ABOUT INTERACTIONS BETWEEN SOL-GEL DERIVED SILICA, TITANIA AND LIVING ORGANISMS

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Abstract. Sol-gel derived silica and titania have a specific interaction with many biological molecules, microbes, algae, cells and living tissue. The specific interactions mean that they differ from common reactions between non-viable materials and biomolecules or living tissues and the interactions are mostly beneficial from the viewpoint of biotechnical applications. Pepetides and proteins may preserve their activity and bacteria, algae and cells may preserve their viability and viruses their infectivity as encapsulated in sol-gel derived silica. Silica and titania are known to form a direct bond with living tissue which can be utilized in the biomaterial applications. Other application areas of silica and titania are in biosensing, tissue engineering, gene therapy, controlled delivery of therapeutic agents and environmental protection.

Keywords: Sol-gel method, silica, titania, biomaterials, virus delivery, tissue bonding.

1. Introduction

Silica is one of the naturally occurring substance in living systems and it is found e.g. in diatoms, plants and in humans. Silica is also a potential candidate for biomaterial applications. Implantable biomaterials can be used for example to replace injured or damaged soft or hard tissue and in drug delivery applications. Silica is the main component in bioactive glass which was originally developed by Larry Hench. Bioactive glass is used as bone replacement material mainly in applications where load bearing properties are not needed.[1] It is known that bioactive glass has the ability to bond to bone which is a good example of the

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suitability of silica in biomaterials. On the other hand titanium metal, with naturally occurring titania thin film on the surface, is used as an implant material in load bearing applications. Titania films can be prepared also with sol-gel method and it has been shown that these films can bond directly to tissue. These two examples on bioactive glass and sol-gel derived titania coatings show that silica and titania are able to bond directly to the surrounding tissue and no capsule formation around the implant is observed after implantation. Typically if a foreign material is implanted in body a capsule layer forms around it, which prevents the higher interaction between the material and living tissue.

Generally biomaterials can be divided in four categories which are (i) toxic materials, which elicits a harmful response and thus causing the death of the host tissue, (ii) biologically inactive, nearly inert materials that are encapsulated by fibrous capsules, (iii) bioresorbable materials, which dissolve during the tissue and body fluid contact and (iv) bioactive materials that can be either bioresorbable or biostable. Furthermore, the bioactive materials can be classified as class A (osteopromotive i.e. material elicits both an intracellular and an extracellular response at its interface) and class B (osteoconductive i.e. material elicits only an extracellular response at its interface). The bioactive material can also be defined as: "a material that elicits a specific biological response at the interface of the material, which results in the formation of a bond between the tissues and the material".[2]

Sol-gel derived silica matrices, which are typically biodegradable, can be used in drug encapsulation and delivery applications. In the beginning of 1990s the interest on the delivery and/or encapsulation of different active agents (therapeutic or biologically active) started to grow for many reasons, one of them was the first biopharmaceuticals or biotechnically produced agents (e.g., therapeutic proteins) that reached the phase where the controlled delivery became a real issue. Choradin et al. have reviewed the use of sol-gel materials in medicinal science and they give many good examples on the encapsulation of proteins as well as DNA, cells, algae and bacteria in silica.[2] They discuss also organic modifications of silica and hybrids materials that further widen the possibilities to use silica-based materials in medical applications. Approximately at the same time in 1980s and to a larger extent in the beginning of 1990s, several groups started to encapsulate enzymes, other proteins and cells in the sol-gel derived silica, not to develop delivery device, but to use silica as a matrix material for, e.g., (bio)catalysis and sensors. Avnir et al., Gill et al. and Livage et al. have made excellent reviews on the topic.[3–6] These studies contain a lot of information on the silica sol-gel formulations as well as on the preservation of the activity of proteins encapsulated in silica.

