Review
Zirconia: Established Facts and Perspectives for a Biomaterial in Dental Implantology

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Abstract: Currently, zirconia is widely used in biomedical area as a material for prosthetic devices because of its good mechanical and chemical properties. Largely employed in clinical area for total hip replacement, zirconia ceramics (ZrO_2) are becoming a prevalent biomaterial in dentistry and dental implantology. Although titanium is used in dental implantology currently, there is a trend to develop new ceramic-based implants as an alternative to monolithic titanium. This article reviews the evolution and development of zirconia through data published between 1963 and January 2008 in English language. Articles were identified via a MEDLINE search using the following keywords: zirconia, zirconia/biocompatibility, zirconia/osseointegration, zirconia/periointegration, zirconia/review, and zirconia/bacterial adhesion or colonization. This review of the literature aims at highlighting and discussing zirconia properties in biological systems for their future use in dental implantology. In conclusion, zirconia with its interesting microstructural properties has been confirmed to be a material of choice for the "new generation" of implants, thanks to its biocompatibility, osseoconductivity, tendency to reduce plaque accumulation, and interaction with soft tissues, which leads to periointegration. However, scientific studies are promptly needed to fulfill gaps like long-term clinical evaluations of "all zirconia implants," currently leading to propose an alternative use of "hybrid systems" (i.e., titanium screw with zirconia collar) and also bacterial colonization of zirconia. Moreover, there is a permanent need for consistent information about topography and chemistry of zirconia allowing easier cross-product comparisons of clinical devices. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 88B: 519–529, 2009

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INTRODUCTION

Found in ores like zircon and baddeleyite, Zircon ($ZrSiO_4$) has been known as a popular gem for ages (in ancient Egypt). The name of zirconium is said to be derived from the Persian "Zar"- "gûn," meaning golden in color. Zirconium (Zr) was originally discovered by the chemist Martin Heinrich Klaproth in Berlin (Germany) in 1789 as an end product of gem heating reaction and was isolated in 1824 by the Swedish chemist Jöns Jacob Berzelius.

The major end-uses of $ZrSiO_4$ are refractories, foundry sands, and ceramic opacification. Impure zirconium oxide, zirconia, is largely employed to make laboratory crucibles, for linings of metallurgical furnaces (high-performance pumps and valves). It is also used as a refractory material by ceramic and glass industries. Zirconium is extensively used by the chemical industry for piping in corrosive environments, especially high-temperature ones. Because of their high temperature ionic conductivity, zirconia ceramics serve as solid electrolytes in oxygen sensors and fuel cells. But mainly, this metal is employed in alloys (with iron, chromium or tin, i.e., Zircaloy) in nuclear industry for the cladding of nuclear fuel rod or in zircon glass for sarcophagus of radioactive wastes (i.e., plutonium) where zirconium

is cleared from Hafnium. Naturally occurring zirconium is composed of four stable isotopes and one extremely long-lived radioisotope (^{96}Zr) useful in geochronology.

Its physical, mechanical (i.e., high strength, hardness, wear resistance, resistance to corrosion, modulus of elasticity similar to steel, coefficient of thermal expansion similar to iron, and elevated fracture toughness) and chemical properties make zirconia a material of interest for biomedical sciences. The first reference concerning its application in medicine appeared in the late sixties with Helmer and Driskell (1969),[1] followed 20 years later by the first publication[2] referring to its use in orthopedic surgery and particularly in total hip replacement to solve the problem of alumina brittleness and potential failure of implants. It is only in the early nineties that zirconia found its application in dental prosthetic surgery with endosseous implants.[3,4] However, to date, the first experimental research on the use of zirconia was published in 1975 by Cranin and co-workers.[5] Zirconia and alumina were used to coat vitallium (alloy of chromium and cobalt mainly) in oral endosteal implants in dogs. This group examined the mobility as well as biological acceptability (via histological sections) of both types of implants after an eight-month survey. The authors already suggested that zirconia used was a coating superior to alumina but could not increase the acceptability of vitallium implants.

The successful incorporation of dental implants strongly depends on firm longstanding adhesion of the tissues surrounding the implant. Moreover, deeper periodontal structures need to be protected from bacterial invasion and subsequent infection. Today, there is a trend in dental implantology to develop new ceramic-based implants for their enhanced capacities of periointegration such as osseointegration, reduction of plaque accumulation leading to an improvement of the soft tissue management, and aesthetic consideration as an alternative to monolithic titanium implants. This review of the literature aims at highlighting and discussing these properties of zirconia in a context of periointegration for the development of clinical researches with this high-value material.

