

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,500

Open access books available

136,000

International authors and editors

170M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Ceramic Biomaterials for Dental Implants: Current Use and Future Perspectives

Federico Mussano, Tullio Genova, Luca Munaron,
Maria Giulia Faga and Stefano Carossa

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/62701>

Abstract

Although titanium implants have the longest traceable record of predictable clinical performance and by far the widest diffusion in the market, some drawbacks have been recently pointed out. Titanium is not a completely bioinert material, since it may elicit allergic reactions and is capable to diffuse not only within the adjacent tissues, which is proven by the elevated concentrations found in peri-implant bone and regional lymph nodes, but also systemically. Ceramic materials for oral application have been used for 40 years. Presently, the material of choice is yttria-stabilized tetragonal zirconia, which presents excellent mechanical and tribological properties together with biocompatibility. Concerns remain about the long-term durability of the material, owing to the report of *in vivo* failures that were caused by the low-temperature degradation of zirconia. To address this issue, research has developed improved oxide-based materials such as alumina–zirconia composites along with non-oxidic ceramics such as silicon nitride.

The proposed book chapter deals with the above-mentioned improved ceramic materials, based on both scientific literature and the authors' direct experience. Particular emphasis is given to the major achievements attained so far in terms of the biological response supported by the interface. Original *in vitro* data regarding alumina-toughened zirconia (ATZ), zirconia-toughened alumina (ZTA), and silicon nitride (Si_3N_4) samples with different surface modifications are shown. Accurate surface characterization was achieved recurring to scanning electron microscopy, non-contact optical profilometry. Protein adsorption on the surface was determined. A mouse pre-osteoblastic cell line, that is MC3T3-E1, was used to examine cellular adhesion and morphology. Viability and proliferation rate of MC3T3-E1 cells were assessed with proper chemiluminescent kits. Cell differentiation was obtained in terms of calcium deposition within the extracellular matrix and quantification of keynote osteogenic markers. Data were analyzed by GraphPad Prism6. For the first time, the behavior of osteoblasts cultured on ATZ and ZTA that underwent a patented hydrothermal treatment was reported. Also, two different surfaces of Si_3N_4 were compared. MC3T3-E1 cells could properly spread in all the

experimental conditions tested. The proliferation rate was consistent with that expected for biocompatible materials. Hydrothermally treated ATZ samples and Si_3N_4 rough surfaces were capable to enhance the osteogenesis in vitro. The biological responses induced in MC3T3 cells were correlated with the surface features. Immediately after seeded, osteoblasts are known to interact with their substrate via integrins that bind to the proteins adsorbed on the biomaterial surface. The interface effect was discussed in light of the literature. The most recent publications suggest that research aims at investigating the effects of surface modifications dictating the chemical characteristics and the nano-/micro-topography that are paramount modulators of the biological response.

Keywords: surface roughness, dental implants, ceramic materials, surface modifications, interface

1. Introduction

Modern oral implantology has been based on titanium since the research line originated by Brånemark's first discovery and subsequent experiments [1]. Titanium implants have the longest traceable record of predictable clinical performance with a cumulative success rate of 98.8% for 15 years [2]. High biocompatibility, favorable tissue response and adequate strength and corrosion resistance rendered titanium implants widely diffused in the market. The number of dental implant brands grew from 45 systems in 1988 [3] to 600 systems produced by 146 manufacturers in 2008 [4]. Currently, worldwide, there are more than 350 dental implant manufacturers producing an estimate of 1600 different systems, 98% of which are titanium implants. Titanium, however, is no longer considered a completely bioinert material, instead it might be an allergen as reported by several studies [5–8]. Elevated titanium concentrations have been found in the vicinity of oral implants [8], in regional lymph nodes [9], serum and urine [10], which is potentially hazardous to human body. Besides these issues, some dental patients are metal-phobic and demand to be treated solely with metal-free dental implants [11].

Only recently, truly viable alternative materials were proposed to titanium, although the first ceramics for oral applications dated back to the 1970s. Historically, indeed, high-density, high-purity aluminum oxide (alumina) was chosen for dental implant manufacturing, as it combined excellent corrosion resistance, good bio-compatibility, high wear resistance, and high strength. Despite these promising features, the material was brittle and prone to fracture under unfavorable load. Thus, the positive preclinical and clinical outcomes of the first studies could not prevent alumina implant systems to be withdrawn from the market [12]. Research and manufacturing technology have greatly improved the offer of bio-ceramics, thanks to the introduction of yttria-partially stabilized tetragonal zirconia polycrystals (Y-TZP), whilst a possible future use of alumina zirconia composites and silicon nitride–titanium nitride composites may further expand the offer of reliable devices on the market. Three distinct sections of the present chapter are dedicated to each of these materials. Specifically, the literature regarding zirconia was thoroughly revised in Section 2, whilst some novel data of our group are exposed and discussed in light of and along with previous work as for alumina

zirconia composites (Section 3) and silicon nitride–titanium nitride non-oxidic ceramics (Section 4).

2. Yttria-partially stabilized tetragonal zirconia polycrystals (Y-TZP)

2.1. Material features

Zirconia (ZrO_2) is a crystalline dioxide of zirconium: as thoroughly reviewed elsewhere [12], unalloyed zirconia can assume three crystallographic forms depending on the temperature, at ambient pressure. At room temperature and upon heating up to 1170°C , the symmetry is monoclinic ($P2_1/c$). The structure becomes tetragonal ($P4_2/nmc$) between 1170 and 2370°C and cubic ($Fm\bar{3}m$) above 2370°C and up to the melting point [13]. Upon cooling at $\sim 950^\circ\text{C}$, during the transformation from the tetragonal (t) phase to the monoclinic (m) phase, a substantial increase in volume ($\sim 4.5\%$) occurs, which is sufficient to lead to catastrophic failure. By alloying pure zirconia with stabilizers such as calcium oxide (CaO), magnesium oxide (MgO), yttrium oxide (Y_2O_3), or cerium oxide (CeO_2), the tetragonal structure is maintained, even at room temperature, and the stress-induced $t \rightarrow m$ transformation is controlled, efficiently arresting crack propagation [14, 15]. Indeed, when a crack develops, tetragonal grains convert immediately to monoclinic form. The propagation of the crack develops sufficient stress within the tetragonal structure to transform also the grains around the crack to stable monoclinic form. Thus, the expansion volume of zirconium dioxide crystals produces compressive stress around the crack and prevents further propagation of crack [16–18]. This mechanism is known as transformation toughening and is influenced by temperature, vapor, particle size, micro- and macrostructure, and concentration of stabilizing oxides [19].

Yttria-stabilized zirconia (Y-TZP) [20] is endowed with excellent mechanical, and tribological properties together with biocompatibility and rightly represent a good choice for preparing dental implants. As yttria decreases the driving force of the t-m transformation [21, 22], biomedical grade zirconia are usually stabilized with 3 mol% yttria (Y_2O_3) (hence 3Y-TZP) [16]. The salient mechanical properties of Y-TZP are reported in **Table 1**, but it is noteworthy that the Weibull modulus is strongly dependent on the type of surface finish and the processing conditions [23].

Notwithstanding the excellent mechanical properties of Y-TZP [19, 24], recent reports of in vivo failures [25–27] have questioned the long-term stability of the material. The low-temperature degradation (LTD) of zirconia [22, 28–31], also known as aging process, plays here a fundamental role. Involving the $t \rightarrow m$ transformation, LTD can be favored, even at room temperature, by the penetration of water radicals into zirconia lattice, thus leading to the formation of tensile stresses in zirconia surfaces. The activation barrier for the transformation is lowered, and the phase transition is promoted. The main consequences of this aging process include surface degradation with grain pullout, microcracking, and strength degradation. As reported by Cattani-Lorente et al. [32] also, Young's modulus and hardness of Y-TZP bars were reduced by 30%, when they were subjected to hydrothermal cycling. The increase of mono-

clinic–tetragonal phase ratio was associated with microcracking and resulted responsible for the decline in mechanical parameters [32].