Preparation of SiO_2 and TiO_2 by sol-gel processing is well studied and it is shown that the final structure of the material depends on the preparation parameters. This is advantageous also in biomaterial applications. Sol-gel technique makes it possible to prepare different formulations like monoliths, films, fibres or powders. Sol-gel processing also offers an alternative for the preparation of bioactive glasses with potential advantages over conventional melt processing, such as higher purity, lower processing temperature, and the possibility to modify pore structure, nanoscale topography, composition, adsorption capacity as well as dissolution rate.[7,8]

In this paper is described the suitability of sol-gel derived silica in encapsulation of viruses and the suitability of titania films in tissue bonding applications. Also the interaction between silica, titania and living tissue are discussed.

2. Silica in Living Tissue

The Nobel Price winner for chemistry, Professor Adolf Butenandt, provided that life can not exist without silica. According to his research conducted in 1972, silica is an essential nutrient and must be supplied continuously from food. Silica is also the most common substance in earth's crust. Silicon is found in the same element group on the periodic chart as carbon. In the past, this close family relationship to carbon has led many scientists to speculate that a realm of silicon chemistry awaited discovery, however silicon was not a replacement for carbon. However silica is an important element in many body functions like in bone and cartilage growth. Silica is also a natural constituent of blood and urine (<1 ppm). The average human body has about seven grams of silica. Since silica is so common in earth's crust and in living systems it has led to the situation that living systems have been adapted to the presence of silica very well.

Silica can be found in crystalline and amorphous forms. It has been reported that crystalline form of silica can cause fibrosis in lungs as inhaled, this is a serious illnesses called silicosis, which was called at the old times "coal workers disease". Crystalline silica has very low water solubility.9 Whereas the amorphous silica is water soluble and biocompatible. The solubility of amorphous silica in water at body fluid pH is most commonly determined to be 130–150 ppm (μg/ml). Amorphous silica is known to dissolve into body fluids as silicic acid and is removed through urine. No silica accumulation is observed in organs.

The interest on the delivery of large biologically active molecules such as proteins, peptides and polysaccharides is growing fast. However the direct administration of these new biotechnically produced drugs is difficult due to intestinal decomposition. The difficulties can be avoided by encapsulating the

administrated molecules into amorphous sol-gel derived silica matrices and by implanting or injection these systems locally into the desired tissue.[10] One parameter, which affects the drug release rate is the silica matrix degradation rate e.g. the release of proteins from silica matrix is totally controlled by the matrix degradation.[11]

During the material development, first the in vitro matrix degradation and drug release are studied and in the second stage the correlation between the in vitro and in vivo matrix degradation is studied. According to our experimental observations, the *in vitro-in vivo* correlation of the biodegradation rates for silica structures with high silanol amounts and more or less encapsulated water (processed at ≤40°C) is about 8–10. This means that already in vitro dissolution rate of few days will result in relatively slow *in vivo* degradation, i.e., several weeks. This correlation has been observed several times for sol-gel derived silica, mostly in subcutaneous implantation in recent studies[12,13] in mice and rats, but also in our studies in intraperitoneal implantation in mice. The same correlation has been observed for relatively different silica implants developed for drug delivery, i.e., for hydrogel implants with 90% (w/w) water in structure and for xerogels (drying up to constant weight at 40°C). Both silica hydrogels and xerogels contain a lot of silanol groups and both are quite porous, only larger difference is in the water amount. The in vitro biodegradation rates (dissolution rates in simulated body fluid or in corresponding medium buffered to pH = 7.4 at 37°C) in sink conditions varied from 2–3 days to 6–7 days, respectively. These resulted in 25–30 and 55–60 day's degradation *in vivo,* respectively. The difference between *in vitro* and *in vivo* degradation rate is suggested to depend on two main reasons, (1) the obvious difference in the fluid flows in vitro and in tissue and (2) the fibrous capsule formed on the silica implants, which may retard any transfer of matter between the implant, also the fluid flow.