MICROSTRUCTURAL PROPERTIES OF ZIRCONIA

Zirconia is a well-studied polymorphic structure present in three crystal forms: monoclinic (M), cubic (C), and tetragonal (T)[6] (for review). At room temperature, zirconia adopts a monoclinic structure and transforms into tetragonal phase at 1170°C, followed by a cubic phase at 2370°C. While cooling, these phases are unstable and break into pieces at room temperature. Addition of oxides like CaO, MgO, and Y_2O_3 (Yttrium) to pure zirconia was proved to stabilize the C-phase resulting in multiphase material called partially stabilized zirconia (PSZ) combining cubic, monoclinic, and tetragonal phases in this order of importance.[7] In 1972, Garvie and Nicholson[8] could improve the mechanical strength of PSZ (CaO-ZrO$_2$) by obtaining an homogenous

and fine distribution of the monoclinical phase within the cubic matrix. Recently, Nath et al.[9] studied the development of stabilized zirconia ceramics in CaO-ZrO$_2$ system using microwave sintering technique and could conclude that with 8 mol % of CaO, the ceramics (Ca-PSZ) exhibited interesting properties (i.e., Vickers hardness and modest fracture toughness) for specific use in implantology. Various other PSZ ceramics where obtained and extensively tested especially Mg-PSZ for biocompatibility (see section on biocompatibility in soft tissues) with encouraging results. However, the use of such Mg-PSZ material in biomedical application should be stopped for the following reasons. Mg-PSZ is characterized by a residual porosity, it sinters at high temperature, which implies special heating equipment, and finally, it is almost impossible to get it free of silicon dioxide and alumina particularly. PSZ could be obtained with the "stabilizing" oxide Y_2O_3 (Yttria) too. Nevertheless, another type of ceramics could also be achieved with yttria at room temperature. This phase, called tetragonal zirconia polycrystals (TZP), contains tetragonal phase only. This structure obtained by adding 2–3% of Y_2O_3 is constituted of tetragonal grains with an average size of hundreds nanometers. The tetragonal fraction retained at room temperature is dependent on the grain size linked to the yttria content and the grade of constraint exerted on them by the matrix.[10] This yttria stabilized TZP (Y-TZP) presents various interesting characteristics as low porosity, high density, high bending, and compression strength, proving that it is suitable for biomedical application and especially in dental implantology. Aging of zirconia, related to mechanical property of ceramics, is due to the progressive spontaneous transformation of metastable tetragonal phase into monoclinic one. This transformation induces microcracking and spalling found to play a major role in wear of Y-TZP.[11] Moreover, yttria mixing method and distribution seems to influence the transformation behavior of zirconia.[12,13] The strength and structural stability of Y-TZP could also be affected by "finishing polishing" (by dental laboratory technicians or suppliers) and "aging" (by intraoral conditions). However, recent *in vitro* works[14–16] demonstrated, in the limit of these experiments, the stability of this Y-TZP ceramics to these treatments by various parameters analysis. Surfaces were evaluated by using scanning electron microscopy (SEM). Information on the chemical composition was obtained by energy dispersive spectroscopy (EDS) and identification of phase transformations were analyzed by X-ray diffraction (XRD). Moreover, zirconia could be colored by different pigments. Cerium (Cr), praseodymium (Pr), and erbium (Er) even added in small quantities influence flexure strength but not hardness and fracture toughness of zirconia.[17] These properties were evaluated by XRD and SEM.

In conclusion, the microstructure of zirconia is an important factor to take into consideration for the stability and perfect aging of ceramics. Moreover, the presence of impurities lead to a loss of stability of the tetragonal phase

and in turn affect the mechanical properties; major attention should be given to the quality of starting powders for the preparation of this ceramics.

ZIRCONIA: A BIOMATERIAL OF CHOICE

Odontology is one of the surgical disciplines using the largest panel of materials: alloys, polymers, surgical cements, ceramics, implants. Owing to different structural, chemical, and physical properties, these materials are in contact with one or more tissues in the oral cavity. Dental implantology implies fixing in the maxillary or mandibular bone a device aiming to replace a missing root and secondly to support a prosthetic element; the endosseous implant is in contact with at least three different tissues: the buccal epithelium, the gingival connective tissue, and the alveolar bone.

Biocompatibility, defined as the capacity of a material to be used with an appropriate host response for a specified application, involves the effects of the material on the medium and *vice versa*. The biomaterial or its degradation products should not be responsible for inflammatory reaction, neither to provoke allergic, immune, toxic, mutagen, or carcinogenic reactions.

In case of bioinert material, which is particular to zirconia, the encapsulation by connective tissue is faint and the release of residues almost undetectable. Moreover, zirconia is known to be osseoconductive,[29] which means that this ceramic facilitates bone formation when in contact with it as analyzed by SEM.

Biocompatibility of dental biomaterials should be defined at investigation levels: *in vitro* and *in vivo* tests as well as clinical trials in human beings.

In Vitro Tests

Zirconia, under different physical forms, was tested *in vitro* onto different cell lines such as fibroblasts, lymphocytes, monocytes, and macrophages and also osteoblasts for its toxic potency.

Biocompatibility Tests on Fibroblasts. Connective tissue, being the most ubiquitous one in the organism, mainly composed of fibroblasts and fibrocytes, was the first target investigated as regards biocompatibility of zirconia.

