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Expert Review

Organic–Inorganic Surface Modifications for Titanium Implant Surfaces

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Abstract. This paper reviews current physicochemical and biochemical coating techniques that are investigated to enhance bone regeneration at the interface of titanium implant materials. By applying coatings onto titanium surfaces that mimic the organic and inorganic components of living bone tissue, a physiological transition between the non-physiological titanium surface and surrounding bone tissue can be established. In this way, the coated titanium implants stimulate bone formation from the implant surface, thereby enhancing early and strong fixation of bone-substituting implants. As such, a continuous transition from bone tissue to implant surface is induced. This review presents an overview of various techniques that can be used to this end, and that are inspired by either inorganic (calcium phosphate) or organic (extracellular matrix components, growth factors, enzymes, etc.) components of natural bone tissue. The combination, however, of both organic and inorganic constituents is expected to result into truly bone-resembling coatings, and as such to a new generation of surface-modified titanium implants with improved functionality and biological efficacy.

KEY WORDS: calcium phosphate; ECM proteins; protein immobilization; surface modification; titanium implants.

INTRODUCTION

The research field of bone tissue engineering applies the principles of biology and engineering to develop functional substitutes for damaged bone tissue (1). To restore, maintain and improve bone tissue function, three key elements are required: (1) a scaffold or carrier material combined with (2) cells and/or (3) bone stimulating molecules (e.g. growth factors). The scaffold provides mechanical support and serves as a substrate upon which cells attach, proliferate and undergo differentiation. In that respect, metallic implants used in plastic and reconstructive surgery, orthopedic surgery, craniofacial surgery, and oral implantology can be regarded as scaffolds for load-bearing, bone-replacing/contacting applications such as joint and tooth replacement, fracture healing, and reconstruction of congenital skeletal abnormalities. For these implants, the ultimate goal is to obtain a life-long secure anchoring of the implant in the native surrounding bone. Commercially pure titanium (cpTi) and Ti–6Al–4V alloys are the most commonly used metallic implant materials, as they are highly biocompatible materials with excellent mechanical properties and corrosion resistance (1–4). The biocompatibility of titanium implants is attributed to the stable oxide layer (with a thickness of 3–10 nm) that spontaneously forms when titanium is exposed to oxygen (5,6). This reaction prevents the formation of fibrous tissue around the implant, and

creates direct contact to osseous tissue. Nevertheless, when applying Ti(O₂) as implant material, a non-physiological surface is exposed to a physiological environment. However, by generating a coating onto a titanium surface that mimics the organic and inorganic components of living bone tissue, a physiological transition between the non-physiological titanium surface and surrounding bone tissue can be established. In this way, the coated titanium implant functions as scaffold for improved bone cell attachment, proliferation and differentiation. Such a coating is supposed to further enhance early and strong fixation of a bone-substituting implant by stimulating bone formation starting from the implant surface. As such, a continuous transition from tissue to implant surface can be induced. Consequently, research efforts have focused on modifying the surface properties of titanium to control the interaction between the implant and its biological surrounding. This paper reviews current physicochemical and biochemical surface modification approaches to enhance bone regeneration at the interface of titanium(-alloy) implants. The first part of this review will present a brief description of the biological processes that occur at the interface of the implant surface upon implantation in bone tissue, followed by an overview of both inorganic (calcium phosphate) and organic (protein) coatings that stimulate bone formation to achieve an improved and accelerated implant fixation.

THE BONE-IMPLANT INTERFACE

Bone

Bone tissue is a living organ, which can be described as a natural composite composed of an organic matrix strength-

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ened by an inorganic calcium phosphate (CaP) phase. The extracellular organic matrix (ECM) of bone consists of 90% collagenous proteins (type I collagen 97% and type V collagen 3%) and 10% non-collagenous proteins (osteocalcin 20%, osteonectin 20%, bone sialoproteins 12%, proteoglycans 10%, osteopontin, fibronectin, growth factors, etc.). Regarding the inorganic component, the most abundant mineral phase in human bone is carbonate rich hydroxyapatite (with a carbonate content between 4% and 8%) (7). The apatite in bone mineral is composed of small platelet-like crystals of just 2–4 nm in thickness, 25 nm in width, and 50 nm in length (7). This calcified matrix embeds bone cells, which participate in the maintenance and organization of bone. Bone is subject to constant remodeling by osteoblasts and osteoclasts, i.e., bone-forming and bone-resorbing cells. Osteoblasts are responsible for the synthesis, deposition, and mineralization of extracellular matrix. They are located at bone surfaces and form a continuous layer. Upon embedding in this matrix, osteoblasts finally transform into quiescent osteocytes. Osteoclasts are large multinuclear cells that are involved in bone resorption. A main feature of this bone cell type is its ruffled border, which acts as a high surface area interface for excretion of proteins and (hydrochloric) acid. The acid decreases the local pH and dissolves CaP bone mineral. This dynamic process of bone formation and destruction accounts for its remodeling, thereby enabling bone regeneration.