To control the aging phenomenon, several factors can be taken into account: from the obvious use of stabilizers to the modulation of residual stress [33]. Likewise, adjusting crystal size and removing impurities during manufacturing was proposed with the same anti-aging scope [34]. Interestingly, surface finishing could affect the aging kinetics of 3Y-TZP, according to Deville et al. [35]. More precisely, rough polishing produced a compressive surface stress layer beneficial for the aging resistance, whilst smooth polishing lead preferential transformation nucleation around scratches, due to elastic/plastic damage tensile residual stresses.

In an extensive review of his, Jerome Chevalier concluded that “although in the 1990s, 3Y-TZP ceramics were considered very promising materials for biomedical applications, long-term follow-up is needed to address the critical problem of aging in vivo. Moreover, most zirconia implants were processed at a time when aging was not yet fully understood. Methods to assess a priori the aging sensitivity of a given zirconia ceramic have been developed and should lead to safer implants. In the meantime, new zirconia or zirconia-based materials that overcome the major drawback of the standard 3Y-TZP are now available” [22].

2.2. Manufacturing methods

Hot isostatic press (HIP) is the most common method used for preparing zirconia dental implant. By subjecting encapsulated powder, or sintered yet porous parts, to inert gas at isostatic pressure at a high temperature, HIPing is deemed an excellent method to obtain high-density homogenous products [36]. HIPing enables the application of an equally distributed pressure in all directions resulting in greater material uniformity and higher strength [2]. HIPing of Y-TZP enhances the strength, eliminates fracture sources such as pores, and reduces the aging phenomenon [37]. The preparation entails many steps as summarized in **Figure 1**. Briefly, Y-TZP blocks are presintered at temperatures below 1500°C to reach a density of at least 95% of the theoretical density. Hot isostatic pressing (HIP) is applied to the blocks at temperatures between 1400 and 1500°C under high pressure. A HIP cycle after sintering is recommended to achieve a full density close to the theoretical values ($d = 6.1 \text{ g/cm}^3 = 100\%$ dense). Since HIPing changes the color of Y-TZP into dark-grey, a heat treatment in air is usually performed to restore the material whiteness by oxidation, prior to be machined using a specially designed milling system. Because of the high hardness of fully sintered Y-TZP, the milling system is to be particularly robust [38–40].

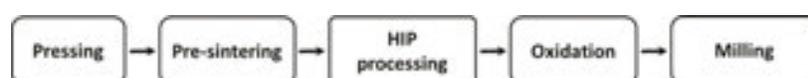


Figure 1. Manufacturing process for Y-TZP.

The relatively recent and yet pervasive introduction of computer-aided design/computer-aided manufacturing (CAD/CAM) technology has provided dentistry with an alternative to HIPing [42]. Usually, dental CAD/CAM systems recur to partly sintered yttria-stabilized

tetragonal zirconia polycrystal (Y-TZP) blanks. The use of this partly sintered state of the Y-TZP ceramic renders the milling process faster and reduces the tools wear, compared to systems employing densely sintered blanks (HIP process). Of course, the final sintering shrinkage must be taken into account during the CAD phase by enlarging the shapes before milling, whilst this compensation is not necessary with the HIPed Y-TZP blanks that are directly ground to the desired dimensions [5]. The salient mechanical properties of Y-TZP subjected to the two manufacturing work-flow described above are compared in the following [43] table (**Table 1**), along with pressed and sintered polycrystalline α -alumina.

	HIPed Y-TZP	Pressure-less sintered Y-TZP	Pressed and sintered polycrystalline α -alumina
Density (g/cm ³)	6.1	6	4
Microhardness (Vickers)	1000–1300	1000–1200	2300
Young's modulus (GPa)	200	200	420
Bending strength (MPa)	1200	800	500
Toughness KIC (MPa m ^{1/2})	9–10	9–10	4

Table 1. Values refer to Duraccio et al. [12].

2.3. Biological properties

In vitro experiments on different cell lines, in vivo studies on animals and clinical studies on humans supported the safety and the high level of biocompatibility of zirconia. In a preliminary in vitro investigation [44], one-piece zirconia implants were proven to possibly fulfill the biomechanical requirements for anterior teeth restoration. In addition, the mean fracture strength of zirconia implants was investigated after chewing simulation and it was found to be within the limits of clinical acceptance. However, the preparation of a one-piece zirconia implant prior to prosthetic finalization may significantly compromise fracture strength. Therefore, long-term clinical data were deemed necessary before one-piece zirconia implants could be recommended for clinical practice [45]. For the same reason, two-piece zirconia implants were considered clinically inadequate due to the increased risk of fracture at the implant head level [46].

Evidence from in vitro studies on osteoblasts supported the possible favorable response of zirconia ceramics in vivo [47, 48]. When implanted in bone or soft tissues, these materials could elicit no inflammatory reactions, nor fibrous encapsulation, according to Hisbergues et al. [41]. Interestingly, Scarano et al. [49] reported the osseointegration of unloaded zirconia implants inserted in rabbit bones without any signs of inflammation or mobility. The possible role of surface roughness was investigated by comparing the removal torque of machined zirconia implants to roughened ones [50]. Notably, the roughened implants performed better than the smooth ones and behaved similarly to the oxidized titanium implants used as control. Loaded zirconia implants were studied and compared to titanium implants by Kohal and co-workers [51], who could find no significant difference in the osseointegration level between the two

groups. Akagwa et al. [52] reported a similar bone to implant interlock in loaded and unloaded zirconia implants, but a crestal bone loss higher around the former group. In favor of the clinical use of Zirconia, it must be cited its maintenance of bending strength of over 700 MPa after immersion in 95°C saline solution for over 3 years [53]. Furthermore, zirconia blanks did not show any significant mechanical detriment even after being embedded in the medullary cavity of the tibia of rabbits for 30 months.

Scarce are the clinical studies dedicated to the long-term performance of zirconia implants. The short follow-up period and the often small sample size hinder their quality of evidence, so that Andreiotelli and coauthors [29] could only include three retrospective cohort studies on one-piece zirconia dental implants in their systematic review, reaching in total 231 patients and 416 implants. The studies by Mellinghoff et al. [54] and Oliva et al. [55] investigated, respectively, 189 and 100 zirconia implants and estimated 1-year survival rates of 93 and 98%. Almost all of the failures occurred during the healing phase, as only one implant failed after prosthetic reconstruction due to fracture. Lambrich and Iglhaut [56] observed 127 zirconia and 234 titanium implants for a mean period of 21.4 months. Notably, in this study, the survival rate of zirconia implants was similar to that of titanium in the mandible (Y-TZP = 98.4% vs. Ti = 97.2%), whilst differed considerably in the maxilla (Ti = 98.4% vs. Y-TZP = 84.4%). Again, all failures occurred during the healing phase owing to increased implant mobility. These findings are consistent with the paper by Depprich et al. [57], where the survival rate of zirconia implants obtained from 17 clinical studies was between 74 and 98% after 12–56 months. Payer et al. [58] followed up for 2 years 19 immediately loaded zirconia implants, reporting a 95% survival rate, as determined clinically and radiographically. These results are in accordance with Oliva et al. [59] who determined the same survival rate at 5 years in 371 patients who received 831 one-piece zirconia implants. Kohal et al. [60] found that immediately restored one-piece zirconia implants have 1-year cumulative survival rate comparable to titanium counterparts. In conclusion, the clinical data currently available for Y-TZP implants may not be sufficient to recommend their routine clinical use. Zirconia, however, may have the potential to be a successful implant material, although this is as yet not fully supported by present investigations and further good-quality research is needed.

3. Zirconia-toughened alumina (ZTA) and alumina-toughened zirconia (AZT)

3.1. Background

The demand of structural ceramics has led to an increased interest in Alumina–Zirconia composites for biomedical [21, 61] and dental implant application [62, 63]. Two composite materials can be prepared: ZrO₂ reinforced with alumina particles, which is denominated alumina toughened zirconia (ATZ), and Al₂O₃ reinforced with zirconia particles, which is known as zirconia-toughened alumina (ZTA). Thus, higher fracture values can be reached if compared with the monophase ceramics [64] (**Table 2**).