In the earlier 90s, Bukat and coworkers,[18] using SEM, observed the adhesion and spreading after direct contact of 3T3 murine fibroblasts onto alumina and sintered zirconia ceramics (Ca-PSZ) disks with 30% of porosity. Later on, the influence of the physical form of materials was tested on *in vitro* biocompatibility by Ito and coworkers[19] comparatively to Ti but also by Li's group.[20] Ito et al.[19] used wear debris of ultra-high-molecular-weight polyethylene (UHMPWE) *versus* Y-PSZ or UHMPWE *versus* Ti-alloy in presence of PECF (pseudo extra cellular fluid as lubricant) on L929 murine fibroblast cell line to analyze cell proliferation. The authors could observe a higher cytotoxicity of

zirconia (Y-PSZ) wear debris than that from titanium alloys in a dose-dependent manner. On the other hand, Li and coworkers[20] compared powders and ceramics of Y-PSZ only on human oral fibroblasts by direct contact (colony forming efficiency), MTT test (methylthiazole sulfate test: a quantitative colorimetric test reflecting the activity of the mitochondrial dehydrogenases), and the dissolution test (ion release at $37°C$ in saline solution). They concluded that zirconia powders were more toxic than ceramics. Finally, zirconia powders were tested for their single toxicity. Dion et al.[21] analyzed zirconia powders on human umbilical vein endothelial cells (HUVEC) and murine 3T3 fibroblasts via indirect contact. The proliferation (MTT test, total cell protein content) and the differentiation via immunofluorescence were assessed. The authors could raise the same conclusion as Harmand et al.,[22] stating that zirconia powders (ZrO_2/Y_2O_3) do not present toxicity on the fibroblast cell line tested.

In conclusion, different physical forms of zirconia and fibroblast cell lines were used. They conduced to distinct conclusions on the toxicity of zirconia but pointed out the evidence that wear products of zirconia could somehow present toxicity. However, it is also worthwhile to note that this *in vitro* data obtained could be partly dubious because of the material characteristics themselves (reactive surface, impurity content, chemical composition). Tateishi et al.[23] pointed out the importance of test conditions. In their Round Robin test for standardization of biocompatibility test with cell lines, the authors observed significant differences between different labs performing the same test with the same materials and cells.

Biocompatibility Tests on Lymphocytes, Monocytes, and Macrophages. Monocytes, lymphocytes, macrophages, and other immune cells are also constitutive but circulating elements of the connective tissue and consequently represent a major class of cells to be *in vitro* tested for biocompatibility. Here again, few physical forms of zirconia, powders or particles, were challenged on cells for their toxicity. Using zirconia powders (Ca-PSZ) on human lymphocytes, Greco et al.,[24] by quantifying the inhibition of cell mitogenesis after phytohemagglutinin (PHA) stimulation, concluded to a dose-dependent cytotoxicity of the powders tested. Ca-PSZ powders and alumina were less toxic than titanium oxide. Moreover, Mebouta-Nkamgeu et al.,[25] in a comparative study with alumina and zirconia powders, could demonstrate the higher cytotoxicity of alumina particles on human monocytes differentiation into macrophages when compared with zirconia one. Cell elemental composition was investigated by X-ray microanalysis, phagocytosis, and respiratory burst of macrophages by flow cytometry. The study of Catelas et al.[26,27] on murine macrophage cell line (J774) with zirconium and alumina oxides (ZrO_2 and Al_2O_3) was focused on macrophage phagocytosis and apoptosis in relation to the particle size and concentration of commercial particles by flow cytome-

try. Their cytotoxicity studies concluded that macrophage mortality increases with size and concentration for sizes greater than 2 μm and that no significant difference in mortality between zirconia and alumina could be observed. Moreover, zirconia and alumina ceramics as well as high-density polyethylene (HDP) particles induce macrophage apoptotic cell death in vitro as recorded by ELISA assays and flow cytometry analysis.[27] Recently, Sterner et al.[28] using the same approach as Catelas (particle sizes) with human monocytic cell line showed that Ti and alumina particles are great inducers of the TNF-α inflammation marker versus zirconia (ZrO_2), which had no effects.

In conclusion, powders and particles of zirconia in vitro tested on different cell lines (human and murine) of lymphocytes, monocytes, or macrophages do not induce high cytotoxicity or inflammation (TNF-α quantification).