3. Encapsulation of Viruses in Sol-Gel Derived Silica

Wet silica gels can be used to encapsulate proteins, cells and viruses. Livage et al. and Avnir et al. discussed the idea to use wet silica gels in the cell and protein encapsulation.[3,6] We have developed silica gel with high water content that preserved the activity of encapsulated adenoviruses that can be utilized in gene therapy or in cancer vaccines.[14] Such silica with encapsulated viruses can then be implanted and the viruses are released by silica dissolution in the living tissue so that the gene transfer occurs.[12]

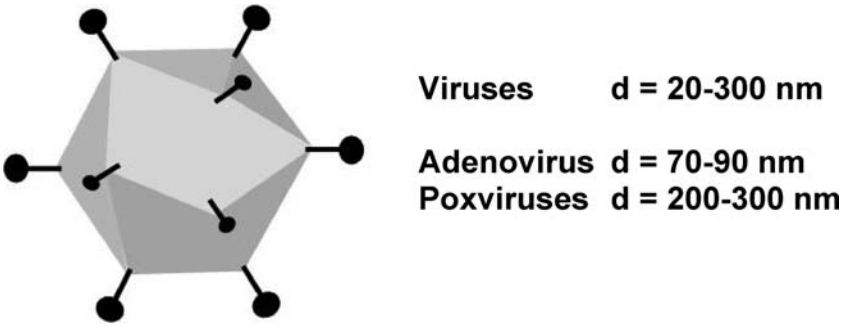

Figure 1. Schematic presentation of a virus and the typical sizes of the viruses.

In order to maintain the functional structure of viruses inside the silica gel, wet gels have to be used. These gels are called silica hydrogels. Water content in silica hydrogels is about 90% (w/w) and silica about 8–9%. Typical precursors in sol-gel process are silicon alkoxides (e.g. tetraethoxysilane, TEOS), acid, base and water. First the hydrolysis of TEOS is done at low pH (pH 1–3) and before adding the viruses into sol the pH of the sol is adjusted to higher level (pH 5–7). After adding the viruses the sol gelation occurs and hydrogel structure with encapsulated viruses is formed. The sols for these hydrogels are typically prepared at high water-to-alkoxide ratios ("R"), typical value of R is between 50–100. Ethanol is the by-product of the hydrolysis and condensation reaction of TEOS (Figure 3), but concentration of ethanol is quit low in the sol because high R-values are used and no harmful effects of ethanol on the viability of adenoviruses has been detected. The hydrolysis reaction of TEOS is closely to 100% because the remaining TEOS amounts in the hydrogels were near the detection limits giving at maximum 0.01 ppm (μg/ml) by GC/MS after extraction of the silica implants as such and as crushed in 5 ml of EtOH at 37–50°C for 3 days, i.e., TEOS content in the ready-made silica hydrogel implant was less than 0.001% (w/w). Ethanol content was maximally 0.5% (w/w). It is quite clear that silica structures that are full of water are also ready to dissolve in water. In addition, it is obvious that the pores are quite large and the porosity is high. The pore structure is difficult to measure directly due to high water amounts, but indirect measurements (dried structures) gave specific surface areas of several hundreds of square meters per gram. This kind of hydrogel silica structures dissolve in sink conditions (in body fluid mimicking water solutions buffered to pH 7.4 at 37°C) totally in 2–3 days.

3.1. STORAGE AND RELEASE

Sol-gel derived silica hydrogels are naturally structurally labile, the preservation of high water content (needed to keep the hydrogel structure virus-compatible) depends on the surrounding conditions, if the hydrogels are stored in dry environment silica matrix will dry and encapsulated viruses may lost their infectivity. This problem was solved by storing the hydrogels in silica-saturated water. We have showed that the labile water-containing silica structure could be stabilized from the viewpoint of virus infectivity by adding the silica implants in water that is saturated with respect to silicic acid or into water of which volume is so low that the slight degradation of the implant will saturate the solution and silica dissolution is terminated. At the same time, the aging of the silica structure in the water storage did not significantly change the dissolution rate of silica.[15] Due to the saturation the silica hydrogel implants do not dissolve in the liquid, but the aging of the silica structure proceeds. It was also observed that it is important to determine a proper aging of the gel prior to storage in silica-saturated water. Too early or too late immersion into water solution may break the implant during the storage. The structure develops during the water storage, dissolution rate decreases slightly within the first month after which it stays rather stable. The water content inside the silica structure may also decrease, but from the point of view of desired virus infectivity, the water storage has been found to be good, e.g., adenoviral vectors preserve their infectivity at least for several months. It is also important to note here that silica hydrogels are primarily developed for encapsulation of large biologically active agents, such as viruses and proteins and other large biomolecules that are not able to diffuse out from the silica structure, but are released by the silica matrix degradation.

