction], simulating ossification (osteoiduction), bone regeneration (osteogenesis), and bone progress (osteopromotion).^[26]

Bone growth in the surface refers to the orientation of formed bone activity to the position or specific surface, like hydroxyapatite coating, which is retained as a framework for making cell connection and growth. Stimulation of ossification includes applying mesenchymal stem cells that will be converted to osteoblasts. Implant surfaces are not stimulating. Bone regeneration is related to the stimulation of proliferation of bone progenitor cells and the stimulation of the biosynthetic activity of osteoblasts. As the fourth modification, although bone progress is relatively a new term, is related to the formation of bone in topical bone positions through using techniques that are related to membrane barriers, and is only utilized in promoting clinical cells.

There are many unanswered questions; however, there is no field of study in implantology that thoroughly investigates osteointegration. The current results and future research projects represent better understanding and a finer transparent image of what is happening in the bone-implant interface, which subsequently provides the required information for implantologists. These findings, after understanding the physiological and biological needs of bone, can supply the patients with the best possible medical treatments.

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