Cellular Interactions with Implant Surfaces

A sequence of complex and strongly interrelated events takes place at the implant surface after implantation of the material (Fig. 1) (8). Immediately after implantation, water molecules bind to the surface and form a water mono- or bilayer. The arrangement of the water molecules depends on

the implant surface properties at the atomic scale. Hydrated ions, such as Cl^- , Na^+ , and Ca^{2+} , are subsequently incorporated into the surface water layer. Blood proteins and tissue specific proteins adsorb and desorb to and from the surface (9). This adsorption process is strongly dependent on the implant surface features, such as its physicochemical, biochemical and topographic characteristics. Inorganic, physicochemical stimuli, such as release of Ca^{2+} and PO_4^{3-} ions from calcium phosphates, can positively affect the cellular response (10). Additionally, implants biochemically modified with biomolecules immobilized on the surface, such as growth factors or cell adhesion motifs, will induce certain cell responses in the physiological surrounding by specific cell signaling pathways. Next to that, implant surfaces that have protrusions, cavities, gullies, etc., on a micro- and/or nano-scale will induce biological interactions different from those with a flat surface (11). As a result, both the exact mixture of adsorbed proteins and their conformational state(s) are largely controlled by the implant surface. This surface-specific adsorbed biofilm subsequently determines cell adhesion, since proteins act as contact for the attachment of cells. This is accomplished by means of integrins, which are specific transmembrane receptors that bind to adhesive proteins on the biomaterials surface and to components of the cytoskeleton through their extra- and intracellular domains, respectively.

In general, the biocompatibility of bone-replacing implant materials is closely related to osteoblast adhesion onto their surface (12–14). Osteoblast attachment, adhesion and spreading will influence the capacity of these cells to proliferate and to differentiate itself upon contact with the implant. These latter processes are quintessential for the establishment of a mechanically solid interface with complete fusion between the implant surface and bone tissue without any intervening fibrous tissue layer.

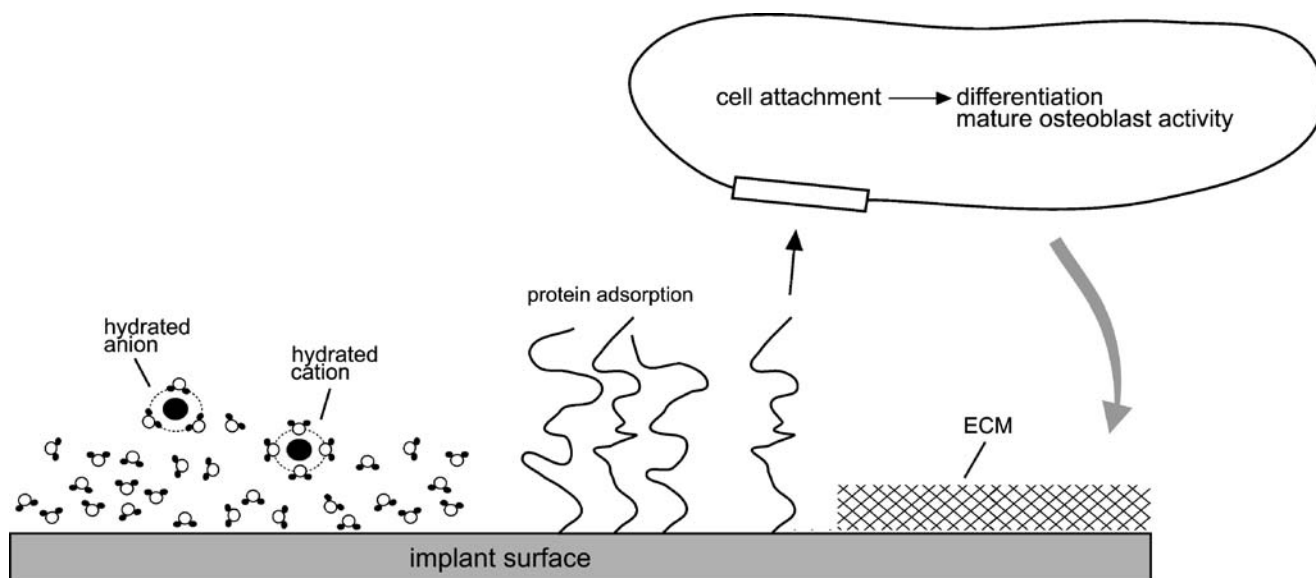


Fig. 1. Schematic representation of events consecutively taking place at the titanium surface after implantation into living bone tissue. Water binds to the surface, followed by incorporation of hydrated ions, adsorption and desorption of proteins, eventually leading to cell attachment. After differentiation, mature osteoblasts produce the extracellular matrix (ECM).