In our previous studies where protein (BSA) was encapsulated into the silica matrix it was shown that the protein release was controlled by the matrix degradation. The protein release was studied in sink conditions where the free dissolution of the matrix was allowed and also in the SiO_2 saturated Tris buffer where no matrix degradation was possible. Results indicate that big molecules like proteins or even bigger viruses, cannot be released from the silica matrix without the matrix degradation. By storing the virus containing silica matrices in silica saturated solution viruses can be kept inside the matrix during the storage time and no matrix degradation occurs.[11]

4. Implant Fixation

Traditionally bioactive glasses and ceramics which are used as bone replacement materials are divided in two categories: class A and B. Class B materials are only osteoconductive, which means that the bone can grow onto the surface of

these materials with a passive process. Where as the class A materials are also osteopromotive which means that they are able to awoke a biological response where chemical signals induce bone formation which leads to direct bone growth and formation of the implant surface, bioactive glass belongs to this class.

4.1. MECHANICAL IMPLANT FIXATION

Metal implants are widely used in load-bearing orthopedic and dental applications. However, these materials are biologically inactive and they do not form a chemical bond with bone but are rather surrounded by a fibrous tissue capsule. In order to overcome the poor tissue bonding of the metal implant, the concept of mechanical implant fixation i.e. biological fixation by bone ingrowth through a metallic cage, is issued already in the early 1900s.[16] Mechanical fixation is still the most widely applied concept for ensuring implant fixation, which is frequently done by either controlling the materials pore size and interconnectivity or by surface roughening techniques such as mechanical grinding, sand-blasting and chemical etching. These techniques are also applicable to porous polymers.[16] The optimal pore size for bone ingrowth has been shown to be approximately 200–400 μm.[17,18] The surface roughening techniques are used to produce surfaces with less than 10 μm of micro-roughness.[19–22] Although improved mechanical interlock between the implants and bone have been achieved, metal implants do not attach to bone through chemical bonding.

4.2. CHEMICAL IMPLANT FIXATION BY SOL-GEL DERIVED COATINGS

Sol-gel derived TiO_2 coatings on metal surface enhance the bone bonding (class B bioactivity) of metel implants. This has been studied *in vitro* and *in vivo.* Based on the *in vitro* experiments in simulated body fluid (SBF) it has been shown that the bone bonding starts with the formation of negative charged surface which attract positively charged Ca^{2+} ions from the body fluid and after that adsorption of phosphate (HPO_4^{2-}/PO_4^{3-}) ions occurs. First the adsorbed layer will be amorphous calcium phosphate and will finally turn in to bone mineral-like hydroxyapatie (HA) through which the material bonds to bone. Thus the following general calcium phosphate formation mechanism on TiO_2 surfaces was suggested[23,24]:

Step 1. Formation of TiO^- groups on the surface. This is followed by a rapid accumulation and adsorption of Ca^{2+} and PO_4^{3-} ions on the surface due to electrostatic interactions.

Step 2. The formation of the initial calcium phosphate nuclei.

Step 3. Organization of the initially formed nuclei to a more stable and larger structure, which is influenced by the surface curvature.

Step 4. Once the nuclei has obtained a more stable structure and the critical size is achieved it will rapidly grow forming a poorly crystalline bone mineral-like hydroxyapatite layer on the surface via dissolution and reprecipitation processes, which is similar to the processes occurring on the surfaces of many bioceramics. This stage is delayed or inhibited in the presence of proteins.