Biocompatibility Tests on Osteoblasts. Provided that bone is the essential structure of implant integration, biocompatibility tests using constitutive elements (osteoblasts) of this tissue revealed to be important. In 1999, Josset and coworkers[29] used human osteoblasts and compared the in vitro biocompatibility of zirconia and alumina. The analysis of the cell viability, their capacity of proliferating, and their growing capacity in contact with these materials raised the following conclusions. Zirconia (ZrO_2) does not present any cytotoxic effect, is able to interact with osteoblasts by intimate contacts, and makes the cells capable of elaborating the extracellular matrix by synthesizing various essential and structural proteins. Zirconia finally does not induce any pseudoteratogenic effects (DNA quantity of cells). The absence of toxic effect and the good biocompatibility of zirconia powder (ZrO_2) on rat osteoblastic cells after direct contact were also reported by Torricelli et al. by analyzing cell proliferation (MTT test) and cell differentiation (alkaline phosphatase activity).[30] This conclusion was reinforced by Lohman et al.[31] and by Bächle et al.[32] Lohman et al.[31] analyzed proliferation with zirconia and alumina particles on MG-63 osteoblast-like cells and could demonstrate a higher reduction of osteoblast proliferation in presence of alumina than with zirconia particles. Bächle and coworkers,[32] using discs with different surface roughening of Y-TZP on CAL-72 osteoblast-like cells, could demonstrate a change in proliferation after three days in relation with the surface. Conversely, they could not observe morphological differences between cell and tissue morphology on the various Y-TZP surfaces tested. Hao et al.[33] analyzed the effect of laser-modified zirconia on human fetal osteoblasts cell adhesion and could demonstrate a better adhesion in vitro after laser treatment most likely because of a change in the wettability characteristics of the Y-TZP. Finally, Wang et al.[34] and Liagre et al.[35] were interested in the influence of wear debris of zirconia (ZrO_2) and the molecular consequence for osteoclast and osteolysis in a context of HIP replacement material. Wang et al.[34] demonstrated a synergic effect of cell activation of

human macrophages and wear particles on O_2^- production by osteoclasts and proposed an involvement of O_2^- in the mediation of osteolysis. Liagre and coworkers,[35] more interested in the inflammation pathway potentially provoked by zirconia (Y-TZP) or alumina particles, could not find any significant differences in the proinflammatory cytokine release (IL-1 & IL-6) or in the metabolism of arachidonic acid in their proposed model.

In conclusion, most of the published results on zirconia in vitro tests report the absence of toxic effects on connective, immunologic, or bone tissues.[18–35] However, biocompatibility of zirconia was assessed few years before the first in vitro tests by implanting different physical and structural forms of zirconia in animal bones.

In Vivo Tests

The literature reported biological reaction to some zirconia ceramics with various animal models (rats, dogs, mice, and monkeys). Various forms have been used including bulk material, particulates, fibers, and coatings.[36–44]

Biocompatibility in Soft Tissues. Several studies in various animals (rabbits, rats, mice, dogs, monkeys) reported on the behavior of zirconia ceramics implanted into soft tissues. These in vivo tests performed with different physical (pins, bars, wear particles) and structural forms (TZP, PSZ, or coatings) of zirconia in different sites of implantation concluded to the analysis of systemic toxicity and/or adverse reactions in the implanted soft tissues.

Only few references dealt with PSZ in rodent muscles compared with alumina. When implanted in the paraspinal muscles of rats for up to 12 weeks, zirconia polycrystals (Y-PSZ) tended to become encapsulated with fibrous tissue as observed for alumina control samples.[36] Similarly, Y-PSZ ceramic elicited a similar response to alumina controls when implanted subcutaneously into rats for periods up to 12 months. Both materials became encapsulated by a thin layer (<80 μm) of fibrous tissue, which was independent from implantation time.[37] In all cases, zirconia did not elicit any form of adverse tissue reaction, suggesting that zirconia was biocompatible. Garvie and coworkers[38] found Mg-PSZ to be also biocompatible when implanted in the paraspinal muscle of rabbit for six months. Zirconia (Y or Mg- PSZ) did not elicit any form of adverse tissue reaction.

Flame sprayed coatings of unstabilized zirconia on stainless steel tubes implanted in trachea of rabbits and dogs did not produce any adverse reaction except for a tendency of the tubes to be occluded by the growth of fibrous tissue.[39]

Another important aspect to be analyzed for the biocompatibility of zirconia in soft tissues was linked to the tribological aspect and the capacity of wear products or powders to induce cytotoxicity or not. No local or systemic

reactions were observed after peritoneal injection of Ca-PSZ powders or Y-PSZ in mice.[40,41]

In conclusion, zirconia, whichever physical forms tested, does not induce cytotoxicity in soft tissues even if fibers[42] were found in lymph nodes after intraperitoneal injection of rat and particles in some macrophages.[26,27]

Biocompatibility in Hard Tissues. To date, the first reference reporting biocompatibility in hard tissue was issued by Helmer and Driskell,[1] who inserted pellet of stabilized zirconia with 6% Y_2O_3 into femur of monkeys. They could not demonstrate any adverse reactions but an apparent ingrowth. The first comparative results with zirconia and another implanted material (alumina) were obtained from Wagner[43] and Christel,[44] who used pins of zirconia (Y-TZP) or alumina inserted into femurs of rabbits and did not observe any difference in bone reaction to implants. Bars and cylinders were also implanted in bones of rats, rabbits, and mice without inducing or causing any local or systemic toxic effects after insertion of yttria-stabilized-zirconia in bones.[6]

Finally, it appeared that the various forms of zirconia tested in hard tissues do not induce any adverse reaction or global toxic effects. Moreover, in the light of these *in vivo* biocompatibility tests, it became evident that zirconia, whichever physical and structural forms tested, is a biocompatible material.