	ATZ	ZTA
Hardness (GPa)	15.3 ± 0.9	21.3 ± 1.5
Young's modulus (GPa)	245 ± 9	363 ± 5
Bending strength (MPa)	633 ± 127	441 ± 24
Toughness KIC (MPa m ^{1/2})	7.1 ± 0.1	3.9 ± 0.05

Table 2. Physical and mechanical properties of ATZ and ZTA data are extracted from Faga et al. [85].

These composites benefit from combining the characteristics of Alumina, namely the high hardness and stiffness, with the superior strength and toughness of Zirconia, which improves remarkably the resistance to crack growth [65]. In addition, alumina increases the hydrothermal stability of tetragonal Zirconia phase [65, 66], owing mainly to the formation of a stiff matrix capable to keep the Zirconia particles in a metastable tetragonal state [67], thus acting as mechanical stabilizer. The only commercially used ATZ oral implant was tested both statically and dynamically for its fracture resistance in different simulated oral conditions with satisfying results [68].

The main features of the aforementioned implant are reported in the following table (**Table 3**).

	ATZ
Density (g/cm ³)	5.5
Average grain size (µm)	<0.5
Microhardness (Vickers)	1000–1200
Young's modulus (GPa)	220
Bending strength (MPa)	2000
Toughness KIC (MPa m ^{1/2})	8

Table 3. Physical and mechanical properties of ATZ values refer to Spies et al. (2015) – [68]

Very recently, a complete powder injection molding process was developed to fabricate cylindrical ZTA parts recurring to a binder system made of high-density polyethylene, paraffin wax, and stearic acid. The effects of sintering temperature on shrinkage, relative density, and hardness of the sintered part were taken into account and proved the technology suitable for the production of ZTA parts with sufficient mechanical properties [69]. However, ATZ and ZTA are usually produced through the classic workflows described above for Y-TZP (see Section 2.2).

The favorable mechanical features and the biological safety of different ZTA and ATZ composites have been the object of several studies in the last years [64, 70–78]. Whilst ATZ materials show increased mechanical stability [79] and improved aging resistance versus Y-TZP, still they exhibit a certain degree of aging [79]. ZTA materials display much better aging resistance than both monolithic Y-TZP and ATZ [21, 79, 80]. In a recent work by our research

group, both ATZ and ZTA were functionalized with two laminins as a preliminary investigation for improving soft tissue healing around implants. The simple adsorption of these two different isoforms was sufficient to induce some of the most important cell kinases in the epithelial cells grown on the surface of the two Alumina–Zirconia composites, supporting the possible advantages of these materials in dental implantology [81]. On this basis, we further studied the behavior of ATZ dental implants treated with a patented hydrothermal process, comparing them to a clinical use titanium surface in a minipig model. Bone healing was assessed through histology and mRNA expression at different time points (8, 14, 28, and 56 days). The most interesting outcome was a statistically significant higher percentage of newly formed bone along ATZ implants, at 56 days, suggesting that the tested material proved to be a promising candidate among the possible ceramic dental implants [82]. Interestingly, by comparing the bone-to-implant contact of moderately roughened ATZ implants ($S_a = 1.51 \mu\text{m}$) to an anodized titanium standard ($S_a = 1.31 \mu\text{m}$) in Sprague–Dawley rats, Kohal et al. [83] found that titanium greatly (58%/75%) outperformed the ceramic implant (24%/41%) after a healing period of 14 and 28 days. In addition, at the same time points, the mechanical interlock measured as push-in values increased from 20 to 39 N for titanium and from 10 to 25 N for ATZ. Although the moderately roughened ATZ implants were well accepted in rat bone, their osseointegration process seemed to proceed more slowly than that of anodized titanium.

However, the concerns raised in light of the *in vivo* data reported by Kohal et al. [51] seemed not to be completely consistent with the promising outcomes of the clinical study conducted by the same research group. Indeed, the cumulative survival rate (94.2%) of one-piece ATZ implants immediately restored with partial fixed prostheses was comparable to that of the loaded titanium implants, in a human clinical trial involving 40 patients after 3 years of observation. In addition to the marginal bone loss (0.79 mm), several soft tissue parameters and patient-reported outcome measures were evaluated suggesting the potential of ATZ for clinical utilization [84]. Notwithstanding the clinical use, little information is still available about the ideal surface treatment that a ceramic dental implant should receive. To better understand whether roughness or hydroxyapatite precipitation capability were more likely to be efficient in terms of surface modifications, we designed a simple *in vitro* pilot study.

3.2. Material and methods

3.2.1. Sample preparation

Two high purity, ready-to-press powders were used to produce the ATZ (ZrO_2 -20 wt% Al_2O_3 , TZ-3Y20AB, Tosoh, Japan) and ZTA (Al_2O_3 -16 wt% ZrO_2 , Taimicron, Taimei, Japan) samples. As reported elsewhere [85], specimens were prepared through linear pressing at 80 MPa followed by cold isostatic pressing at 200 MPa. The process parameters for sintering were as follows: heating up to 700°C at a rate of 50°C/h, followed by a 2-h dwell; heating up to 1500°C at a rate of 100°C/h, followed by a 2-h dwell. The resulting fully dense materials were 12-mm disks with thickness ranging between 4 and 5 mm.

As reported in the diagram below, both ATZ and ZTA discs were either mirror polished with diamond suspension in ethanol with decreasing granulometry to the final surface roughness

of <1 micron. Also as-fired samples were used to evaluate the influence of the surface roughness on the biological response. Subsequently, the samples were either bioactivated with phosphoric acid under hydrothermal conditions (patent numbers: TO2012A000029 and PCT/IB2013/050425) or left untreated (**Figure 2**).

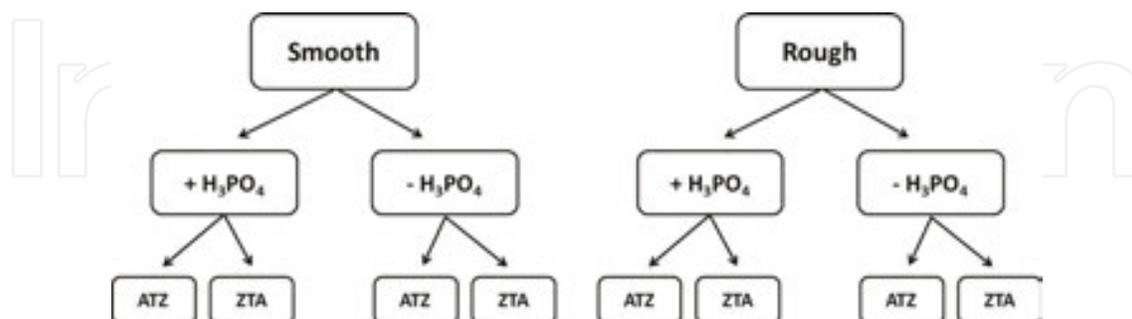


Figure 2. Schematic representation of samples treatments.

3.2.2. Surface characterization

Microstructure was studied by means of a scanning electron microscope Zeiss EVO 50 with energy dispersion spectroscopy analyzer for elemental composition detection. Surface roughness was measured with a non-contact profilometer, Talysurf CCI 3000A. The tests were performed in an air-conditioned laboratory, where temperature is kept at 20°C, on a representative surface of 90 μm^2 . To quantify the amount of protein adsorbed, fetal bovine serum (FBS) was diluted in phosphate-buffered saline (PBS) at a concentration of 2% and was used to incubate the samples at 37°C for 30 min. After two wash in PBS, the adsorbed protein was eluted from the disks using Tris Triton buffer (10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10% Glycerol, and 0.1% SDS) for 10 min. Finally, the total protein amount was quantified using Pierce™ BCA Protein Assay Kit (Life Technologies, Milan, Italy) following the manufacturer's instructions.

3.2.3. Biological response

Pre-osteoblastic murine cells MC3T3-E1 (ECACC, Salisbury, UK) were used to characterize the biological response in vitro. Cells were maintained in alpha MEM supplemented with 10% FBS (Life Technologies, Milan, Italy), 100 U/ml penicillin, 100 mg/ml streptomycin, under a humidified atmosphere of 5% CO₂ in air, at 37°C. To prevent contact inhibition, cells were always passaged at subconfluency. When required, to differentiate MC3T3 cells, the culture medium was supplemented with 10 mM β -glycerophosphate and 50 $\mu\text{g}/\text{ml}$ ascorbic acid.