SURFACE MODIFICATION OF TITANIUM IMPLANTS

Several reviews have summarized a wide variety of surface modification approaches for titanium and titanium alloys in the biomedical field (3,11,15,16). Traditionally, these approaches focused on the modification of the implant surface topography and morphology (17,18). These surface modifications mainly included mechanical methods such as machining (19,20), grinding, polishing (21) and blasting (22,23), and chemical methods such as acid etching (24,25), alkali etching (26,27) and anodization (28,29) to alter the topography of the titanium surface. Another approach towards the creation of a biologically active implant surface involves the application of an additional coating onto the titanium surface by means of physicochemical and biochemical deposition techniques (30,31). In the following sections an overview will be given of the physicochemical and biochemical methods to provide titanium with components of the ECM as a surface coating aimed at implant fixation within living bone tissue. First, calcium phosphate coatings that are similar to the mineral phase in natural bone will be reviewed on their use for biomedical implant materials (“Inorganic Calcium Phosphate Coatings”). Thereafter, coating methods to immobilize various organic biomolecules onto implant surfaces will be evaluated (“Organic Biomolecule Coatings”), whereas organic-inorganic composite coatings, which mimic the composition of natural bone even more, will be discussed (“Organic-Inorganic Composite Coatings”).

Inorganic Calcium Phosphate Coatings

Calcium Phosphates

CaPs are often used in the biomedical field due to their similarity with the mineral phase present in bone and teeth (32). Hydroxyapatite, or more specifically carbonate apatite, is by far the most abundant inorganic phase in the human body. Apatites have the formula $\text{Ca}_5(\text{PO}_4)_3\text{X}$, where X may represent several mono- and/or divalent anions such as F^- , OH^- , or carbonate. The name apatite is derived from the Greek $\alpha\pi\alpha\tau\alpha\omega$ (Eng. “to deceive”), because the mineral was frequently confused with other compounds such as aquamarine, amethyst, etc. The apatite structure is very tolerant for ionic substitutions. For example, Ca^{2+} ions can be partly or completely replaced by Ba^{2+} , Sr^{2+} or Pb^{2+} . The exact lattice parameters—and many other properties of

apatites—depends slightly on the mode of preparation because of the frequent occurrence of nonstoichiometry. Table I lists the chemical names, compositions and frequently used abbreviations of the most important CaP phases (33).

Carbonate apatite comprises a chemical composition closer to bone and dental enamel than that of hydroxyapatite. The relation between carbonate apatite and hydroxyapatite is important, because carbonate increases the chemical reactivity of apatites. This occurs by an increase of the solubility of the product and rate of dissolution in acids, and by reducing the thermal stability (34). Since carbonate is known as an effective crystal growth inhibitor, carbonate apatite consists of smaller crystals than hydroxyapatite (7).

Bioactivity of Calcium Phosphates

Calcium phosphate (CaP) ceramics are known for their bioactive properties (35,36). Generally, bioactive materials interact with surrounding bone, resulting into the formation of a chemical bond to this tissue (“bone-bonding”). This phenomenon of bioactivity is determined mainly by chemical factors—such as the crystal phase and molecular structure of the material—as well as physical factors, such as surface roughness and porosity.

Bone-bonding occurs through a time-dependent kinetic modification of the surface, triggered by their implantation within the living bone (8,37). An ion-exchange reaction between the bioactive implant and surrounding body fluids results in the formation of a carbonate apatite layer on the implant that is chemically and crystallographically equivalent to the mineral phase in bone. The bone healing process is therefore enhanced by this biological apatite layer (38,39). The correlation between bioactivity and the formation of a carbonate apatite layer is often inverted for preliminary *in vitro* testing of the potential bioactivity of biomaterials. The capacity to nucleate CaP formation under *in vitro* conditions is then interpreted as a first indication of possible bioactivity *in vivo* (40).

Calcium Phosphate Coatings

CaP ceramics are too brittle for use as bulk material under loaded conditions, which makes that CaP ceramics are frequently applied as coatings onto the surface of metallic

Table I. Ca/P Ratios, Composition, Names and Abbreviations for Various Calcium Phosphates

Ca/P ratio	Formula	Name	Abbreviation
0.5	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	Monocalcium phosphate monohydrate	MCPM
0.5	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	Monocalcium phosphate anhydrous	MCPA
1.0	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	Dicalcium phosphate dihydrate	DCPD
1.0	CaHPO_4	Dicalcium phosphate anhydrous	DCPA
1.33	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	Octacalcium phosphate	OCP
1.5	$\text{Ca}_3(\text{PO}_4)_2$	Tricalcium phosphate	TCP
1.67	$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	Hydroxyapatite	HA/OHAp
1.67	$\text{Ca}_5(\text{PO}_4)_3\text{F}$	Fluorapatite	FA/FAp
≥ 1.67	$\text{Ca}_5(\text{PO}_4)_3(\text{CO}_3)_y$	Carbonate apatite	CA/ CO_3 Ap
2.0	$\text{CaO} \cdot \text{Ca}_3(\text{PO}_4)_2$	Tetracalcium phosphate	TetCP

implant materials in order to combine the mechanical strength of metals with the excellent biological properties of CaP ceramics.