TiO_2 is negatively charged at pH 7.4. In addition to the charge complementary of the negatively charged TiO_2 surface (at pH 7.4) and the firstly adsorbed Ca^{2+} ions, the nucleation property (or the lowering of the nucleation activation energy) is also dependent on the surface topography, which is one of the things that can be adjusted by the sol-gel method.

4.3. SURFACE MODIFICATIONS OF METAL IMPLANTS

Titanium (Ti) metal and its alloys are used in dental and orthopaedic applications. The biocompatibility of titanium metal is related to its surface oxide layer, which naturally occurs on the metal surface. In addition to biocompatibility, the bioactivity of the metal can be increased by introducing thicker titania coatings by different surface treatment techniques. One method is to use sol-gel technique to produce bioactive titania films on metal surface and an other method is to use simple chemical surface modification methods. Such methods were well known in conventional metallurgy, but their use for implant surface functionalization was novel. First the titanium metal was treated with mild H_2O_2 solutions as a chemical surface cleaning procedure leading to Ti-peroxide (TiO_2^{2-}) and Ti-superoxide (TiO_2^{-}) formation on their surface.[25–28] However it has been shown that H_2O_2 treatments using higher concentrations produce a thick titania gel layer with microporous structure on the surface of titanium implant enhancing the bioactivity and biocompatibility.[29–38] The most widely used and studied bioreactive metal implant of such type has been obtained by soaking of metal implant in either NaOH or H_2O_2 solutions with subsequent heat-treatments.

An other approach is to employ a bioceramic coating on the metal. Various bioceramic coatings have been used including calcium phosphates and hydroxyapatite coatings.[42–48] Techniques like sputtering and plasma spraying have been applied for preparation of these coatings on metal implants. Bioactive ceramic coatings have been widely applied to ensure direct chemical implant-bone contact and to reduce the time required for osseointegration. In this respect the plasma-sprayed calcium phosphate coatings are the most widely applied[33–35] although the composition, structure and the adhesion to the substrate are difficult

to control. One potential risk connected to the clinical use of any bioactive ceramic coatings is delamination or fragmentation. For example, recent studies have suggested that a breakdown at the metal-ceramic interface may occur[36,37]

Recent findings also suggest that bioactive surfaces (in terms of cell adhesion and activation) can also be obtained by controlling the surface roughness in the nanometer scale.[38–41] The surface nanoscale dimensions were shown to be important with respect to the interaction between the nanostructured surfaces and proteins as well as cells affecting the soft and hard tissue formation. Both nanostructured ceramics and polymeric surfaces showed similar enhanced cell adhesion and activation effects compared to conventional materials. These studies showed that there are specific dimensions between 1–100 nm that have a direct influence either on the adsorption of proteins (e.g., vitronectin) that are important with respect to adhesion and growth of bone tissue forming cells (osteoblast) or on the gene activation of soft-tissue forming cells, fibroblasts.

Surface dimensions between 1–100 nm can be achieved by sol-gel derived TiO_2 coatings that together with titanium metal provide a potential material for good tissue attachment.

4.4. SOL-GEL DERIVED TITANIA COATINGS

The sol-gel systems of TiO_2 can be adjusted to form versatile coatings structures and the applying techniques are quite simple. The sol-gel reactions are induced by polymerizing Ti-alkoxides via hydrolysis and condensation reactions (Figure 2). The polymerization stages can be described as, (i) polymerization of monomers to oligomers, (ii) condensation of polymers to primary particles, (iii) growth or agglomeration of primary particles to larger particles and (iv) linking of particles into chains followed by extension of their network eventually transforming into a gel.[7,9] In the preparation of sol-gel derived coatings by dip-coating, the substrates are immersed into a dilute sol and the gel-like coating is formed during substrate withdrawal.

$$\equiv\text{M-OR} + \text{H}_2\text{O} \leftrightarrow \equiv\text{M-OH} + \text{ROH}$$

Hydrolysis → ← Esterification

$$\equiv\text{M-OR} + \text{HO-M}\equiv \leftrightarrow \equiv\text{M-O-M}\equiv + \text{ROH}$$

Alcohol condensation → ← Alcoholysis

$$\equiv\text{M-OH} + \text{HO-M}\equiv \leftrightarrow \equiv\text{M-O-M}\equiv + \text{H}_2\text{O}$$

Water condensation → ← Hydrolysis

Figure 2. Hydrolysis and condensation reactions of (IV)-metal alkoxides. M=Ti or Si.