To examine cell morphology, MC3T3 cells were seeded at a concentration of 5000 cells/well in a 24-well plate. After 1 day, cells were fixed in 4% paraformaldehyde in PBS. Rhodamine-Phalloidin and Dapi (Life Technologies, Milan, Italy) were, respectively, used to stain cytoskeleton and cell nuclei, thus evaluating cell adhesion and morphology.

Alkaline phosphatase activity was quantified using the Alkaline Phosphatase Assay Kit (Abcam, Cambridge, UK). Following the manufacturer's instruction, the OD was measured at a wavelength of 405 nm. The calcium deposited within the extracellular matrix was quantified colorimetrically through the Calcium Assay Kit (Cayman Chemical, Michigan, USA). Absorbance of the lysates was measured at 570 nm.

Data were analyzed recurring to GraphPad Prism6 (GraphPad Software, Inc., La Jolla, CA, USA). Each experiment was repeated at least three times. Statistical analysis was performed using the Student t-test. A p value of <0.05 was considered significant.

3.3. Results and discussion

The success of dental implants is directly related to the bone implant interlock, which can be experimentally evaluated in animal living bone, by histomorphometry and/or biomechanical testing [50]. A moderately rough surface topography is known to positively affect the interfacial tissue reaction [86]. Surface modification of zirconia and its composites is, however, challenging. Among the roughening techniques used to attain proper bone-implant interfaces, it is convenient to remember the apposition of sintering particles, nano-technology, sandblasting and acid etching, and laser technology [50, 87–90]. In recent animal studies, in vivo evidence was found that alumina-toughened-zirconia is a suitable candidate for dental implantology [82], which was further supported by very recent clinical data at the University of Freiburg [84, 91]. Following Dohan Ehrenfest's classification [92], surface roughness was moderate ($S_a = 1.51 \mu\text{m}$) in case of Ziraldent implants or very high ($S_a = 5.4 \mu\text{m}$) for our research group. Here, the microstructure of ATZ and ZTA was determined by SEM (**Figure 3**).

The materials show an almost defect-free surface, with a homogeneous distribution of both zirconia and alumina. Submicrometric grains are present in both composites, the darkest representing alumina phase. It is noteworthy that similar dimensions can be observed for alumina and zirconia grains only in ATZ material, whilst ZrO_2 growth is inhibited by the predominant alumina content in ZTA composite.

Figure 3. Scanning electron micrographs of ATZ (A) and ZTA (B).

Surface roughness was measured via profilometry (**Table 4**). As it can clearly be seen, polished and as-fired samples were, respectively, endowed with a very smooth and a highly rough surface, according to the expected values.

	ATZ		ZTA	
	Mirror polished	As fired	Mirror polished	As fired
Sa (μm)	0.043990	0.376492	0.051174	0.485283
SD	0.003604	0.018970	0.005554	0.079454

Table 4. Surface roughness measured via profilometry.

Interestingly, from **Figure 4**, it can be inferred that the only condition capable to affect significantly the protein adsorption was surface roughness. No statistically significant difference was found among materials (ATZ vs. ZTA) or chemical treatment (hydrothermal cycle present + vs. absent -), although a trend in facilitating protein adsorption could be noted in roughened-treated surfaces.

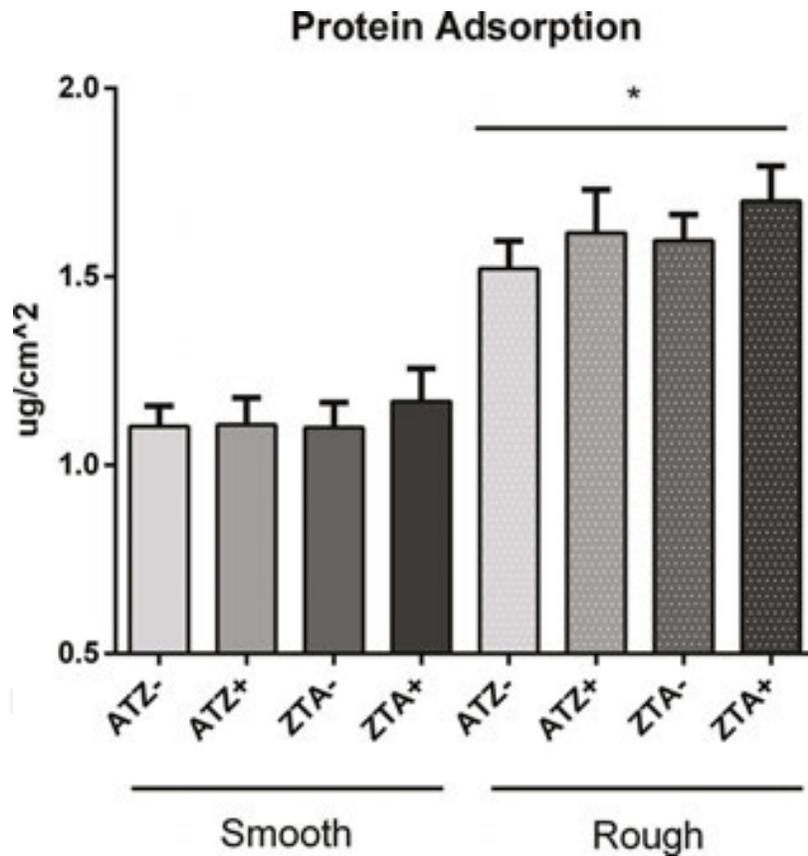


Figure 4. Quantification of adsorbed bovine serum albumin (BSA) by samples, as measured through BCA assay (see Methods). The rough surfaces significantly increase the amount of adsorbed proteins.

The same trend described for protein adsorption was observed in the other cell-based assays whether they were focusing on the early cell response as in the focal adhesion density (**Figure 5**), or they were dealing with intermediate and late stages of osteogenic differentiation in vitro such as alkaline phosphatase activity (**Figure 6A**) and calcium deposition within the extracellular matrix (**Figure 6B**).

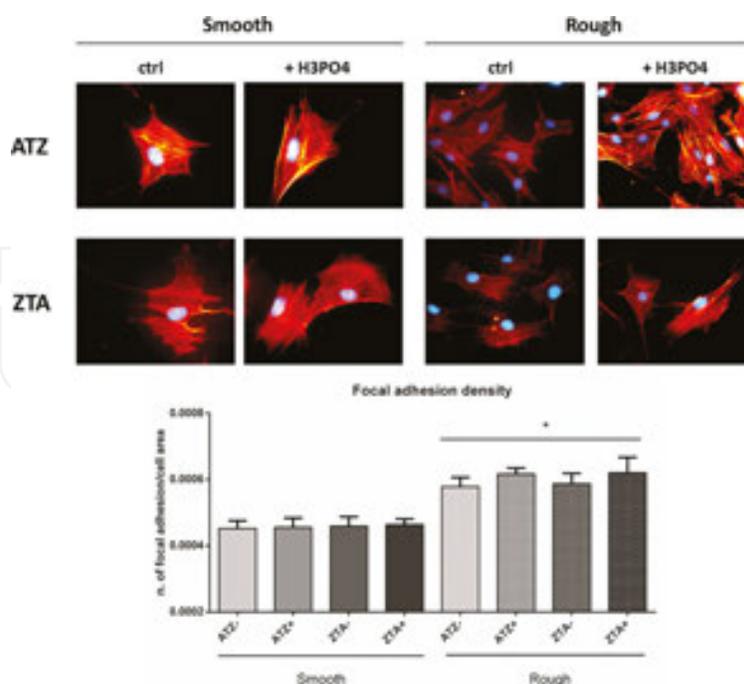


Figure 5. Morphology of MC3T3 cells seeded on different surfaces and stained with phalloidin–rhodamine and DAPI to visualize, respectively, the cytoskeleton and nucleus (see Methods). (A) Quantification of focal adhesion density measured by normalizing the number of focal adhesions on cell area (see Methods). (B) The rough surfaces significantly increase the density of focal adhesion.