CaP coatings for orthopaedic and dental implants were introduced by de Groot and Geesink (41,42). Since then numerous reports have been published about the osteoconductive properties of CaP-coated implants (osteoconduction refers to the ability of a biomaterial to support the growth of bone over its surface). These CaP coatings are described to induce an increased bone-to-implant contact (38,43–45), to improve the implant fixation (46), and to facilitate the bridging of small gaps between implant and surrounding bone (47,48). As an example of the osteoconductive properties of CaP coatings, Fig. 2 shows the light micrographs of histological sections of implant gaps either with or without CaP coating. The CaP-layer guides bone growth along the implant surface, and as a result bone formation now occurs from both the surrounding tissue and the implant surface, in which CaP functions as a physiological transition between the non-physiological titanium surface and surrounding bone.

Calcium Phosphate Coating Techniques

From a commercial point of view, the most successful method to apply CaP coatings to implants has been the plasma-spraying technique, due to its high deposition rate and the ability to coat large areas. Although the osteoconductive and bone-bonding behavior of plasma-sprayed coatings is confirmed by numerous studies (49–51), still some serious concerns are related to the plasma-spraying technique (52):

- Plasma-sprayed coatings must be at least 50 μm thick to completely cover the implant. As a consequence, the adhesion of the thick plasma-sprayed coatings tends to be quite weak, which necessitates a pre-treatment of the substrates such as grit blasting to roughen the substrate and to increase the mechanical interlocking of the coating–substrate system.

- Phase changes in the CaP powder particles during the coating process are unpredictable due to the high temperature differences in the plasma, leading to the formation of undesired phases such as tetracalcium phosphate, calcium oxide, and α -tricalcium phosphate. Moreover, particularly promising phases such as carbonate apatite (which is close to bone composition) (32) and biological agents such as growth factors cannot be deposited using plasma-spraying.
- Particle release and delamination are specific drawbacks for the plasma-spraying technique. The crystallinity of plasma-sprayed coatings is not uniform, as the coatings consist of crystalline and amorphous regions. When CaP material is released from these heterogeneous coatings, the resultant particles may initiate inflammation in surrounding tissues.
- Poor control over thickness and surface morphology.

Therefore, researchers have been continuously inspired in the past two decades to explore alternative or complementary techniques for deposition of CaP coatings onto an implant surface. To overcome the above mentioned drawbacks of plasma-sprayed coatings, various deposition methods have been proposed, including magnetron sputtering, electrophoretic deposition, hot isostatic pressing, sol–gel deposition, pulsed laser deposition, ion beam dynamic mixing deposition, electrospray deposition, biomimetic deposition, and electrolytic deposition. Table II presents the CaP coating thickness and the most relevant advantages and disadvantages of different CaP coating techniques. Clinically, each application demands specific requirements, and in that respect the wide range of available coating techniques offers the possibility to select the most appropriate deposition method for each specific implant application.

Summary and Outlook

Currently, a large variety of deposition methods is available for application of CaP coatings onto titanium implants. Generally, the properties of the produced coatings differ considerably in terms of chemical structure, composition, thickness, mechanical properties, etc. (see Fig. 3 for an illustration of the large variation in surface morphology of three common CaP coating techniques). Therefore, caution should always be taken when directly comparing the success rates of these coating techniques without a proper understanding of the physicochemical nature of the specific CaP coatings. Generally, it should be realized that conclusions about the biological/clinical performance of CaP coatings cannot be made without a complete set of characterizations that enable correlation of material properties to biological response.

Despite the proven efficacy of CaP-coatings for bone-bonding purposes, universal acceptance of CaP-coated systems has not been achieved. Several factors are supposed to be responsible for this phenomenon, such as commercially based pricing strategies which determine that cemented devices are currently cheaper. Still, the effect of marketing efforts and national habit are suggested to be the main determinants (95). Also, the large variability in quality of hydroxyapatite coatings from different companies and even between different batches has caused concerns about the long-term reliability of CaP-coated systems. Therefore, qual-

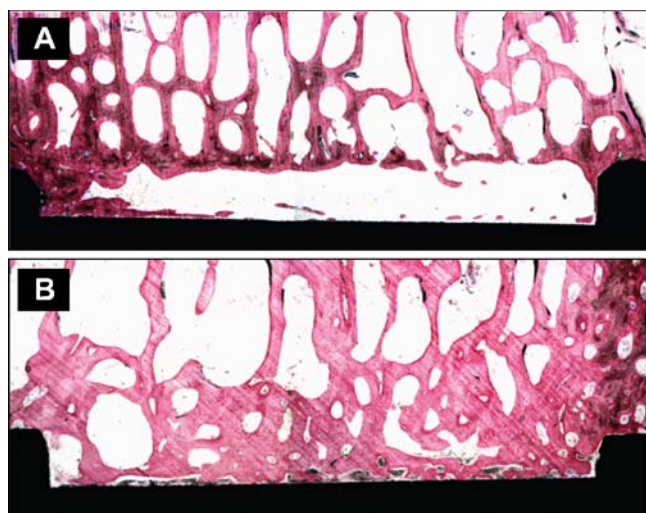


Fig. 2. Light micrographs showing bone ingrowth at **A** uncoated titanium implant and **B** an electro sprayed CaP coating, gap 1 mm (original magnification $\times 2.5$) (185).