ZIRCONIA AND ITS PERIOINTEGRATIVE PROPERTIES

The notion of periointegration implies two integration counterparts: the bone integration and the soft tissue integration. Both integrations are equally important for a successful long-lasting survival of implant. Both are dependent on various local and systemic parameters such as physicochemical and structural properties of the biomaterial, characteristics of tissues (bone tissue and gingival), localization of implants, quality of surgical interventions (surgical trauma), and individual characteristics.[45]

Implants and Soft Tissue

Improvement of peri-implant soft tissue is an essential factor in implant success. The orientation of peri-implant tissue is different from that of periodontal tissue because of periodontal ligament fibers, whose absence makes the implant–bone interface weaker than that of natural dentition.[46] As in periodontal tissue, the integrity of the attached gingiva, and its gingival contour, color, shape, size, consistency, and bleeding upon probing, is an indicator of bacterial activity that will potentially lead to gingivitis and periodontitis (see section Zirconia: A Material of Choice for Reduced Bacterial Colonization). As a consequence, the type of material (its characteristics, treatments: i.e., roughness, surface free energy, and coating methods) and the

bacterial ecosystem are paramount factors influencing the healing and success of the implant. Extensive investigations of soft tissue responses to oral implant have shown that the surface treatments (coatings or physical treatment) of implant influences the attachment of oral fibroblast and epithelial cells especially with titanium surfaces.[47] Using different surface treatments (polished titanium, TiN coating, thermal oxidation, laser radiation), and by analyzing material surfaces, growth, and proliferation (MTT test and total proteins), this study suggests that TiN coating would be a convenient method favoring cellular growth on implant surfaces. Moreover, *in vitro* biocompatibility of zirconia (sections Biocompatibility Tests on Fibroblasts and Biocompatibility Tests on Lymphocytes, Monocytes, and Macrophages) could be an evidence in favor of better maintenance and healing of soft tissue (i.e., cellular behavior such as adhesion and proliferation). Very recently, two studies remarkably analyzed soft tissue integration of zirconia by two different approaches.[48,49] The attachment of the gingiva to dental implants/or natural teeth is mediated by the junctional epithelium. Cells of this tissue attach to tooth by means of hemidesmosomes, which are specialized in adhesion structures. In culture, most cells adhere by focal adhesion contacts (FACs) that are restricted zones close to the basal membrane and the substrate. They are identified by the presence of the actin-binding protein vinculin. The mechanical attachment to the extracellular matrix and signal transduction processes are then facilitated. The localization, organization of FAC, and hemidesmosomes are good indicators of cell adhesion. Groessner-Schreiber and coworkers[48] used surfaces with various roughness and coatings (TiN and ZrN applied by physical vapor deposition) to analyze FAC formation by human gingival fibroblasts in *in vitro* experiments. These authors could demonstrate that the highest number of counted FACs was observed on the lowest roughness surfaces (i.e., Ti, TiN or ZrN). By immunogold-labeling methods to visualize the extracellular fibronectin and vitronectin as well as the intracellular actin and vinculin in FAC areas, Groessner-Schreiber et al.[48] linked the highest number of gold particles counted on surfaces with the lowest roughness again. The authors finally stated that these surfaces, and particularly zirconium nitride coating, favor the attachment of human gingival fibroblasts. In a previous study,[50] the hard coating with ZrN has been shown to reduce bacterial adhesion.

In periodontal tissue, angiogenesis and particularly vascular endothelial growth factor (VEGF) appear to be of importance for the tissue maintenance but also in chronic inflammatory periodontal diseases.[51,52] Nitric oxide (NO) synthesized by three isoforms of NO synthases in humans is also well known in inflammatory processes. The second approach to analyze soft tissue integration realized by Degidi et al.[49] was focused on the inflammatory response analysis on peri-implant soft tissues around titanium and zirconium oxide healing caps (Y-TZP) in human beings. The authors could highlight with biopsy of soft tissue from

patients receiving zirconia oxide healing caps that (1) the inflammatory infiltrate present in the peri-implant soft tissues (sub-mucosa mainly) around zirconium oxide healing cap was lower than that present around titanium one; (2) the microvessel density was significantly lower than that with titanium caps; and (3) both NOS1 and NOS3 expression intensities, indicative of the activity of NO synthases, were also significantly lower in tissue surrounding zirconium oxide healing caps. The authors finally concluded that tissues around zirconium healing caps underwent a lower rate of inflammation-associated processes mostly related to a lower inflammation. Moreover, as bacterial infection generally induced production of large quantities of NO by neutrophils particularly, the lower activity of NO synthesis observed in tissues around zirconia oxide healing caps could be indicative of a lower bacterial colonization on this surface. However, no experimental evidences were given by the authors.