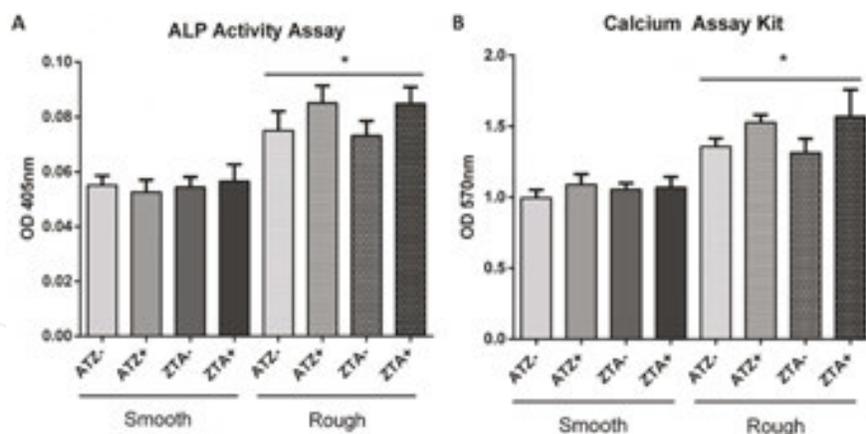


Figure 6. Colorimetric quantification of ALP activity (A) and calcium deposition (B) (see Methods). The rough surfaces significantly increase the level of either ALP activity (A) and calcium deposition (B).

Although surface chemistry is known to play a role in cueing the biological systems [81], the present experimental data showed that roughened surfaces were more efficient in inducing an osteogenic response *in vitro* independently of the application of the chemical treatment. In other terms, roughness *per se* seemed to overpower the effect of the chemical treatment which was deemed bioactive on the ground of the Kokubo tests previously performed (i.e., the capacity to induce hydroxyapatite precipitation) [85]. Within the obvious limits of this

experimental setting, our results support the importance of roughening modifications over the chemical treatment.

4. Silicon nitride–titanium nitride

4.1. Background

Silicon nitride (Si_3N_4) is a high-strength and tough ceramic used as a viable implant material [93–95]. Since the first clinical trial in 1986 [96], over two decades have passed before the introduction of Si_3N_4 to the biomedical market of the US and EU. Since 2008, it has been used as a fusion cage for arthrodesis of the cervical and thoracolumbar spine [97], with few adverse reported events [98]. Silicon nitride has been shown to possess favorable cell interaction characteristics [94, 95, 99–104], along with bacteriostatic properties [105, 106]. Also, porous or unpolished Si_3N_4 osseointegrates with adjacent bone [104, 105, 107–109].

Silicon nitride derives its strength and toughness through its microstructure, which is composed of asymmetric needle-like interlocking grains surrounded by a thin (<2 nm) refractory grain-boundary glass [110]. Unlike other ceramics, no phase transformation is involved. Thus, similar to alumina, Si_3N_4 exists as an irreversibly stable phase at room temperature, but an advancing crack must navigate a high energy path through the ceramic, and bridging grains within the crack wake restrict its continued propagation [111–113].

Industrial standards have been adopted for Si_3N_4 composition, processing, and properties [114, 115]. However, sintered Si_3N_4 is usually machined by hard grinding with diamond tools and the high hardness of Si_3N_4 makes the production of complex shapes through conventional mechanical machining difficult and expensive. To address this issue, electrically conductive reinforcements, such as TiN, TiC, TiB_2 , ZrB_2 , were added to the Si_3N_4 matrix, generating composites suitable to be wrought by electrical discharge machining (EDM) [116]. The EDM has been introduced with encouraging results, achieving complex shapes from dense electroconductive bulks with high densification [94]. Accurate semi-finished Si_3N_4 -TiN surfaces may be either used as they are, or further finished through diamond polishing [116]. Some preliminary data comparing in vitro the osteogenic behavior of two different surface modifications of a silicon nitride–titanium nitride (Si_3N_4 -TiN) composite are here presented. The two surfaces were, respectively, the very product of the EDM process (henceforth Si_3N_4 -TiN_A) and the result of partial polishing with diamond suspensions (henceforth called Si_3N_4 -TiN_B). For material and methods please refer to Sections 3.2.2 and 3.2.3.

4.2. Results and discussion

A detail of the two silicon nitride–titanium nitride surfaces is reported in **Figure 7**.

IntechOpen

Figure 7. Surface electron micrographs of $\text{Si}_3\text{N}_4\text{-TiN}_A$ (A) and $\text{Si}_3\text{N}_4\text{-TiN}_B$ (B) at 10,000 magnifications.

$\text{Si}_3\text{N}_4\text{-TiN}_A$ showed an interesting coalesced structure derived from the melting generated during the manufacturing process, whilst, in $\text{Si}_3\text{N}_4\text{-TiN}_B$ the microstructure of silicon nitride–titanium nitride is clearly appreciable along with the remnants of the peaks after polishing. The tridimensional analysis of $\text{Si}_3\text{N}_4\text{-TiN}_A$ and $\text{Si}_3\text{N}_4\text{-TiN}_B$ is graphically depicted in **Figure 8**, whilst S_a values were, respectively, 2.92 ± 0.07 and $0.88 \pm 0.06 \mu\text{m}$. Thus, $\text{Si}_3\text{N}_4\text{-TiN}_A$ resulted rougher than $\text{Si}_3\text{N}_4\text{-TiN}_B$.

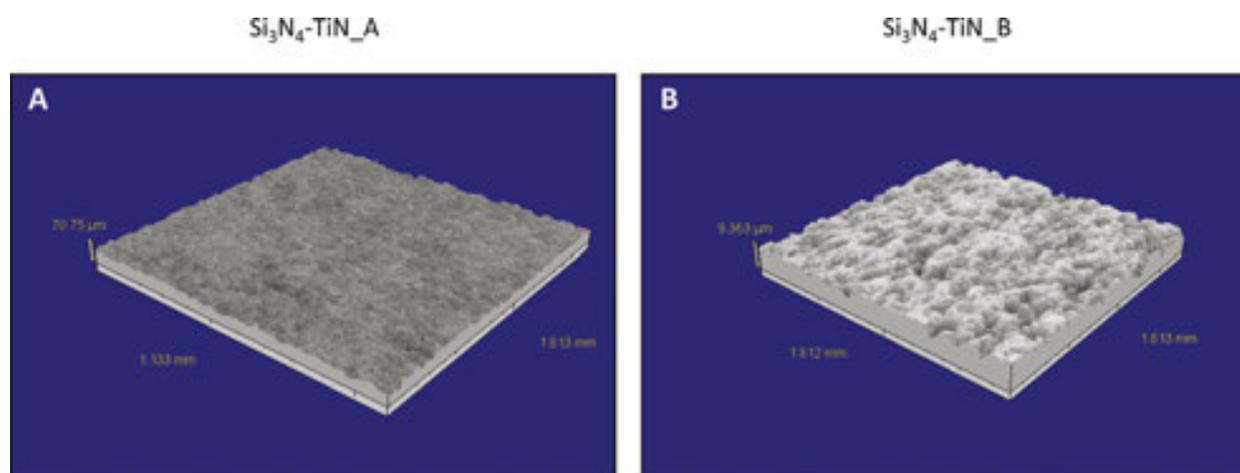


Figure 8. Tridimensional graphical representation of $\text{Si}_3\text{N}_4\text{-TiN}_A$ (A) and $\text{Si}_3\text{N}_4\text{-TiN}_B$ (B).

MC3T3 cells grew well on both samples. Notably, fluorescent images of adherent cells at 24 h (**Figure 9A**) clearly show that $\text{Si}_3\text{N}_4\text{-TiN}_A$ induced a more complex morphology with more tapered shape cells than $\text{Si}_3\text{N}_4\text{-TiN}_B$, as expected for rougher surfaces. Consistently, a higher density of focal adhesions was quantified on the $\text{Si}_3\text{N}_4\text{-TiN}_A$ surface [117] (**Figure 9B**).