Table II. Techniques for Producing Calcium Phosphate Coatings onto Titanium Implants

Technique	Coating thickness	Advantage	Disadvantage	References
Plasma spraying	50–250 μm	High deposition rates	Non-uniform coating crystallinity; line of sight technique	(41,49–62)
RF magnetron sputtering	0.5–5 μm	Uniform and dense coating; strong adhesion	Line of sight technique; time consuming; low deposition rates	(63–67)
Electrospray deposition	0.1–5 μm	Co-deposition of biomolecules; control over coating composition and morphology	Low mechanical strength; Line of sight technique	(68–74)
Pulsed laser deposition	0.05–5 μm	Control over coating chemistry and morphology	Line of sight technique	(75–78)
Hot isostatic pressing	0.2–2 mm	Dense coatings	Thermal expansion mismatch; differences in elastic properties	(79)
Ion beam dynamic mixing deposition	0.05–1 μm	High adhesive strength	Line of sight technique; requires high sintering temperatures	(80–82)
Sol-gel deposition	<1 μm	Coating of complex geometries; low processing temperature	Requires controlled atmosphere processing; expensive raw materials	(83–85)
Dip coating	0.05–0.5 mm	Coating of complex geometries; quick method	Thermal expansion mismatch; high sintering temperatures	(86,87)
Biomimetic deposition	<30 μm	Coating of complex geometries; co-deposition of biomolecules	Time consuming; requires controlled pH	(38,88–90)
Electrophoretic deposition	0.1–2 mm	Uniform coating; coating of complex geometries; high deposition rates	Difficult to produce crack-free coatings; low adhesive strength	(91–94)

ity reports should be available for each batch in order to avoid the use of coatings of poor quality (96).

Organic Biomolecule Coatings

In addition to the physicochemical and morphological surface modifications, biochemical methods to immobilize proteins, enzymes and peptides on implant materials have currently generated a great deal of interest (97–101). Many different biologically functional molecules can be immobilized onto titanium surfaces to enhance bone regeneration at the interface of implant devices. In contrast to inorganic calcium phosphate coatings, biomolecule surface modification utilizes purely organic components of bone to affect tissue response. Currently available organic coating approaches include (1) immobilization of ECM proteins (such as collagen) or peptide sequences as modulators for bone cell adhesion; (2) deposition of cell signaling agents (bone growth factors) to trigger new bone formation; (3) immobilization of DNA for structural reinforcement; (4) enzyme-modified titanium surfaces for enhanced bone mineralization.

Immobilization Approaches

Three major methods can be used to immobilize biomolecules onto titanium surfaces: (1) physical adsorption (via van der Waals or electrostatic interactions); (2) physical entrapment (use of barrier systems); (3) covalent attachment.

Adsorption is a very simple immobilization method performed under mild conditions, and therefore hardly disruptive to the biomolecules. However, by dipping titanium implants into a solution of proteins, biomolecule linkage is highly dependent on experimental parameters such as pH, temperature and solvent. Furthermore, surface loading is very low compared to methods as covalent coupling. In addition, biomolecules desorb from the surface in an uncontrolled manner. Using the approach of physical entrapment of biomolecules, the biomolecule is retained by a barrier but not chemically bound to it. Therefore, this technique is extremely mild and universal for any biomolecule. However, barriers are often fragile, and tearing or eroding can cause loss of biomolecules. Besides, this method is mostly used to biosensor applications (102). For the delivering of biomolecules to the implant interface, biomolecules are incorporated into coatings made of materials such as poly(D,L-lactide) (PDLLA), ethylene vinyl acetate (EVA) and collagen (103–105). In this way, biomolecule release from the implant surface can be controlled, which makes it an attractive approach for the immobilization of bone growth factors. For the immobilization of peptides, enzymes and adhesive proteins onto titanium surfaces, covalent attachment is widely used, even though this approach is more complicated and time consuming than other immobilization methods. Covalent binding is advantageous over biomolecule adsorption and entrapment due to very high surface loading and low protein loss. Using covalent attachment, the titanium surface is derivatized into reactive groups, such as amino groups or aldehyde groups (106). Subsequently, the biomolecules are conjugated to the surface by reacting with these groups. The most commonly covalent immobilization methods use silane chemistry.

