Biochemical-Modification of Titanium Oral Implants

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Biochemical Modification of Titanium Surfaces (BMTiS) is the process that immobilize proteins, enzymes, or peptides on biomaterials for the purpose of inducing specific cell and tissue responses or, in other words, to control the tissue implant interface with molecules delivered directly to the interface. Biochemical surface modification utilizes critical organic components of bone to affect tissue response. The purpose of implant surface functionalization by BMTiS derives from the supposition that the ability to imitate bone tissue's characteristics may increment implant surface performances, thus promoting the initial biological response. Therefore BMTiS, strictly speaking, refers only to the use of molecules normally present in the human body.

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1. Introduction

When a titanium implant is placed in bone tissue, its surface immediately reacts with several water molecules, creating a coat that surrounds the entire implant surface. In a short time it is covered by ions and biomolecules, followed by the non-specific adsorption of plasmatic proteins as consequence of the so called "Vroman effect", that reaches over time an equilibrium between the phases of adsorption and desorption. The entire process is influenced by implant surface properties, such as chemical composition, surface energy and charge, that are determined prevalently by implant treatment processes during the industrial production. Protein layer stabilization on the implant surface allows the interaction with the host tissue's cells, that can recognize and bind proteins by cytoplasmic protrusions and membrane proteins. The specificity of cells-surface interaction is mainly due to the composition and organization of the protein layer, that in turn depends on the modalities with the implant surfaces earlier bonded water, ions and biomolecules[1].
Based on above, in the last decades increased research aimed at the functionalization of implant surfaces to obtain stimulatory effects on the biological tissues, to improve tissue's response and by this increase the osseointegration process other than the long-term stability of the implant therapy. First attempts have been performed mainly through surface topography's modification, starting from the first machined titanium implants to get to the most recent micro- and nano-modified surfaces [2][3][4]. One of the most fascinating fields of research concerns the Biochemical Modification of Titanium Surfaces (BMTiS), defined by Puleo and Nanci in 1999 as a process that "utilize current understanding of the biology and biochemistry of cellular function and differentiation. (…) The goal of biochemical surface modification is to immobilize proteins, enzymes, or peptides on biomaterials for the purpose of inducing specific cell and tissue responses or, in other words, to control the tissue implant interface with molecules delivered directly to the interface. (…) Biochemical surface modification utilizes critical organic components of bone to affect tissue response" [5]. The purpose of implant surface functionalization by BMTiS derives from the supposition that the ability to imitate bone tissue's characteristics may increment implant surface performances, thus promoting the initial biological response. Therefore BMTiS, strictly speaking, refers only to the use of molecules normally present in the body. In implant dentistry field, the studies concerning BMTiS have been carried out considering 4 main classes of biomolecules: (i) Peptides; (ii) Bone Morphogenetic Proteins (BMPs); (iii) non-BMPs growth factors; (iv) Extracellular matrix components (ECM). The purpose is to promote specific adhesion and differentiation of osteogenic cells on titanium implant surface, lending it improved osteogenic and osteoconductive properties.

2. Techniques that can be used to produce BMTiS

2.1. Adsorption of biomolecules by immersion

As it should be realized basically immersing the implant in a solution containing the selected biomolecule, physical adsorption is one of the simplest techniques to immobilize biomolecules on implant surfaces. In this method interactions like Van der Waals or electrostatic forces ensure the bond between the two interfaces. Because of the weakness of those interactions, one of the limits of the technique is the impossibility to handle the fixing and releasing processes of

biomolecules on the implant surfaces. As consequence, the biomolecules that have been initially adsorbed can quickly detach from the surface. The technique has been exploited to immobilize biomolecules such as BMP-2, non-BMPs growth factors, peptides and ECM components.

2.2. Covalent bonding

Covalent bonding technique determines a solid fixation of biomolecules on implant substrates. It is frequently employed to fix biomolecules such as ECM components on titanium implant surfaces. The mechanism involves implant surface functionalization through amine, hydroxyl and carboxyl groups that reacts with biomolecules and stabilize them to the surface.

The major advantage of the technique is the opportunity to fix a known amount of a determined biomolecule and to control its orientation on the substrate. To obtain covalent bonding, silanization represents the most common process to immobilize biomolecules on titanium implant surfaces; these are coated with silane or derivates molecules that work as connection tool between the implant substrate and the biomolecules. Concerning titanium oral implant surfaces, the present technique has been mainly employed to immobilize ECM components such as type I collagen or hyaluronic acid, the latter both on animals and humans[6].

2.3. Anodic Polarization

High potential's anodic polarization can be exploited to augment the thickness of the oxide (TiO_2) layer present on the implant surfaces, till increments up to 100 nm. Anodic titanium-oxide ingrowth consists in a two-substrates system: the inner substrate corresponds to the metal/oxide interface, while the outermost corresponds to the oxide/electrolytes interface. The process occurs because of the reaction between metallic ions with water and electrolytes. Due to this interaction ions, molecules and nanoparticles previously adsorbed to the oxide/electrolytes interface may be incorporated in the growing anodic oxide layer.

