PUTATIVE EARLY RISK FACTORS AT BONE-IMPLANT INTERFACE IN IMMEDIATE LOADING

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Abstract: during osseointegration a dental implant early develops special spatial and molecular relationships with surrounding tissues. The surface roughness and microtopography are closely related to a long-term clinical success. A better understanding of various but relevant molecules, and even of genes, that encodes biological factors involved in tissue healing should be considered useful in particular cases. Biocompatibility of implant is not yet considered as a solved problem, at infrastructural and molecular level, at least on long-term. Various biological factors, already in use, such as platelet-rich plasma, and growth factors, should be under control in order to follow a more efficient pathway towards a reliable osseointegration. A paramount attention has to be done, on long-term, to the functional modulation of bone tissue-dental implant, especially in immediate occlusal loading of dental implants.

Key words: osseointegration, implant surface topography, titanium wear, immediate loading.

The main goal in contemporary implant dentistry is to obtain a morphological and functional efficient direct bone-implant interface[11]. Concerning the immediate occlusal loading of a dental implant, Misch and Scortecci (2008) recommend, as prosthetic treatment, a restoration in occlusal contact, completed during first 14 days after the surgical implant insertion[12].

As a tooth substitute, a dental implant requires not only biocompatibility as any other allogenic insert into the human body tissues, but particularly a physiological distribution of chewing forces at its interface with bone, and especially normal cellular and molecular relationships with the surrounding bone tissue.

Conventionally, the consequence of a biological and functional success of an implant insertion is called osseointegration, that means, according to the definition given by Brånemark *et al.* (1969), a "direct contact between living bone and a functionally loaded implant surface without interposed soft tissue at the light microscope level"[3].

To aiming a successful osseointegration, for a clinician, first of all, is mandatory to understand the dynamic background of bone, its development, physiology and biomechanics in mature state, that render this mineralized tissue extremely adaptive to its permanently changing environment[1,17].

Numerous studies revealed that this interface of an osseointegrated implant is not a static, but a dynamic one, because it has to face to both, the healing process and the tissue-strain induced by occlusal load, which together are responsible for the quality and compositional structure of bone-implant interface.

Some other recent studies also emphasized that the beneficial bone remodeling taking place during osseointegration of a functional loaded implant in fact represents the feed back of some chemical and biological effects that the mechanical forces triggered in a strain sensitive tissue component, namely the bone cells and extracellular matrix proteins distributed at the bone-implant interface[16]. Now is almost established that osteocytes are capable, in their position of mechanosensors, to convert the strain, as physical stimulus, in biochemical signals[17].

Osseointegration plays the role of a gradually process that involves both, the recruitment of mesenchymal stem cells and their further differentiation in osteoblasts. It has been demonstrated that implant surface microtopography and immediate or early loading may influence, among others, such as extracellular matrix proteins and the group of transmembrane proteins, the outcome of osseointegration process[8].

The surface microtopography of a dental implant is characterized by its degree of roughness and the orientation of irregularities occurring on its surface. In that respect, of surface roughness, dental implants may have an isotropic or anisotropic structure.

An isotropic surface is obtained by abrasive blasting, plasma spraying, etching, and oxidizing, whereas an anisotropic one results as a consequence of turning or milling processes. That is the reason why an isotropic structure has any dominating directions unlike the anisotropic surface structure that has a regular pattern[14,22].

Masaki *et al.* (2005) demonstrated that implant surface microtopography may alter the gene expression of bone-related factors in osseointegration. They found, through a real-time PCR, a significant quantitative increase of alkaline phosphatase gene expression in osteoblasts grown on titanium discs large grit Al_2O_3 blasted, etched and rinsed under N_2 protection, as compared to those odontoblasts grown on titanium discs prepared through various technological procedures, such as: a) titanium dioxide grit blasted, b) titanium grit blasted and electrochemically etched in hydrofluoric acid, or c) large grit Al_2O_3 blasted and H_2SO_4 / HCl hot liquid etched[8].

The expression of mRNA level of type I collagen has also been increased on discs prepared by large grit Al_2O_3 blasted, etched and rinsed under N_2 protection versus same Al_2O_3 blasted discs following procedure "c"[8].

It seems that have also been registered some minor morphological differences of osteoblasts. Cells cultured on titanium dioxide grit blasted discs with or without etching were more flattened and had less cytoplasmic extensions and lamellipodia in comparison with cells grown on large grit Al_2O_3 blasted discs.