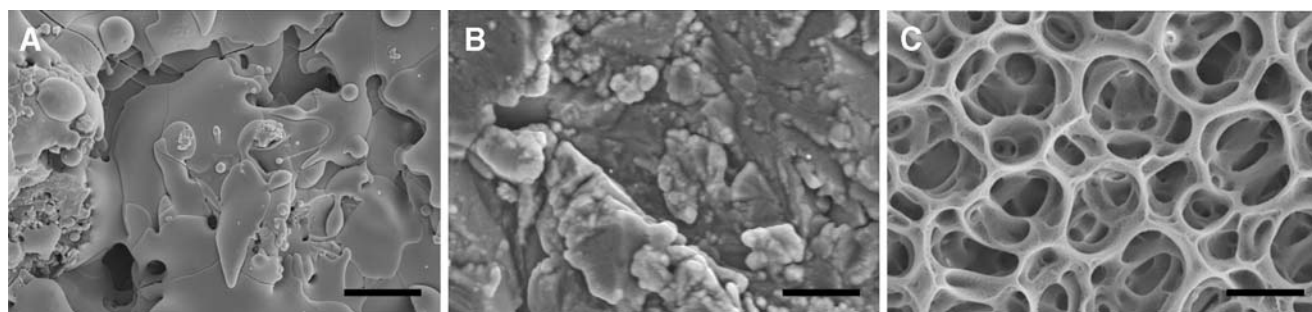


Fig. 3. Scanning electron micrographs of CaP coating morphologies of **A** plasma-spray coating, **B** RF magnetron sputter coating, and **C** electrospray deposition coating (bar represents 10 μ m).

The preferred method of immobilization depends on the working mechanism of the specific biomolecules, which dictates for instance a short-term, transient immobilization for growth factors and a long-term immobilization for adhesion molecules and enzymes. Biomolecules immobilized onto the implant surface have to interact with surrounding cell populations for a period of time to initiate cellular events. Moreover, the concentration of biomolecule must exceed the threshold levels for cellular activity (107). However, exact data regarding the required duration of exposure and concentration of biomolecule for optimal cell and tissue response are still lacking.

ECM Proteins and Peptide Sequence Immobilization

Because of the crucial role of extracellular matrix-mediated adhesion in osteoblast functions, extensive studies have been performed to functionalize titanium implant surfaces with elements of ECM proteins. Contact of cells with adjoining cells and the surrounding ECM are mediated by cell adhesion receptors. The cell membrane receptor family of integrins is involved in cell adhesion to ECM proteins. These integrins bind to specific amino acid sequences within ECM molecules. In particular, the amino acid sequence arginine–glycine–aspartic (RGD) has been identified as a cell adhesion motif in many ECM proteins, including fibronectin, vitronectin, type I collagen, osteopontin and bone sialoprotein. Thus, by immobilizing ECM proteins or peptide sequences onto titanium implant materials, bio-functional surfaces are produced that bind adhesion receptors and promote cell adhesion. Additionally, the ECM also takes an active part in regulating the cellular processes and responses, influencing not only adhesion, but also proliferation, migration, morphological change, gene expression and cell survival by intracellular signaling. As such, the biological acceptance of implants can be improved by modifying implant surfaces

with ECM components, thereby mimicking the natural interface and influencing the response of osteoblastic cells.

Although surface immobilization of entire proteins, such as fibronectin and vitronectin, is demonstrated to be effective in enhancing cellular attachment (97,108,109), research has focused on the design of materials representing only short peptide fragments of ECM proteins. These peptide sequences can possess similar functionalities, for example, receptor specificity, binding affinity, and signaling of cell responses, compared to their native proteins (110). A major opportunity in using peptide sequences is to target specific cellular interactions to a given sequence, while eliminating possible undesired responses of an intact protein. Peptide sequences can be produced synthetically, allowing precise control over their chemical composition and avoiding issues related to concerns on proteins from animal sources. As compared to the long chain proteins, the short peptide sequences are generally more resistant to denaturizing insults (30,111). Furthermore, an entire ECM protein tends to be randomly folded upon adsorption to the biomaterial surface, resulting in a less effective availability of the receptor-binding domains as compared to short peptides (112). By linking peptide sequences to implant materials, an artificial ECM can be generated onto the titanium surface providing suitable biological cues to guide new tissue formation.

The most commonly used peptide sequence for surface modification is the above mentioned cell adhesion motif RGD (113–115). Additionally, various other peptide sequences have been immobilized onto implant materials (Table III) (99,112,116–120). To provide a stable link, peptide sequences are usually covalently attached to the titanium surface, e.g. via functional groups like hydroxyl-, amino-, or carboxyl groups. RGD-functionalized materials are reported to improve early bone ingrowth and matrix mineralization in implanted constructs (113,121) and to induce more bone contact to the implant (114,122).

Table III. Peptide Sequences of Extracellular Matrix Proteins Used for Implant Surface Modifications

Peptide sequence	Origin	Function	References
RGD	Fibronectin, vitronectin, collagen type I, bone sialoprotein	Cell adhesion	(112,117,118,123)
YIGSR, IKVAV	Laminin	Cell adhesion	(108,124)
FHRRKA	Heparin binding domain	Improve osteoblastic mineralization	(120)
KRSR	Heparin binding domain	Osteoblast adhesion	(116)

Growth Factor Immobilization

Growth factors are proteins that serve as signalling agents for cells, and are secreted by cells that act on the appropriate target cell or cells to carry out a specific action. They promote replication, differentiation, protein synthesis and/or migration of proper cell types. Once a growth factor binds to a target cell receptor, it induces an intracellular signal transduction system that produces a biological response. Growth factors released from an implant surface can increase the osteoblastic activity of the bone tissue and therefore favour bone regeneration (125). Critical to the success of growth factors is the ability to deliver the molecules so that they will induce the desired biological effect. The kinetics of release of growth factors from the implant varies depending on the chemistry of both growth factor and implant surface (influenced by factors such as adsorption, roughness, electrostatic interactions, etc.). Optimum growth factor dosage, release kinetics and duration are highly dependent on the specific clinical situation and therefore still subject to much debate (106).