IntechOpen

Figure 9. Morphology of MC3T3 cells seeded on $\text{Si}_3\text{N}_4\text{-TiN}_A$ and $\text{Si}_3\text{N}_4\text{-TiN}_B$ and stained with phalloidin–rhodamine and DAPI to visualize, respectively, the cytoskeleton and nucleus (see Methods). MC3T3 cells seeded on $\text{Si}_3\text{N}_4\text{-TiN}_B$ display a more complex shape with a lower spreading level than $\text{Si}_3\text{N}_4\text{-TiN}_A$ (A). Quantification of focal adhesion density measured by normalizing the number of focal adhesions on cell area (see Methods) (B). $\text{Si}_3\text{N}_4\text{-TiN}_B$ significantly increase the density of focal adhesion.

The osteogenic differentiation was evaluated based on the alkaline phosphatase activity as well as the deposition of bone matrix on the specimens. A statistically significant difference between $\text{Si}_3\text{N}_4\text{-TiN}_A$ and $\text{Si}_3\text{N}_4\text{-TiN}_B$ was determined in favor the former, when ALP activity was determined (**Figure 10A**).

IntechOpen

Figure 10. Colorimetric quantification of ALP activity (A) and calcium deposition (B) (see Methods). $\text{Si}_3\text{N}_4\text{-TiN}_B$ surface significantly increase the level of either ALP activity (A) and calcium deposition (B).

The rougher surface promoted a greater osteogenic response than the smooth surface in terms of calcium deposition (**Figure 10B**).

The biological responses induced in MC3T3 cells, a widely diffused osteoblast model, were correlated with the surface roughness, even in this case. The effect of roughness on osteoblast adhesion has been mainly attributed to an increased surface-to-volume ratio that may provide more sites for cell attachment [118]. Consistently, the rougher surface tested ($\text{Si}_3\text{N}_4\text{-TiN_A}$) could promote better cell viability, higher density of focal adhesions and more pronounced calcium deposition than the smoother one ($\text{Si}_3\text{N}_4\text{-TiN_B}$). Taken together, these data confirmed the biocompatibility of silicon nitride–titanium nitride composites in accordance with the literature, which has indeed so far explored preferably the pristine Si_3N_4 material [93, 94, 99, 119]. The possible application of surfaces directly obtained by EDM to $\text{Si}_3\text{N}_4\text{-TiN}$ is therefore noteworthy. Further research should be oriented at investigating the *in vivo* effects of such surface finishing, as well as the importance of the texture in the pattern recognition operated by cells.

5. Concluding remarks

As stated in previous sections, even though titanium and titanium alloys are the material of choice for dental implants, they are not without drawbacks. Among the possible issues, for instance, the hypersensitivity in allergic patients and some aesthetic concerns deserve attention. To address these problems, ceramics have been introduced to the market in the last decades. Y-TZP was first proposed owing to its biocompatibility, white *root-like* color and low plaque affinity. More recently, oxidic composites containing variable amounts of zirconium oxide such as zirconia-toughened alumina (ZTA) and especially alumina-toughened zirconia (AZT) were recently considered an improved alternative to Y-TZP. These implants seem very suitable to replace the anterior teeth to avoid the formation of dark shimmer in the presence of thin gingival biotype. However, one-piece ceramic implants may be more difficult to place than two-pieces titanium implants if angulated abutments are required.

Nevertheless, the demand of non-metallic materials endowed with high mechanical features is prompting research and industry to explore also ceramics such as silicon nitride. This non-oxidic material, whose use is almost completely limited to orthopedics in the biomedical field, possesses really promising quality even for dental application. The possibility to dope silicon nitride with titanium nitride, thus rendering it electroconductive, enables a range of manufacturing processes like the electro discharge machining. This opens compelling perspectives in the future as biomaterials are supposed to be increasingly customizable, maneuverable, and adaptable to the particular necessity of the single case, possibly entering the digital *work-flow*.

Author details

Federico Mussano^{1*}, Tullio Genova^{1,2}, Luca Munaron^{1,2}, Maria Giulia Faga³ and Stefano Carossa¹

*Address all correspondence to: federico.mussano@unito.it

1 CIR Dental School, Department of Surgical Sciences, Turin, Italy

2 Department of Life Sciences and Systems Biology, Turin, Italy

3 IMAMOTER-National Council of Research, Turin, Italy

References

- [1] Albrektsson T, Sennerby L. State of the art in oral implants. *J Clin Periodontol* [Internet]. 1991;18(6):474–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1890231> [cited 2016 Jan 31].
- [2] Lindquist LW, Carlsson GE, Jemt T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. *Clin Oral Implants Res* [Internet]. 1996;7(4):329–36. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9151599> [cited 2016 Jan 31].
- [3] English CE. Cylindrical implants. Parts I, II, III. *California Dent Assoc J*. 1988;16:17–38.
- [4] Jokstad A. How many implant systems do we have and are they documented? In: Jokstad A, editor. *Osseointegration and Dental Implants* [Internet]. Wiley Blackwell; 2009. Available from: http://media.wiley.com/product_data/excerpt/17/08138134/0813813417-1.pdf
- [5] Evrard L, Waroquier D, Parent D. Allergies to dental metals. Titanium: a new allergen. *Rev Med Brux*. 2010;31(1):44–9.
- [6] Pigatto PDPD, Guzzi G, Brambilla L, Sforza C. Titanium allergy associated with dental implant failure. *Clin Oral Implants Res* [Internet]. 2009;20(8):857. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19604283> [cited 2016 Jan 31].
- [7] Sicilia A, Cuesta S, Coma G, Arregui I, Guisasola C, Ruiz E, . Titanium allergy in dental implant patients: a clinical study on 1500 consecutive patients. *Clin Oral Implants Res* [Internet]. 2008;19(8):823–35. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18705814> [cited 2016 Jan 31].
- [8] Koutayas SO, Vagkopoulou T, Pelekanos S, Koidis P, Strub JR. Zirconia in dentistry: part 2. Evidence-based clinical breakthrough. *Eur J Esthet Dent*. 2009;4(4):348–80.

- [9] Onodera K, Ooya K, Kawamura H. Titanium lymph node pigmentation in the reconstruction plate system of a mandibular bone defect. *Oral Surg Oral Med Oral Pathol.* 1993;75(4):495–7.
- [10] Jacobs JJ, Skipor AK, Patterson LM, Hallab NJ, Paprosky WG, Black J, . Metal release in patients who have had a primary total hip arthroplasty: a prospective, controlled, longitudinal study. *J Bone Joint Surg Am.* 1998;80(10):1447–58.
- [11] Bågedahl-Strindlund M, Ilie M, Furhoff AK, Tomson Y, Larsson KS, Sandborgh-Englund G, . A multidisciplinary clinical study of patients suffering from illness associated with mercury release from dental restorations: psychiatric aspects. *Acta Psychiatr Scand* [Internet]. 1997;96(6):475–82. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9421345> [cited 2016 Jan 31].
- [12] Duraccio D, Mussano F, Faga MG. Biomaterials for dental implants: current and future trends. *J Mater Sci.* 2015;50:4779–812.
- [13] E. C. Subbarao, “Zirconia-an overview”; pp. 1–24 in “Science and Technology of Zirconia”, *Advances in Ceramics*, Vol. 3. Edited by A. H. Heuer and L. W. Hobbs. The American Ceramic Society, Columbus, Ohio, 1981
- [14] Heuer AH, Lange FF, Swain MV, Evans AG. Transformation toughening: an overview. *J Am Ceram Soc* [Internet]. 1986;69(3):i–iv. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1986.tb07400.x> [cited 2016 Feb 1].
- [15] Nicholson G. Phase analysis in zirconia systems. *J Am Ceram Soc* [Internet]. 1972;55(6):303–5. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1972.tb11290.x> [cited 2016 Feb 1].
- [16] Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. *Biomaterials.* 1999;20(1):1–25.
- [17] Christel P, Meunier A, Heller M, Torre JP, Peille CN. Mechanical properties and short-term in-vivo evaluation of yttrium-oxide-partially-stabilized zirconia. *J Biomed Mater Res* [Internet]. 1989;23(1):45–61. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2708404> [cited 2016 Jan 14].
- [18] Morena R, Lockwood PE, Evans L, Fairhurst CW. Toughening of dental porcelain by tetragonal ZrO₂ additions. *J Am Ceram Soc* [Internet]. 1986;69(4):C–75–C–77. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1986.tb04756.x> [cited 2016 Feb 1].
- [19] Kelly JR, Denry I. Stabilized zirconia as a structural ceramic: an overview. *Dent Mater* [Internet]. 2008;24(3):289–98. Available from: <http://www.sciencedirect.com/science/article/pii/S0109564107001121> [cited 2015 Aug 25].
- [20] Piconi C, Burger W, Richter HG, Cittadini A, Maccauro G, Covacci V, . Y-TZP ceramics for artificial joint replacements. *Biomaterials* [Internet]. 1998;19(16):1489–94. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9794524> [cited 2016 Feb 1].
- [21] Deville S, Chevalier J, Fantozzi G, Bartolomé JF, Requena J, Moya JS, . Low-temperature ageing of zirconia-toughened alumina ceramics and its implication in biomedical