Thus, zirconia seems to actively interact with soft tissues by inducing different cellular pathways aiming at periointegration process. However, the physical and chemical surface treatments of implant appeared to be of paramount importance in cell growth, cell adhesion, inflammation process, and bacterial colonization.

Implants and Hard/Bone Tissue

A parameter of major importance for the clinical success of endosseous implants is the formation of a direct contact between the implant and the surrounding bone. Implant surface topography is thought to influence the implant-bone response. Since the last two decades, a large number of publications have focused on bone- titanium interactions either in *in vitro*, animal studies or in clinical trials.[53] The osseointegration rate of titanium dental implants is related to their composition and surface roughness. Most of surfaces available on the market have proved clinical efficiency (>95% over five years). However, the precise role of surface chemistry and topography on the early events in dental implant osseointegration remains still debated in spite of the growing number of publications.[54–58] In addition, comparative clinical studies with different implant surfaces are rarely performed.[59] The literature concerning osseointegration and zirconia is sparser even if a recent effort has been achieved in the last years. The underlying reason is most likely that, until now, only few implant system companies propose full zirconia implants, maybe because of their lower mechanical resistance compared with titanium. However, few reports stated the *in vitro* and *in vivo* (animals and humans) analysis of zirconia osseointegration. As previously mentioned (section Biocompatibility Tests on Osteoblasts), *in vitro* interaction of zirconia with osteoblasts has been for years referenced in a biocompatibility point of view. Bächle et al.[32] survey comes to the conclusion that Y-TZP showed a good surface attachment and cell proliferation of osteoblastic cells and is consequently

considered as osseoconductive. Furthermore, the roughness of the material seems to be crucial in this process as depicted by Hao et al.[33] (section Biocompatibility Tests on Osteoblasts) for Y-TZP and for other biomaterials.

In animals, various studies were conducted with different bones in rabbits, pigs, or monkeys (tibia, femur, or maxilla) to compare the osseointegration of few different surfaces of zirconia comparatively with machined zirconia or titanium. In monkeys, Kohal et al.[60] compared custom-made zirconia (Y-TZP) sandblasted and custom-made titanium sandblasted and subsequently acid-etched. After nine months of healing and five months of loading, the authors could not report any significant difference in osseointegration or in soft tissue dimension between both types of implants. In rabbits, most of the studies engaged were focused on osseointegration in tibiae or femur. Chang et al.[61] challenged three types of biomaterials (alumina, zirconia, and hydroxyapatite) in different and adjacent anatomical regions of the bone: periosteum, endosteum, and marrow cavity. The bone formed around the implants after a long time period (24 weeks) was more abundant in regions adjacent to the periosteum, then by the endosteum and the marrow cavity. It means that the connective tissue constitutive of the first two regions is of major importance in the osseointegration process. In conclusion, bone formation around these materials is related to their specific osteoconductivity and to the osteogenic capacity of the tissues. Scarano and coworkers[62] inserted as well zirconia ceramic implants in tibia of rabbits for a four-week period. They already reported a great quantity of newly formed bone in close contact with zirconia ceramic surfaces and even in some areas, the presence of osteoblasts directly on zirconia. Finally, Sennerby[63] and Sollazzo[64] groups went further in the investigation of zirconia integration. Sennerby et al.[63] implanted in tibiae and femur of rabbits for a short healing period (six weeks) different surface-treated zirconia ceramics compared with machined zirconia to investigate the relationship between osseointegration and roughness of the material by resistance torque. The authors demonstrated that the higher the surface roughness is, the better and more stable the osseointegration is. Sollazzo and coauthors[64] directed their research on the capacity of zirconia oxide coating of surfaces with colloidal suspension to improve osseointegration. They illustrated this by histological analysis of bone tissues around their test implant. Moreover, the authors completed their study by an *in vitro* analysis on osteoblast-like cells (MG-63) in contact with this zirconia oxide coating surface to analyze for the first time the expression of 20,000 genes by DNA microarrays.[65] Finally, Sollazzo et al.[65] gave the first map of the genetic regulatory processes happening in osteoblastic cells in contact with zirconium oxide. This strategy appeared to be highly encouraging even if the coating of surfaces in dental implantology raised some questions of stability because of mechanical forces in the mouth for instance.

Clinical trials using zirconia oxides in a context of osseointegration are very scarce. The majority of published human clinical trials deals with zirconia use as crown or fixed partial dentures mainly. In 2004, Glauser and coworkers[66] analyzed in humans an experimental self-made zirconia abutment in a context of peri-implant hard and soft tissue reaction as well as fracture resistance over time (four years). While observing that no fractures occurred, with a mean index plaque nearly identical to that of teeth and a marginal bone loss reduced (1.2 mm), the authors assumed that zirconia abutments could be used in single tooth reconstruction in anterior and pre-molar regions. Since this experimental pilot study, a large number of improvements and controls in the process of zirconia abutments preparation, as well as physical and mechanical properties, were published.[67,68] Bianchi et al.[69] published a human trial showing the advantages of a transmucosal titanium implant with a bioactive zirconia (Y-TZP) collar (i.e., hybrid system) on soft and hard tissues in a nonsubmerged approach (one time surgery) for a two-year period. By analyzing various parameters such as plaque index, bleeding on probing, and measures of mucosal sulcus depth around implant via clinical and radioscopic analysis, the authors stated that zirconia collar type implant offers a better tissue stabilization than titanium. Their observation was also corroborated by *in vitro* adhesion, spreading and proliferation of fibroblasts, and osteoblast showing that zirconia-coated titanium improves all three cell parameters for both cellular types. However, in humans, with this one time surgical intervention, the collar composed of zirconia is not directly in contact with bone tissue compared with titanium but is closer to soft tissue. On that basis, the authors demonstrated an improvement of biocompatibility of zirconia.