During film deposition, the structure of the film forms rapidly (as compared to bulk structures) and is influenced by several complex factors. The film thickness can be controlled, for example, by sol viscosity and withdrawing speed, where the increasing viscosity and/or increasing withdrawing speed increases film thickness. In addition to film thickness, the pore volume, pore size and surface area of the final film can be controlled by controlling the size and extent of branching of the reacted species in the sol prior to film deposition. Also the contribution of the competition between evaporation and continuing condensation reactions is crucial for the final film structure. If the rate of evaporation is significantly higher than the condensation, it results in a more compact film structure and vice versa. After the film deposition films are heat-treated, typically the heat-treatment temperature for sol-gel derived films is between 80–500˚C. Dip-coating can be done at room-temperature which allows the preparation of ceramic coatings at lower temperatures.

Since the ceramic coatings are deposited from colloidal sols, film thickness, surface area, porosity etc. can be controlled. In Figure 3 is schematically presented how the colloidal structure in the sol has an effect on the film surface topography. Furthermore, sol-gel dip-coating method is cost effective and technically simple giving the possibility to coat substrates with difficult geometries.

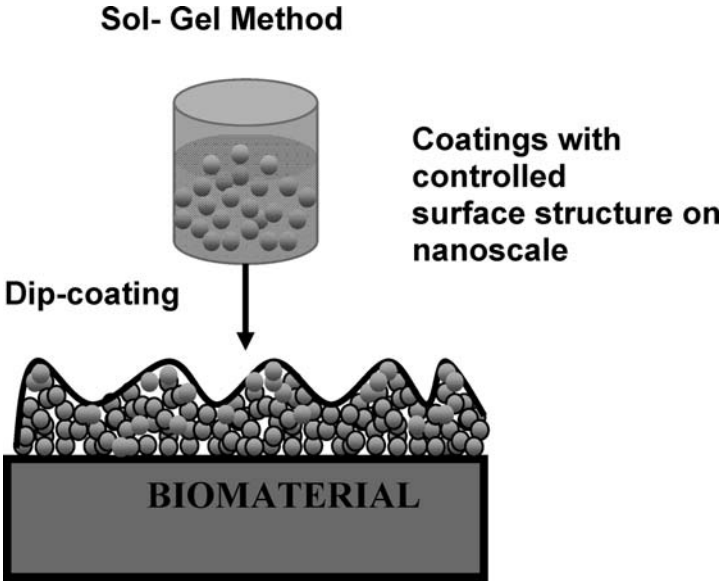

Figure 3. A schematic presentation of sol-gel dip-coating method.

It is now well established that the sol-gel derived titania coatings promote bone bonding (class B) via the formed bone mineral-like calcium phosphate on their surfaces *in vitro* and *in vivo*.[49–51] The use of sol-gel-derived titania coatings is also motivated by the fact that a nonresorbable, reactive coating might guarantee a direct and immediate contact between the implant and the tissues, whereas for resorbable reactive coatings, the underlying inert substrate might be exposed during long-term implantation. Although, titanium metal and metal alloy implants have been shown to leach metal ions during long-term implantation[52,53] the titania (TiO_2) is not degraded and it is thought to be stable in the body environment. Furthermore, coatings thinner than 1 μm are known to possess self-healing properties due to crack and dislocation annihilation, providing enhanced material toughness as for the whiskers and glass fibers, which get more elastic as they get thinner[54] Also the energy required for coating self-healing becomes smaller the thinner the coating. One of the attractive features of the application of TiO_2-sol-gel derived coatings lays in the possibility of local processing, for example with a focused CO_2-laser beam.