The abovementioned researchers, using same quantitative analysis, also revealed a significant increase of *Cbfa1/RUNX-2* and *Osterix* gene expression in osteoblasts grown on titanium dioxide grist blasted discs as compared to Al_2O_3 blasted titanium discs.

Core-binding factor (*Cbfa1/RUNX-2*) is an transcription factor protein having an essential role in regulating osteoblast differentiation and expression of bone extracellular matrix protein, chondrocytes differentiation and bone resorption by osteoclasts. *Osterix* is also an transcription factor protein which involved in osteoblast differentiation choosing another pathway, of down-streaming *RUNX-2* in order to control the shifting of osteogenesis from chondrogenesis.

According to this study of Masaki *et al.*(2005), it means that surface topography might induce transcriptional regulation or gene expression that encode osteogenesis, including synthesis of bone extracellular matrix protein, such as bone sialoprotein (BSPII), osteocalcin and type I collagen[8].

A putative hypothesis of the way the microtopography of implant surface might control odontoblasts differentiation is *via* protein kinase A and phospholypase A_2, and likely, by collagen-$\alpha 2\beta 1$ integrin interaction[2,20] . The surface topography is also in charge of triggering the expression of some growth factors and cytokines, such TGF-β and IL-1β, and might be responsible for increasing of the phenotypic markers of osteoblasts differentiation like osteocalcin and alkaline phosphatase activity[4,8,20].

The increased alkaline phosphatase activity expressed on titanium discs large grit Al_2O_3 blasted, etched and rinsed under N_2 protection, might also be related to the cell shape acquired on this hydrophilic surface. It is worth to remind that also a higher expression of type I collagen gene was related to this hydrophilic implant surface.

Phenotypic differentiation of osteoblasts associated to different pathways to regulating *Cbfa1/RUNX-2* and alkaline phosphatase expression suggest a possible involvement of implant surface microtopography in gene control of cell behavior, and further differentiation.

Several *in vivo* animal studies reported that rough surface implants generated a faster healing and an increased bone synthesis at bone-implant interface as compared to smooth surface implants[19]. According to Wennerberg, an optimal average surface roughness (S_a) for a reliable osseointegration should be on the order of 1-1,5 μm[18,21,22]. Sammons *et al.* (2005) suggest that a porous surface promotes cell attachment and spreading, whereas grit blasting or plasma spraying would facilitate mechanical interlocking[19].

The onset of healing after surgical insertion of an implant depends on adhesion that develops between osteoblast and allogenic implant, mediated by extracellular matrix proteins. Osseontegration is a long and complex process that assume the participation of bone cells, specific adhesion proteins such as fibronectin and vitronectin, transmembrane proteins like integrins, calcium-dependent cadherins, selectins, osteoblast secreted proteins such as osteopontin, and many other factors.

Fibronectin is an extracellular glycoprotein wearing multiple binding sites for osteoblast membrane integrins, collagen fibrils, fibrin proteoglycans, and numerous extracellular matrix molecules. Fibronectin promotes osteoblasts spreading on implant surface, its further differentiation and finally stabilizes the cell. The main fibronectin receptor is α5β1 integrin[4].

Vitronectin is an attachment factor localized in cells lining bone surfaces, like osteoblasts. Its receptor is αvβ5 integrin[4].

Cadherin is a transmembrane glycoprotein localized intra- and extra-cellular that participates, as classic E-cadherin, in adherens junctions, and ,as desmosomal cadherin, in desmosomes[4].

Integrins are a family of cell surface transmembrane proteins that form various cell surface receptors, coupling to extracellular matrix adhesion molecules, such as collagen, fibronectin, vitronectin, vascular adhesion molecule 1 (VCAM-1), etc[4].

For example, integrin receptor α3β1 binds to collagen, fibronectin, and laminin; integrin receptor α4β1 and α4β7 binds to fibronectin, and VCAM-1; integrin receptor α5β1 binds to fibronectin; αvβ5 binds to fibronectin, and vitronectin; integrin receptor αvβ6 binds to fibronectin, etc[4].

The attachment of osteoblast to implant material is mediate by integrins[6]. The substrate, regardless of its chemical composition is essential for cell migration and organization of the extracellular matrix. Osteoblast cells express α5β1, αvβ3, α3β1, and α1β1 integrins that are distributed in plasma membrane attachment plaques. Essential for normal osteogenesis is the coupling of β1 integrin subunits to collagen fibrils and fibronectin. It seems that self-secreted fibronectin plays the trigger role in initial attachment of osteoblasts[4].