Bone regeneration around implants can be strongly enhanced by immobilizing growth factors such as bone morphogenetic protein (BMP), transforming growth factor-beta (TGF- β), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) to the titanium surface (Table IV) (125–127). The most common osteogenic growth factors used for biomedical purposes are the members of the TGF- β superfamily, including the BMP family (126). In particular BMP-2, BMP-7 and TGF- β 1 are promising growth factors for enhanced bone formation around the implant (127–133). Growth factors can be adsorbed or covalently bound to the titanium surface (129,134), but are commonly added to CaP or collagen-coated implants (103,132,135–137). Growth factors immobilized on titanium implants pre-coated with collagen or CaP were found to be more effective in inducing bone formation than growth factors bound to untreated titanium surfaces (138–140). This may be due to a sustained delivery profile or a higher stability of the growth factor (103,141). Overall, loading implants with growth factors has shown to accelerate bone formation (129,131,142,143) and to facilitate the bridging of small gaps between implant and surrounding bone (130,144,145). In summary, coating implants with locally acting growth factors can improve the remodelling process at the tissue-implant interface, and is therefore a promising option for establishing an improved integration of implants into healing bone.

Deoxyribonucleic Acid (DNA) Coatings

Another possibility for the surface modification of implants using organic components of native bone tissue is the generation of DNA-containing coatings. The structural properties of DNA show high potential for this unique biomolecule to be used as a biomaterial coating, regardless of its genetic information. Vertebrate DNA, a natural polymeric material, possesses non- or low immunogenic properties unlike bacterial DNA, which is a potent stimulator of immune reactions (146,147). Additionally, DNA can be used as a drug delivery since its functional groups allow incorporation of growth factors.

The structure of DNA enables its interaction with other molecules via groove binding and intercalation (148–150). In view of this, DNA loaded with molecules that elicit specific cellular responses (cytokines, growth factors, antibiotics, etc.) can deliver these signals at an implantation site. Further, the high phosphate content in DNA may beneficially affect the deposition of calcium phosphates due to the high affinity of phosphate for calcium ions (151,152). Finally, DNA-lipid complexes, depending on composition, may exert antibacterial activities (153). Since infections are common problems associated with implantation procedures, a coating that possesses antibacterial activity may diminish the incidence of peri-implantitis.

The high solubility of DNA in water and susceptibility to degradation by nuclease enzymes, hampers coating applications without modifications. Since the introduction by Decher, the electrostatic self-assembly (ESA) technique, also known as the layer-by-layer (LbL) assembly, has received a great deal of attention as a versatile and simple coating technique (154,155). Further, this technique has the advantage that it is applicable on many different materials without limitations regarding implant geometry. The LbL technique is based on electrostatic interactions between positively (cationic) and negatively charged (anionic) polyelectrolytes. The coatings generated by this process are stable through electrostatic interactions between anionic phosphate groups in the DNA and cationic polyelectrolytes. Multilayered coatings with DNA as the anionic component have been produced for sensors or transfection purposes (156–158), but van den Beucken *et al.* were the first to examine LbL applied DNA coatings for biomaterial purposes (101). Their studies demonstrated that DNA-based coatings improved the deposition of CaP, favorable for direct apposition of bone tissue to the implant surface (159). Furthermore, DNA-based coatings proved to be eligible for functionalization with biologically active growth factors, and hence can modulate cell response (160,161). These beneficial effects on cell and tissue response show potential for DNA-based surface modifications with respect to immunology, drug-delivery, and apposition of bone mineral.

Enzyme Coatings

A novel approach for surface modification utilizes enzyme-modified titanium surfaces to enhance bone mineralization along the implant surface. Biologists have been extensively investigating enzymes with respect to the mechanism of bone mineralization, but their potential for biomedical applications is rather unexplored. The enzyme alkaline phosphatase (ALP) is known to play an important role in the mineralization process of bone and cartilage. ALP appears to act both to increase the local concentration of inorganic phosphate (P_i), required for physiological mineralization of hard tissues, and to decrease the concentration of extracellular pyrophosphate (PP_i), a potent inhibitor of mineralization (162). Until now, ALP was mainly of interest for tissue engineering purposes to predict neo-tissue mineralization by means of the enzyme expression. De Jonge *et al.* described the electrospray deposition of ALP on titanium surfaces to enable enzyme-mediated mineralization onto the implants (article submitted to *Advanced Functional Materials*). The

Electrospray deposition technique has proven a very successful method for the deposition of biomolecules (163–166). Due to fast dehydration upon electrospraying, a thin biofilm can be deposited onto implant surfaces without the occurrence of detrimental effects on biomolecule bioactivity. Under physiological conditions, ALP coatings accelerated mineralization onto the titanium surface (167). These newly developed enzyme coatings seem promising for an early and improved implant fixation.