4.4.1. *Modification of Titania Coatings with Laser Processing*

Sol-gel derived TiO_2 coatings can be locally treated with CO_2 laser beam, achieving local modification of the bioactive properties of the surface.[56–58] The use of a CO_2 laser is based on the fact that the radiation on the 10.6 μm wavelength is absorbed by the titania film. Besides, CO_2 lasers are rather common and inexpensive devices. The output of the laser can be controlled to obtain the desired effect on the surface.[58–60] Unlike the traditional furnace firing direct laser densification of sol-gel derived TiO_2 coatings allows the selective treatment of the surface of the coating. Further, the surface is only heated locally in the proximity of its surface. This permits the manufacture of the coatings with different areas of the same coating having different properties and coatings of implant materials other than titanium, including those that do not withstand extensive heat-treatment. The CO_2 laser treatment on the sol-gel-derived TiO_2-coatings has been successfully applied in inducing *in vitro* calcium phosphate formation either as post treatment method or as direct densification after dip-coating.[55,56]

4.5. BIOACTIVE PROPERTIES OF SOL-GEL DERIVED TITANIA COATINGS

The heat-treatment of sol-gel derived TiO_2 coatings at different temperatures produces titanates with different structures i.e. amorphous, anatase or rutile (in the order of increasing calcination temperature). The amount of hydroxyl groups on the TiO_2 film depends also on the heat-treatment temperature, the higher the heat-treatment temperature less OH-groups will remain on the film.[61–64] It has

been shown that hydroxyl groups and the negatively charged surface at pH 7.4 are properties which enhance the chemical bonding of TiO_2 coatings to bone. In addition, there is also evidence of strong surface structural dependence to the calcium phosphate formation properties on these coatings. The surface topography at the nanometer level has been shown to influence the *in vitro* bioactivity. This phenomenon was related to the charge density and the topographical matching of the titania surface and calcium phosphate crystal size found in bone (i.e. not with the matching of the atomic distances in single crystals) as shown in Figure 4.[65–67] It was shown that the anatase structure favours bone minerallike hydroxyapatite formation compared to rutile when the rutile is produced by increased heat-treatment temperatures [70,71]. One possible reason could be that the crystallographic lattice match of anatase and hydroxyapatite (HA) is closer to each other than that of rutile and HA.[68,69]

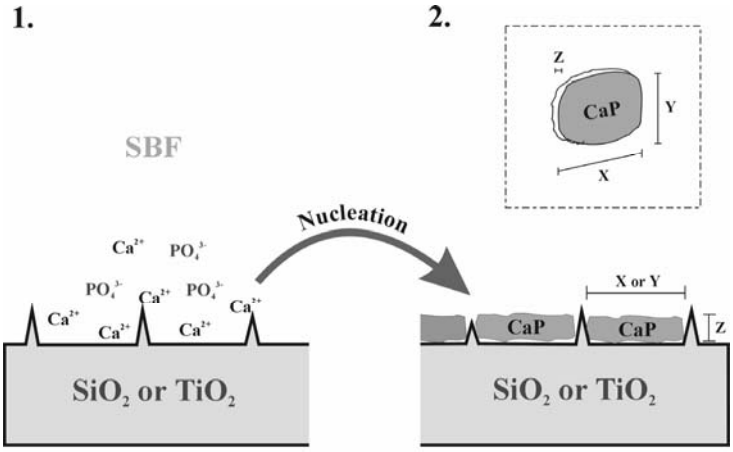

Figure 4. The most favourable dimensions on the titania surface for the formation of calcium phosphate match the natural size of calcium phosphate crystals in bone (plate-like calcium phosphate crystals, X = 20 nm, Z,Y = 3–7 nm).[73–74]