Meyer *et al.* (1998) revealed that *in vitro* vinculin is a mandatory prerequisite for osteoblast attachment[9]. The attachment of osteoblast to the metallic substrate of dental implant, and extracellular matrix as well, is mediated by cell surface receptors for collagen and fibronectin. The intracellular segment of integrin receptors binds to the peripheral cytoplasm protein

talin, which interacts further with another protein, which is vinculin[4,19].

Subsequently, vinculin undergoes conformational changes and attaches to actin microfilaments. Finally a molecular bridge is borne, which connects cytoskeletal microfilaments (*cell's contractile apparatus*) and fibronectin in the extracellular matrix. Through further binding of fibronectin to collagen fibrils cell contractile apparatus is linked to the extracellular collagen network[4,19].

Once attached, is important to evaluate to what extent surface topography of the implant modifies both, osteoblast cell shape and its subsequent differentiation. There are four stages in the attachment progression of cells onto surfaces, as described by Rajaraman *et al.* (1974)[15].

1. initial contact mediated by filopodia, narrow finger-like protrusions of cytoplasm;
2. extension of lamellipodia, flat folds of cytoplasm sent out across a broad area;
3. spreading of the cytoplasm between the lamellipodia;
4. full spreading to a round or polygonal shape.

Several local mechanisms might guide the spreading process of osteoblastic cells on inserted implant surface. One of them is haptotaxis, which allow the cell to move along a concentration gradient of an extracellular matrix molecule, such as fibronectin, which is established to induce a direct migration. Investigating the effect of magnetic field on the fibronectin adsorption, Kim *et al.* (2005) noticed that in those magnetic fields under 10 mT the mentioned effect did not occur, but a significant influence was produced on cell attachment and proliferation[7].

Even the surface roughness of the implant plays an important role in osseointegration, some other factors such as oxide thickness, oxide crystallinity and ions might also be involved in controlling the quality of osteoblasts adhesion at interface and extracellular matrix laid down proteins[18].

Sader *et al.* (2005) studied the attachment and spreading of osteoblasts on various titan surfaces, such as ground, blasted, and, blasted and etched. In first 2 weeks osteoblasts proliferated faster on ground titan surfaces, but after one month cell number was similar for all three kind of treated surfaces. Metabolic activity of alkaline phosphatase was unmodified and similar too[18].

Since the influence the microtopography of implant surface plays in bone cell shape, orientation and adhesion, the acquired morphology, related to different treated surfaces, of these environmental sensitive cells, has also been distinct. On smooth surfaces the cells appeared flattened, with large and well-defined lamellipodia, like bone-lining cells, whereas on rough surfaces the cells did not spread completely[18].

Sader *et al.* (2005) suggest that a rough topography facilitates differentiation of reactive fibroblast-like osteoblasts which are supporting osseointegration through a high secretion of extracellular matrix proteins as compared with smooth surface implants promoting a lining cell layer that isolate the implant rather than integrate[18].

It seems that of paramount importance for the osteoclast spreading speed is related to the microtopography or an associated surface property, such as surface wettability of the implant, rather than its surface roughness induced by grit blasting[19].

In that respect, of osteoconductive properties, some controversies are discussed in literature between rough calcium phosphate-coated and smooth native titanium oxide-coated implants. It seems that the effect of surface microstructure exceed that of surface chemical composition. It also appears that in long term the rough titanium surfaces

became better osseointegrated as compared to a smooth turned one[23].

It has to be emphasized that chemical composition, and crystallinity as well, might be decisive. Xiropaidis *et al.* (2005) found that a modified titanium oxide-coated implant achieved better osteoconductive properties than a calcium phosphate-coated one. The higher level (71%), and the accelerated rhythm of osseointegration that resulted in modified titanium oxide-coated implant at 8 weeks, as compared to the other one (57%) are of paramount significance when implants will be stimulated through an immediate occlusal load.

An increased hydrophilic ability promotes protein adhesion, and cell proliferation as well as the speed of cell attachment and spreading. Rapid cell attachment and spreading is crucial for bone-implant interface healing, efficient osseointegration, and finally, for clinical success of immediate-loaded implants[19].

Sammons *et al.* (2005) noticed *in vitro* after first 30 minutes of cell attachment all stages described by Rajaraman, but the majority of osteoblats reached the 2nd, and 3rd ones[15,19]. Sader *et al.* (2005) found that in 2 hours odontoblasts actively adhered, presenting a typical polygonal morphology, and exposing numerous filopodias in all directions. Same researcher revealed surface microtopography-dependent adhesion and spreading rate, which were quite different for ground with parallel grooves, blasted with irregular rough morphology, and blasted-etched with uniform-smooth titan surfaces. Concretely, on blasted plates there were unattached round osteoblasts, and on blasted-etched ones few almost flat cells[18].