- implants. *J Eur Ceram Soc* [Internet]. 2003;23(15):2975–82. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221903003133> [cited 2016 Jan 31].
- [22] Chevalier J, Gremillard L, Deville S. Low-temperature degradation of zirconia and implications for biomedical implants. *Annu Rev Mater Res* [Internet]. Annual Reviews; 2007;37(1):1–32. Available from: <http://www.annualreviews.org/doi/abs/10.1146/annurev.matsci.37.052506.084250> [cited 2016 Jan 31].
- [23] Kosmac T, Oblak C, Jevnikar P, Funduk N, Marion L. The effect of surface grinding and sandblasting on flexural strength and reliability of Y-TZP zirconia ceramic. *Dent Mater* [Internet]. 1999;15(6):426–33. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10863444> [cited 2016 Jan 22].
- [24] Denry I, Kelly JR. State of the art of zirconia for dental applications. *Dent Mater*. 2008;24(3):299–307.
- [25] Clarke IC, Manaka M, Green DD, Williams P, Pezzotti G, Kim Y-H, . Current status of zirconia used in total hip implants. *J Bone Joint Surg Am* [Internet]. 2003;85-A Suppl: 73–84. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14652396> [cited 2015 Sep 17].
- [26] Chevalier J, Gremillard L. Ceramics for medical applications: a picture for the next 20 years. *J Eur Ceram Soc* [Internet]. 2009;29(7):1245–55. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221908004391> [cited 2015 Nov 13].
- [27] Chevalier J. What future for zirconia as a biomaterial? *Biomaterials* [Internet]. 2006;27(4):535–43. Available from: <http://www.sciencedirect.com/science/article/pii/S0142961205007039> [cited 2016 Jan 7].
- [28] Deville S, Guénin G, Chevalier J. Martensitic transformation in zirconia. *Acta Mater* [Internet]. 2004;52(19):5709–21. Available from: <http://www.sciencedirect.com/science/article/pii/S1359645404005269> [cited 2016 Feb 1].
- [29] Deville S, Chevalier J. Martensitic relief observation by atomic force microscopy in yttria-stabilized zirconia. *J Am Ceram Soc* [Internet]. 2003;86(12):2225–7. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.2003.tb03639.x> [cited 2016 Feb 1].
- [30] Chevalier J, Cales B, Drouin JM. Low-temperature aging of Y-TZP ceramics. *J Am Ceram Soc* [Internet]. 2004;82(8):2150–4. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1999.tb02055.x> [cited 2016 Feb 1].
- [31] Lawson S. Environmental degradation of zirconia ceramics. *J Eur Ceram Soc* [Internet]. 1995;15(6):485–502. Available from: <http://www.sciencedirect.com/science/article/pii/S095522199500035S> [cited 2015 Oct 19].
- [32] Cattani-Lorente M, Scherrer SS, Ammann P, Jobin M, Wiskott HWA. Low temperature degradation of a Y-TZP dental ceramic. *Acta Biomater* [Internet]. 2011;7(2):858–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20854937> [cited 2016 Feb 1].

- [33] Lughì V, Sergo V. Low temperature degradation aging of zirconia: a critical review of the relevant aspects in dentistry. *Dent Mater* [Internet]. 2010;26(8):807–20. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20537701> [cited 2016 Feb 1].
- [34] Vagkopoulou T, Koutayas SO, Koidis P, Strub JR. Zirconia in dentistry: part 1. Discovering the nature of an upcoming bioceramic. *Eur J Esthet Dent* [Internet]. 2009;4(2):130–51. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19655651> [cited 2016 Feb 1].
- [35] Deville S, Chevalier J, Gremillard L. Influence of surface finish and residual stresses on the ageing sensitivity of biomedical grade zirconia. *Biomaterials* [Internet]. 2006;27(10):2186–92. Available from: <http://www.sciencedirect.com/science/article/pii/S0142961205010379> [cited 2016 Jan 31].
- [36] Naito Y, Jimbo R, Bryington MS, Vandeweghe S, Chrcanovic BR, Tovar N, . The influence of 1 α .25-dihydroxyvitamin d3 coating on implant osseointegration in the rabbit tibia. *J Oral Maxillofac Res* [Internet]. 2014;5(3):e3. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4219862&tool=pmcentrez&rendertype=abstract> [cited 2016 Feb 1].
- [37] Andreiotelli M, Wenz HJ, Kohal R-J. Are ceramic implants a viable alternative to titanium implants? A systematic literature review. *Clin Oral Implants Res* [Internet]. 2009;20 Suppl 4:32–47. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19663947> [cited 2016 Jan 24].
- [38] Covani U, Bortolaia C, Barone A, Sbordone L. Bucco-lingual crestal bone changes after immediate and delayed implant placement. *J Periodontol* [Internet]. 2004;75(12):1605–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15732861> [cited 2016 Jan 7].
- [39] Cosyn J, Hooghe N, De Bruyn H. A systematic review on the frequency of advanced recession following single immediate implant treatment. *J Clin Periodontol* [Internet]. 2012;39(6):582–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22509794> [cited 2016 Feb 1].
- [40] den Hartog L, Slater JJRH, Vissink A, Meijer HJA, Raghoobar GM. Treatment outcome of immediate, early and conventional single-tooth implants in the aesthetic zone: a systematic review to survival, bone level, soft-tissue, aesthetics and patient satisfaction. *J Clin Periodontol* [Internet]. 2008;35(12):1073–86. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19040585> [cited 2016 Feb 1].
- [41] Hisbergues M, Vendeville S, Vendeville P. Zirconia: Established facts and perspectives for a biomaterial in dental implantology. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2009;88B:519–29
- [42] Esposito M, Maghaireh H, Grusovin MG, Ziounas I, Worthington HV. Interventions for replacing missing teeth: management of soft tissues for dental implants. *Cochrane Database Syst Rev* [Internet]. 2012;2:CD006697. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22336822> [cited 2016 Jan 22].