Calcium phosphate nucleation ability of TiO_2 coatings with different crystal structure is studied in simulated body fluid and it is shown that *in vitro* bioactivity was enhanced on the coatings with existence of surface OH-groups and anatase crystal phase. It was shown that the anatase structure favours bone minerallike hydroxyapatite formation compared to rutile when the rutile is produced by increased heat-treatment temperatures.[70,71] However, when the rutile phase on the coatings coatings were produced by the laser induced thermal "shock" treatment (i.e. the surface OH-groups are not removed by extensive heat-treatment) it was observed that the as prepared rutile phase favors the bone mineral-like hydroxyapatite formation compared to the anatase phase prepared by conventional heat treatment.[57] It should be noted that the bone mineral-like hydroxyapatite formation

experiments were done in a competing system where the coatings having anatase and rutile structures were in the same flask and these results are not interpreted so that the anatase coatings produced by laser treatment are not bioactive at all. These results indicate that the precise lattice matching of anatase and HA is not a crucial factor in the in vitro properties of TiO_2 based coatings.

A possible reason for the low bioactivity of amorphous TiO_2 may be that particle size on the film surface is smaller than what is required for the optimal topographical features. During the film densification (e.g. by heat- or laser-treatment) the deposited particles form particles and/or aggregates, and the final surface structure is formed. Furthermore, during densification the mechanical and chemical stability of the coating is enhanced. Following this reasoning bioactive amorphous TiO_2 coatings could be obtained if the initial deposited particles and/or aggregates are large enough although their mechanical properties and chemical stability probably remains too poor for practical application.

The influence of topography on calcium phosphate formation on sol-gel derived TiO_2 coatings is shown experimentally using AFM image analysis.[65,66] By controlling the particle size and particle size distribution in the dipping sol, the surface topography on the nanometer scale can be varied. It was concluded that the optimal topographical features of the outermost surface for calcium phosphate formation was in the range of 2–50 nm (as obtained from the line section analysis of the AFM images in vertical and lateral directions). It should be noted that also the highly bioactive rutile containing coatings exhibited such topographical features.[57] The aspect of topography on nucleation of inorganic solids has been well studied in the context of organic matrix-mediated biomineralization processes. It has been stated that "electrostatic accumulation of ions on organic surfaces is influenced by the localized clustering of ligands and their spatial charge distribution, which in turn depend on the surface structure and topography". For example, surfaces with concave-like topographical features give rise to a high spatial charge density and 3-D clustering of ions and are thus good nucleation sites.[72] This theory has been applied in one model of bone mineralization where the calcium phosphate nucleation is proposed to occur in regiospecific hole zones of collagen having a distinct size and topography.

A firm bond between the soft tissue and implant is also important for the performance of many medical devices like stents, canyls and dental implants. It is shown that the sol-gel derived titania coatings can bond also to soft tissue. A direct attachment between soft tissue and sol-gel derived titania coating was found in vivo after 2 days of implantation in rats, where as the titanium control implants showed no evidence of soft tissue attachment. The coated implants were in immediate contact with connective tissue, whereas the titanium controls formed a gap and a fibrous capsule on the implant-tissue interface. The good soft tissue attachment of titania coatings may result from their ability to initiate

calcium phosphate nucleation and growth on their surfaces in vitro, thus the formation of bone mineral-like calcium phosphate layer is not crucial for their integration in soft tissue.[73]

5. Conclusions

Both silica and titania based materials can be produced with sol-gel method and can be used as biomaterial in different applications. Sol-gel derived silica and titania are biocompatible and can be used in direct contact with different organisms e.g. microbes and living tissue. The main parameter controlling the beneficial interactions between silica and living organisms is nanoscale and molecular structure of sol-gel derived silica combined with a large amount water in structure during encapsulation in silica structure. Whereas the most important driving force for calcium phosphate nucleation on sol-gel derived TiO_2 coatings is the electrostatic accumulation of ions in the localized regions (grooves and/or pockets) of high spatial charge density. This can be achieved with the negatively charged TiO_2 surface at pH 7.4 and also the surface topography has to be suitable the calcium phosphate nucleation. It is shown that sol-gel derived titania films can bond to living bone and soft tissue.

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