After 24 hours, on ground titanium plates, the odontoblasts were well attached, and widely spread, oriented following the grooves, having a flattened morphology. On blasted plates, and blasted-etched ones, the cells were still polygonal, expressing an uncomplet spreading[18].

During their migration along the bone-implant interface, odontoblasts make close and focal contacts with the implant surface. Close contacts express the initial association of specific cell membrane attachment proteins to the extracellular matrix. A space of 20-30 nm separates cell membrane from implant surface. Focal contacts represent the outcome of the maturation of close contacts by recruitment of integrin receptors and other membrane-associated proteins. In focal contact, a narrower space, of only 10-15 nm separates cell membrane from implant surface[4].

In this stage of attachment, under the molecular control of integrins, which take in charge the transmembrane binding of osteoblats cytoskeletal proteins to the extracellular matrix, the already mentioned talin, actin, and vinculin are intensively involved in focal adhesion expression[4].

For the normal course of osseointegration, in parallel with extracellular matrix synthesis and maturation and the onset of mineralization, the development of an early efficient blood irrigation is essential. The osteoblast contact with implant rough surfaces might promote a mechanical interlocking, followed by a penetration of newly formed vascular tissue[19]. Osteoblasts facilitate the formation of new blood vessels since they are capable to elaborate the vascular endothelial growth factor (VEGF), which has a strong mitogenic activity[4].

An active osteoblastic cell is a strong builder of bone matrix, producing numerous proteins, both collagenous and noncollagenous, and proteoglycans. Once the maturation of the extracellular matrix proteins, such as type I collagen, fibronectin, vitronectin, and osteopontin, the next step to follow at bone-implant interface is the onset of mineralization[8,13].

Type I collagen is the major component of the principal fibers,

accounting for approximately 80% of the total collagen content. Bone sialoprotein (BSP) is a calciumbinding protein with high affinity for both, hydroxyapatite and cells. It may promote bone mineralization, when bond to a solid tissue substrate or inhibit the mineral laying down, while in solution[4].

Another calciumbinding protein involved in bone mineralization, soft tissue organization, and cell-mediate immunity is osteopontin. Localized in small globular areas in bone matrix and highly expressed in differentiating osteoblasts, osteopontin is supporting the cell attachment to bone matrix. Osteopontin may facilitate or inhibit bone mineralization in a similar manner to bone sialoprotein[4].

Among the noncollagenous proteins, osteocalcin is one of the most representative, providing coupling sites involved in bone matrix mineralization and control of mineral crystals growth. Accordingly to its biological role, osteocalcin mRNA is found in osteoblasts and mineralized bone matrix as well. Combined with bone sialoprotein and collagen type I fibers promotes local mineral precipitations of previously induced high calcium and phosphate concentration[4].

The most spread noncollagenous protein in bone matrix is osteonectin, which has the capability to couple different collagen and substrate adhesion molecules. It seems that ostenectin, even highly expressed by osteoblasts, play a generalized function in calcium-modulated organization of extracellular matrix in numerous body tissues, soft and mineralized, as well[4].

A special attention is wise to be directed towards the understanding of all local processes elicited by healing when additional biological products are delivered during surgical step of implant insertion.

Local use of platelet-rich plasma allows a direct access of a growth factors concentrate at bone-implant interface. Graziani *et al.* (2006), at platelet concentration of 2,5 times, communicate an increased synthesis of osteocalcin, a marker of intense differentiation of osteoblasts, and a simultaneously decrease of osteoprotegerin. High osteocalcin and decreased protegerin values are a reliable indicator of increased osteoclastic activity[5].

The same team also relieved that at maximal values of platelet-rich plasma TGF-β1 increased too. Concluding the significance of osteocalcin, osteoprotegerin, and TGF-β1 it means that the surgical use of platelet-rich plasma may modulate the early stage of osseointegration by stimulating osteoclastogenesis and osteoblasts differentiation[5].

Takeuchi *et al.* (1997) demonstrated that coupling of type I collagen with α2β1-integrin receptors controls gene expression of bone specific proteins, and subsequently mineralization[20]. Experimental studies indicates that integrins mediated attachment of osteoblasts to components of the extracellular matrix induce signaling cascades through activation of focal adhesion tyrosine kinase (FAK)[2,4,8,20]. It is well-established that osteoblasts display high number of integrin-receptors and phosphotyrosine[8]. In that respect, it might be an plausible explanation that downstream transcription of *Cbfa1*and *Osterix* osteoblast-specific differentiation genes is regulated by integrin-adhesion mediated pathway[8].