According to this very recent research, it can be concluded that surface roughness and thus the "finishing" (polishing) of zirconia is of major importance for osseointegration of this biomaterial. However, for humans, available hybrid systems for dental implantology composed of titanium and zirconia collar, would improve the periointegration by preserving both mucosal and bone levels.

ZIRCONIA: A MATERIAL OF CHOICE FOR REDUCED BACTERIAL COLONIZATION

The mouth being a humid milieu, with a practically constant temperature of 36.6°C, offers a multitude of ecological niches for the buccal flora. This flora is essentially composed of commensal microorganisms whose abundance and virulence are individually dependent and in constant evolution from birth to death.

Different factors influence this buccal flora rich of more than 500 species.[70] The adhesion capacity of bacteria that are able to secrete a slime layer or glycocalix mainly composed of extracellular insoluble polysaccharides and is of major importance. Composition of the saliva, the anaerobiosis, the diet (acting on pH variations), and the immune system constitute the other major factors by which fluctuations generate changes in quantity and quality of the buccal flora. Thus, this flora should be considered as a dynamic and complex ecosystem in a dynamic equilibrium between adhesion capacity of microorganisms and the removal forces active in the mouth. This microbial community is able to constitute an open architecture similar to other biofilms with channels and voids and constitutive of the dental plaque.[71] Teeth, crowns, fixed partial dentures, or endosseous implants provide nonshedding surfaces facilitating the formation of thick biofilms generally in equilibrium with the host. However, loss of control (accumulation/metabolism) of these biofilms on such surfaces is the main source of dental pathologies (i.e., gingivitis, periodontitis, peri-implantitis, or stomatitis) and failure in implantology. The adhesion process is reviewed in detail by Teughels and coworkers[70] and is regarded either as a biochemical or physicochemical point of view dependent on the material characteristics and surface topography. The authors highlight that a surface roughness (above the Ra threshold of 0.2 μm), surface free energy (wettability), and chemical composition *per se* of the biomaterial are dominant factors influencing the formation of biofilms at the supra- and subgingival levels of restorative materials.

The microflora around implants, being similar to that of natural teeth, microbial pathogens (i.e., *Actinobacillus actimomycetemcomitans*, *Porphyromonas gingivalis*, or *Prevotella intermedia*) associated with periodontitis, may also contribute to implant failure.[72] The adhesion/colonization of bacteria on titanium has already been described both *in vivo* and *in vitro*.[73–78] One major conclusion from these studies was that the degree of colonization of titanium implants was highly related to surface roughness and that surface irregularities facilitate plaque accumulation *in vivo*. Mabboux et al.[79] using two saliva-coated titanium implant materials and two streptococci bacterial strains (hydrophilic and hydrophobic) confirmed that the physicochemical properties of oral bacterial strains play an important role in bacterial retention to implant material in the presence of adsorbed proteins. Unfortunately, no comparative study is at present available for zirconia. Other authors[50,80] reported the use of hard titanium coating (TiN & ZrN) for the analysis of microbial adhesion and colonization. They concluded that the use of titanium nitride coating on titanium implant can reduce bacterial colonization and proved that ZrN is the most efficient. Reduction observed is probably important in the decrease of inflammation of peri-implant soft tissues. Recently, Zhou and coworkers[81] open a new way of reducing bacterial adhesion by specific grafting of titanium. These reports show that improvements of titanium implant surface are under constant investigation, particularly for reducing the adhesion of oral bacteria, potentially harmful in peri-implant areas.