2.4. Layer-by-layer technique (LBL)

One of the options to immobilize peptides or proteins exploits the deposition of molecular layers which bind electrostatically to the implant surface. The mechanism consists in the alternate adsorption of opposite-charged polyelectrolytes, which because of attraction forces are stabilized creating a self-assembled polyelectrolytic multilayer (PEM). As the combination of PEMs and biomolecules is able to imitate native ECM and to furnish adequate osteoconductive properties, LBL has been used to functionalize titanium surfaces by immobilizing organic bioactive molecules.

3. Biomolecules tested in in vivo studies

3.1. Peptides

The development of the tissues goes through the adhesion of the cells to the extracellular matrix and the proliferation and organization of the matrix itself, giving rise to a functional evolution of the tissues. In particular, a membrane receptor's family known as integrins was identified for cellular adhesion to ECM. Integrins react with short aminoacidic sequences present inside the matrix; among them, Arg-Gly-Asp sequence (RGD sequence) plays a central role in mediating cellular adhesion to both plasmatic and ECM proteins, such as fibronectin, vitronectin, type I collagen, osteopontin and bone sialoprotein. Those simple aminoacidic sequences can be used to confer specific adhesion properties to the implant surfaces. Early as 1990, peptides bonding to biomaterials surface was studied to promote the interaction between the own surfaces and cells[7]. According to this approach, a single, few Daltons (Da) weight peptide can mediate cellular adhesion in a similar manner to that carried out by the same parental molecule, characterized by both higher dimensions and molecular weight. Since peptides can be synthetically produced, a better control of their chemical composition is possible.

3.2. Growth Factors

Growth factor (GF) is a protein able to promote the mechanism of replication, differentiation and migration of certain cellular populations, with which interact by specific membrane receptors. When a GF binds to a target cell's receptor, it induces an intracellular signal transduction system that determines a precise biological response. In the context of endosseous titanium implants, an increase of osteoblastic precursors and mesenchymal cells differentiation in active phenotype brings significative benefits to bone healing.

The major growth factors in bone tissue are: Transforming Growth Factor β (TGF-β), Plasma-Rich Growth Factor (PRGF), acid and basic Fibroblast Growth Factor (aFGF, bFGF) and Insulin-like Growth Factor (IGF-I and IGF-II). The most important osteogenic GF belong to TGF-β superfamily, of which BMPs represents the most studied category, consisting of almost 18 different proteins. Among them, BMP-2 possesses a high osteoconductive potential, that makes it widely used in implant bio-functionalization field.

3.3. Extracellular matrix components

Extracellular matrix is a major component of cellular environment, and it is mainly formed by collagen, glycoproteins, proteoglycans and glycosaminoglycans, organized to create a highly site-specific system. ECM are not only actively involved in osteoblast's adhesion mediation, but also in the mechanism of migration, proliferation and morphologic evolution and in the expression of specific genes. Therefore, the immobilization of the ECM components on the implant surface should mimic the native interface and influence the osteoblastic activity, increasing the biological response. Among the ECM components immobilized on titanium implant surfaces, type I collagen showed promising evidence both for its fundamental structural function in bone tissue and for the well-known role of osteoblast's functions mediator in the cellular processes of adhesion, differentiation and in ECM secretion.

4. Conclusion

Biochemical modifications of titanium dental implants demonstrated benefits for the osseointegration process. To immobilize biomolecules on titanium implant surfaces have been proposed different techniques, of which the most commonly used are physical adsorption, covalent bonding, anodic polarization and LBL technique. Different bioactive molecules were investigated for that purpose: concerning growth factors, the most frequently employed is rhBMP-2, although even the effect of ErhBMP-2, PRGF, bFGF, IGF-1, FGF-FN, TGF-β1 and rhVEGF were studied. Regarding peptides, the effects on osseointegration were considered for RGD sequences, rhOPN, OC-1016, VnP-16 and Ln2-P3. Among the ECM components, the most frequently used in BMTiS field were type I collagen, alone or in combination with GF or chondroitin-sulphate, but also the effects of fibronectin, an elastin-like protein (ELP) and hyaluronic acid, the latter both on animal and human models, were studied.

The most significative applications of BMTiS involve BIC parameter, which demonstrated the greatest benefits from the immobilization of biomolecules on implant surfaces. Moreover, even RTQ , ISQ and newly formed bone density values provided positive evidence in favor of biochemically coated titanium implants.

Except for the mentioned randomized clinical trial about the usage of covalently-linked hyaluronan surfaces, the application of BMTiS in humans has not been found in the literature[6].

Based on the above, BMTiS represents a promising application for the characterization of titanium dental implants surfaces, although more studies are necessary, especially in humans, to confirm the evidence obtained in vivo on animal models.[1]

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