Masaki *et al.* (2005) suggest that the expression of bone sialoprotein BSPII and osteocalcin is regulated when osteoblasts reached a more mature state of differentiation. It also seems that 3D display of integrin-receptors mediates the 3D expression of extracellular matrix proteins, especially of bone sialoprotein[8].

Experimentally, osteoblasts differentiation was associated with extracellular matrix protein synthesis,

more intense produced by mature cells. Extracellular matrix proteins were preferentially deposited on implant surfaces and among cellular layers[19].

After 4 weeks have been detected mini areas (10 μm diameter) consisting in mineral phosphates within the microenvironment of grit blasting or plasma spraying cavities, in association with extracellular matrix proteins, mainly collagen-like fibrils, previously lead down, which facilitate crystal nucleation. Calcium / phosphate ratio was similar to octacalcium phosphate and amorphous calcium phosphate (1,3-1,4) as compared with tooth hydroxyapatite (1,67)[19].

Another factor playing a definite role for apatite formation on implant surfaces is the titanium-oxide crystallinity, which promote similar bone formation to that demonstrated by calcium phosphate-coated implants. It might be assumed that apatite laid down *in vivo* on crystalline titanium dioxide-coated implant is chemical similar to a hydroxiapatite surface-coated implant[23].

Osseointegration also assumes the biocompatibility of dental implants. Concerning this in medicine essential issue definitely is important to have a real insight at the bone tissue-dental implant processing infrastructural, biochemical, and immunohistochemical events. Furthermore, it has also to be considered the functional connection of an allogenic tooth-substitute with vital surrounding tissues.

Biocompatibility behavior of implant material, and strain forces developed at the newly in-growing formed interface are particularly significant in immediate occlusal-loading, when superposed factors, namely the bite forces which generate micromotions and microstrains in surrounding bone adjacent to implant surface structure, early modulate adaptive events evolving during osseointegration.

At microscopic level, for long-term the implant stability roughly depends on morphological, infrastructural and histochemical state of peri-implant bone. Under loading conditions, more frequent than in submerged quiescent state, a dental implant may release material particles and ions, as well.

There are some studies on titanium implant that retrieved titanium wear in peri-implant bone as a result of an accumulation from bulk implant disrupted particles during dental implant moving subsequent clinical occlusal-loading. Another plausible explanation of titanium wear adjacent to implant in tissue collected particles are the shear-forces generated during the implant insertion[10].

It might be raised the question to what extent, the clinically long-term success is influenced by dissolution or release of titanium in surrounding bone tissue structures. Experimental evidence supports the fact that titanium wear particles may induce peri-implant osteolysis *via* inflammatory cytokines or phagocytosis[10].

Of extreme risk consequence of titanium debris is to encourage the fibroblast local proliferation during healing. Instead an osseointegration, around the implant a fibrous membrane is generated and the outcome will be the loosening of the implant[10].

Using SEM-EDS (scanning electron microscope - energy dispersive spectroscopy) technology to studying the bone-implant interface on minipigs, and working on nanosize scale, Meyer *et al.* (2006) succeeded to visualize the surrounding structures of the implant, and its disrupted particles up to the resolution of 10 nm.

The highest contamination has been found around TPS implants. Adjacent titanium particles were large and oval-shaped. Around SLA and ILI implants there were less frequent particles, looking small-angular or round-elongated[10].

Meyer *et al.* (2006) noticed that the titanium particles release depends to an important extent to surface roughness and

microtopography of the implants. The amount of disrupted titanium particles was highest around the titanium-plasma-sprayed surfaces. Intermediary values were found for sand-blasted large grit acid-etched. The amount of particles was minimal adjacent to smooth titanium surfaces[10].

The insertion of rough implants generated more disrupted titanium particles than a smooth turned implant. In that respect, it is also relevant that the highest accumulation of particles has been observed at the crestal part of peri-implant bone, while around the tip their concentration was minimal[10].

Even Meyer *et al.* (2006) noticed no morphological alterations in cells adjacent to disrupted titanium particle after a surveying period of 24 hours, they concluded that the unintended titanium release in peri-implant tissues might of a biological concern as far as its effect on long-term is insufficient known[10].

Altogether, the accuracy of surgical technique of the implant insertion, surface roughness and microtopography of a variety of commercially available dental implants are extremely meaningful to achieve, on long-term, a reliable osseointegration particularly in cases of immediate occlusal loading, because we are not yet in position to control the intimate molecular processes developing during the bone tisuue-implant interface conformation, and healing.

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