However, with the emergence of zirconia, sometimes used in orthopaedics and in dental implant market, only

few studies investigated the adhesion capacity and/or colonization of oral bacteria on this biomaterial. Rimondini et al.[82] analyzed comparatively to titanium the inhibition of growth and adhesion (slime production) of selected oral bacteria *in vitro* on zirconia (Y-TZP) and concluded that differences in adhesion could be observed for some of the selected bacteria. In the *in vivo* part of this study, the authors used discs of zirconia or titanium (with approximately the same roughness) fixed onto the buccal region of the molar and premolar in volunteers for 24 hours. The SEM analysis enables them to conclude that both zirconia-tested surfaces accumulated significantly fewer bacteria than Ti one. Moreover, the prevalence of cocci, few short rods, and no long rods on ZrO_2 (Y-TZP) surfaces were suggestive of an immature plaque. So, the early colonization of zirconia is reduced when compared with titanium and would conduce to immature plaque. A human follow-up of commercially pure titanium and zirconium oxide discs (Y-TZP) conducted by Scarano and coworkers[83] focused on the early bacterial adhesion to these surfaces. The authors demonstrated that the early adhesion/colonization of bacteria on zirconia surfaces was significantly reduced comparatively with titanium one. It was finally suggested that this result probably lies in the superficial structure of zirconium oxide (i.e., its electric conductivity). Recently, Scotti et al.[84] evaluated in a pilot study the effect of glazing and polishing Y-TZP ceramic on early dental plaque formation but also the effect of dental hygiene by brushing to reduce bacterial deposits. The authors could not show significant difference in bacteria presence between polished and glazed ceramics. However, the glazed surface accumulated more bacteria probably because of irregularities in the surface and in possible relation with their other observation that brushing did not significantly reduce the bacterial cell count on this surface.

Infection in implantology could be one of the two paramount factors of implant failure as reviewed by Bowen-Antolin and coworkers.[85] To date, occlusal overloading is the second cause of failure. The literature concerning infectious disease in implantology is rich. Infections could occur before, rarely during, and postoperatively. Various factors should also been considered such as immunological state of patient, diseases, and traumatic and elapsed time of intervention. Contradictory results have been obtained with antibiotherapy pre- or post-operatively. However, Quirynen et al.[86,87] indicate that the use of chlorohexidine or other single washes can efficiently reduce the number of germs present in the mouth.

In the light of the small number of reports on bacterial adhesion/colonization of zirconia surface, it can be concluded that zirconia would be able to reduce the bacterial charge on this surface. However, a need for complementary studies is currently observed. Moreover, the huge amount of investigations on improvements of titanium surface for this purpose (adhesion/colonization) should serve as template for zirconia evolution.

CONCLUSIONS

Titanium, as a biomaterial of choice, has been and is still largely employed in dental implantology. However, its corrosion products and individual sensitivities to it[88] are still controversial. A huge amount of researches involving biocompatibility, improvement by coatings for osseointegration, bacterial adhesion, or infectious diseases in implantology with titanium has also been engaged in the last two decades. The question arising about titanium use and zirconia (Y-TZP) slowly takes importance in the "new generation" of dental implants, particularly in hybrid systems. Studied for its biocompatibility, osseointegration, and bacterial adhesion/colonization revealed the interesting properties of this ceramics as outlined in this review. In brief, zirconia has been proved to be biocompatible *in vitro* and *in vivo*; it has very interesting microstructural properties; and it is osseoconductive. Physical and chemical treatments of zirconia were shown to largely influence its soft tissue interactions (mainly fibroblastic ones). Moreover, few studies highlighted that zirconia and its derivatives (ZrN) have the capacity to reduce plaque on implant and surrounding tissues and consequently should be important in soft tissue healing and implant success at bone level. It probably avoids the resorption of peri-implant bone as well. Finally, the capacity of zirconia to be colored to match natural teeth tint[17,89] appeared to be a beneficial property compared to titanium in aesthetical demanding regions.

Various studies also tried to improve adhesion to cells or osseointegration in animal models by *in vitro* coating of either zirconia or titanium. This strategy of material improvement is exciting for *in vitro* studies or in animal models as there is until now a trend to improve the deposition methods.[90,91] However, another futurist strategy of dope osseointegration or periointegration would be grafting of extracellular matrix proteins or growth factors, which could accelerate the healing and anchoring of these biomaterials. This strategy would also go through an improvement of knowledge as regards gene expression profile of biomaterials with cells in contact. This was started by Sollazzo and coworkers,[65] thanks to DNA microarrays. Furthermore, periointegration is related to roughness, wettability of the biomaterial, and also the tissue in contact. These factors seem to influence bacterial adhesion and further on healing and stability of implant. The future of dental implantology should aim at developing a serious codification of production zirconia processes to get surfaces with controlled and standardized topography or chemistry. This approach will be the only way to understand the interactions between proteins, cells and tissues, as well as implant surfaces. This strategy should ultimately enhance the osseointegration process of dental implants for their immediate loading and long-term success. Finally, new zirconia-based composite bioceramics are under investigation, that is, hydroxyapatite-zirconia[92] or titania-Y-TZP[93] graded

for their biocompatibility. They would perhaps open new developments in implantology biomaterials.

Since 2004, all zirconia implants have been distributed and certified on the market. A need for references concerning resistance to failure in long-term clinical trials is of paramount importance for such systems.[94]

Until now, a good compromise to improve the periointegration of biomaterials appeared to be the use of hybrid systems (i.e., composed of titanium screw and zirconia collar[69]) using the pre-cited properties of zirconia. However, a larger amount of data should be collected to confirm the putative superiority at long term of such "hybrid systems" in dental implantology.

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