Organic–Inorganic Composite Coatings

Since bone is composed of an organic matrix (of which 90% are collagenous proteins) strengthened by an inorganic CaP phase (carbonated hydroxyapatite), research during the last decade has focused on the development of bio-inspired composite coatings that resemble the unique nano-composite structure bone tissue, thereby offering an added value over coatings consisting of merely organic or inorganic components. Composite coatings made of both collagen and CaP have therefore generated a great deal of interest for implant surface modification. Moreover, CaP coatings have been combined with biomolecules that elicit specific cellular responses (cytokines, growth factors, antibiotics, etc.) to enhance bone formation at the implant surface.

Most techniques used to prepare inorganic CaP coatings are performed either at extremely high temperatures or under extremely non-physiological conditions (Table II), which preclude the incorporation of biomolecules (41,52,57,63,70). Investigations have attempted to circumvent this difficulty by adsorbing biological agents onto the surfaces of preformed inorganic layers (168–170). However, these superficially adsorbed molecules will be rapidly released in an uncontrollable single burst upon implantation (132,171). Hence, coating procedures that incorporate biomolecules into the CaP coating create a more sustained release profile and are therefore of high interest. In this way, the molecules can both sustain their biological activity for a considered period of time and support the mechanical properties of the coating in case of structural ECM components such as collagen. Both the biomimetic and electrospray deposition process (Table II) are among the most promising techniques for generating organic–inorganic composite coatings on implant materials due to their physiological process conditions (172–174).

Collagen–CaP Composite Coatings

A composite coating composed of collagen protein and CaP minerals is considered to be bioactive and may enhance bone growth and fixation of titanium implant materials. Collagen, being the main organic component of the ECM, induces positive effects concerning cellular adhesion, proliferation, and differentiation of many cell types in culture (175–177). Furthermore, collagen exhibits high *in vivo* biodegradability and excellent biocompatibility (178).

Uniform, homogeneous collagen–CaP coatings were generated by adding collagen to electrolytic (ELD) and biomimetic coating deposition procedures (179–181). Biomimetic growth induced a denser and thicker coating with higher crystallinity compared to ELD (180). These composite coatings improve early bone ingrowth in implanted constructs, however, in the same amount as implants coated with only calcium phosphate (181). Nevertheless, the composite of collagen type I and hydroxyapatite behaved mechanically in a superior way than the individual components (182). The ductile properties of collagen increased the poor fracture toughness of hydroxyapatites.

Growth Factor–CaP Composite Coatings

Improvement of the osteoconductivity of CaP coatings can be achieved by the addition of bone growth factors. Bone regeneration around CaP-coated implants can be strongly enhanced by immobilizing growth factors such as BMP-2 and TGF- β to the implant surface (Table IV) (168,170,183). Growth factors immobilized on CaP resulted in a delayed delivery and a higher stability of the growth factor (103,141). For obtaining sustained release of the biologically active agents, the biomimetic coating process proved to be a successful method (184). Compared to growth factor adsorption onto CaP-coated surface, this technique incorporates the growth factors directly into the inorganic layer. In this way, the molecules were shown to be conducive to a sustained biological activity for a considered period of time.

Incorporation of growth factors into CaP coatings was found to be very effective in enhancing bone formation at the tissue–implant interface (183,184). Additionally, the continuous release of bone-stimulating agents is of great promise for the integration of implants into healing bone.

Table IV. Growth Factors for Bone Repair

Growth factor	Origin	Function
Transforming growth factor-beta (TGF- β)	Platelets, bone extracellular matrix (ECM)	Stimulates undifferentiated mesenchymal cell proliferation and osteoblast proliferation
Bone morphogenetic protein (BMP)	Osteoprogenitor cells, bone ECM	Promotes differentiation of mesenchymal stem cells and osteoprogenitor cells to osteoblasts
Fibroblast growth factor (FGF)	Macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes replication of mesenchymal stem cells and osteoblasts
Insulin-like growth factor (IGF)	Bone ECM, osteoblasts, chondrocytes	Promotes proliferation and differentiation of osteoprogenitor cells
Platelet-derived growth factor (PDGF)	Platelets, osteoblasts	Promotes replication of osteoblasts

CONCLUSIONS

The biological performance of titanium implants can be significantly improved by modifying the non-physiological surface of these metallic implants through the application of biologically active coatings. Therefore, various approaches have been extensively investigated that use inorganic (CaP) and organic (ECM components, growth factors, enzymes, etc.) components of natural bone tissue, in that way directly influencing the local response of surrounding tissues and improving the apposition of newly formed bone. In that respect, the combination of both organic and inorganic constituents into composite coatings is believed to result into truly bone-resembling coatings, and as such to a new generation of surface-modified titanium implants with improved functionality and biological efficacy.

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