- [43] Cooper LF, Zhou Y, Takebe J, Guo J, Abron A, Holmén A, . Fluoride modification effects on osteoblast behavior and bone formation at TiO₂ grit-blasted c.p. titanium endosseous implants. *Biomaterials* [Internet]. 2006;27(6):926–36. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16112191> [cited 2016 Feb 1].
- [44] Kohal R-J, Klaus G, Strub JR. Zirconia-implant-supported all-ceramic crowns withstand long-term load: a pilot investigation. *Clin Oral Implants Res* [Internet]. 2006;17(5):565–71. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16958698> [cited 2016 Feb 1].
- [45] Andreiotelli M, Kohal R-J. Fracture strength of zirconia implants after artificial aging. *Clin Implant Dent Relat Res* [Internet]. 2009;11(2):158–66. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18657150> [cited 2016 Feb 1].
- [46] Kohal R-J, Finke HC, Klaus G. Stability of prototype two-piece zirconia and titanium implants after artificial aging: an in vitro pilot study. *Clin Implant Dent Relat Res* [Internet]. 2009;11(4):323–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18783418> [cited 2016 Feb 1].
- [47] Josset Y, Oum'Hamed Z, Zarrinpour A, Lorenzato M, Adnet JJ, Laurent-Maquin D. In vitro reactions of human osteoblasts in culture with zirconia and alumina ceramics. *J Biomed Mater Res* [Internet]. 1999;47(4):481–93. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10497283> [cited 2016 Feb 1].
- [48] Bächle M, Butz F, Hübner U, Bakaliniš E, Kohal RJ. Behavior of CAL72 osteoblast-like cells cultured on zirconia ceramics with different surface topographies. *Clin Oral Implants Res* [Internet]. 2007;18(1):53–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17224024> [cited 2016 Feb 1].
- [49] Scarano A, Di Carlo F, Quaranta M, Piattelli A. Bone response to zirconia ceramic implants: an experimental study in rabbits. *J Oral Implantol* [Internet]. 2003;29(1):8–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12614079> [cited 2016 Feb 1].
- [50] Sennerby L, Dasmah A, Larsson B, Iverhed M. Bone tissue responses to surface-modified zirconia implants: a histomorphometric and removal torque study in the rabbit. *Clin Implant Dent Relat Res* [Internet]. 2005;7(Suppl. 1):S13–20. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16137083> [cited 2016 Feb 1].
- [51] Kohal RJ, Weng D, Bächle M, Strub JR. Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment. *J Periodontol* [Internet]. 2004;75(9):1262–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15515343> [cited 2016 Feb 1].
- [52] Akagawa Y, Ichikawa Y, Nikai H, Tsuru H. Interface histology of unloaded and early loaded partially stabilized zirconia endosseous implant in initial bone healing. *J Prosthet Dent* [Internet]. 1993;69(6):599–604. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8320646> [cited 2016 Feb 1].

- [53] Shimizu K, Oka M, Kumar P, Kotoura Y, Yamamuro T, Makinouchi K, . Time-dependent changes in the mechanical properties of zirconia ceramic. *J Biomed Mater Res* [Internet]. 1993;729–34. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/?term=Time-dependent+changes+in+the+mechanical+properties+of+zirconia+cerami> [cited 2016 Feb 1].
- [54] Mellinghoff J. Erste klinische Ergebnisse zu dentalen Schraubenimplantaten aus Zirkonoxid. *Zeitschrift für Zahnärztliche Implantologie*. 2006; 22:288–93
- [55] Gittens RA, Olivares-Navarrete R, Tannenbaum R, Boyan BD, Schwartz Z. Electrical implications of corrosion for osseointegration of titanium implants. *J Dent Res* [Internet]. 2011;90(12):1389–97. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3215755&tool=pmcentrez&rendertype=abstract> [cited 2015 Feb 12].
- [56] Lambrich M, Iglhaut G. Vergleich der Überlebensrate von Zirkondioxid-und Titanimplantaten. *Zeitschrift für Zahnärztliche Implantologie*. 2008; 24:182-91
- [57] Depprich R, Naujoks C, Ommerborn M, Schwarz F, Kübler NR, Handschel J. Current findings regarding zirconia implants. *Clin Implant Dent Relat Res* [Internet]. 2014;16(1): 124–37. Available from: <http://doi.wiley.com/10.1111/j.1708-8208.2012.00454.x> [cited 2016 Feb 1].
- [58] Payer M, Arnetzl V, Kirmeier R, Koller M, Arnetzl G, Jakse N. Immediate provisional restoration of single-piece zirconia implants: a prospective case series – results after 24 months of clinical function. *Clin Oral Implants Res* [Internet]. 2013;24(5):569–75. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22335358> [cited 2016 Feb 1].
- [59] Oliva J, Oliva X, Oliva JD. Five-year success rate of 831 consecutively placed Zirconia dental implants in humans: a comparison of three different rough surfaces. *Int J Oral Maxillofac Implants*. 2010;25(2):336–44.
- [60] Kohal R-J, Knauf M, Larsson B, Sahlin H, Butz F. One-piece zirconia oral implants: one-year results from a prospective cohort study. 1. Single tooth replacement. *J Clin Periodontol*. 2012;39(6):590–7.
- [61] Guazzato M, Albakry M, Quach L, Swain MV. Influence of surface and heat treatments on the flexural strength of a glass-infiltrated alumina/zirconia-reinforced dental ceramic. *Dent Mater* [Internet]. 2005;21(5):454–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15826702> [cited 2016 Feb 1].
- [62] Fabbri P, Piconi C, Burrelli E, Magnani G, Mazzanti F, Mingazzini C. Lifetime estimation of a zirconia–alumina composite for biomedical applications. *Dent Mater* [Internet]. 2014;30(2):138–42. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24246473> [cited 2016 Feb 1].
- [63] Hallmann L, Ulmer P, Reusser E, Louvel M, Hämmerle CHF. Effect of dopants and sintering temperature on microstructure and low temperature degradation of dental Y-TZP-zirconia. *J Eur Ceram Soc* [Internet]. 2012;32(16):4091–104. Available from:

- <http://www.sciencedirect.com/science/article/pii/S0955221912004281> [cited 2016 Jan 11].
- [64] De Aza AH, Chevalier J, Fantozzi G, Schehl M, Torrecillas R. Crack growth resistance of alumina, zirconia and zirconia toughened alumina ceramics for joint prostheses. *Biomaterials*. 2002;23(3):937–45.
- [65] Shimada MTK. Thermal stability of Y₂O₃-partially stabilized (Y-PSZ) and Y-PSZ/Al₂O₃ composites. *J Mater Sci Lett*. 1985;4:857–61.
- [66] Tsubakino H, Nozato R, Hamamoto M. Effect of alumina addition on the tetragonal-to-monoclinic phase transformation in zirconia-3 mol% yttria. *J Am Ceram Soc* [Internet]. 1991;74(2):440–3. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1991.tb06905.x> [cited 2016 Feb 1].
- [67] Gutknecht D, Chevalier J, Garnier V, Fantozzi G. Key role of processing to avoid low temperature ageing in alumina zirconia composites for orthopaedic application. *J Eur Ceram Soc* [Internet]. 2007;27(2–3):1547–52. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221906001750> [cited 2015 Nov 25].
- [68] Spies BC, Sauter C, Wolkewitz M, Kohal R-J. Alumina reinforced zirconia implants: effects of cyclic loading and abutment modification on fracture resistance. *Dent Mater* [Internet]. 2015;31(3):262–72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25582058> [cited 2016 Feb 1].
- [69] Md Ani S, Muchtar A, Muhamad N, Ghani JA. Fabrication of zirconia-toughened alumina parts by powder injection molding process: optimized processing parameters. *Ceram Int* [Internet]. 2014;40(1):273–80. Available from: <http://www.sciencedirect.com/science/article/pii/S0272884213006500> [cited 2016 Jan 30].
- [70] Kim DJ, Lee MH, Lee DY, Han JS. Mechanical properties, phase stability, and biocompatibility of (Y,Nb)-TZP/Al₂O₃ composite abutments for dental implant. *J Biomed Mater Res* 2000; 53: 438–43.
- [71] Maria Cecilia Corrêa de Sá e Benevides de Moraes I; Carlos Nelson Elias I; Jamil Duailibi Filho II; Leandra Guimarães de Oliveira Mechanical properties of alumina-zirconia composites for ceramic abutments. *Mater Res* [Internet]. *Materials Research*; 2004;7(4): 643–9. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-14392004000400021&lng=en&nrm=iso&tlng=en [cited 2016 Feb 1]
- [72] Affatato S, Testoni M, Cacciari GL, Toni A. Mixed-oxides prosthetic ceramic ball heads. Part II: effect of the ZrO₂ fraction on the wear of ceramic on ceramic joints. *Biomaterials* [Internet]. 1999;20(20):1925–9. Available from: <http://www.sciencedirect.com/science/article/pii/S0142961299000939> [cited 2016 Feb 1].
- [73] Nevarez-Rascon A, Aguilar-Elguezabal A, Orrantia E, Bocanegra-Bernal MH. On the wide range of mechanical properties of ZTA and ATZ based dental ceramic composites

- by varying the Al_2O_3 and ZrO_2 content. *Int J Refract Metals Hard Mater.* 2009;27(6):962–70.
- [74] Nevarez-Rascon A, Aguilar-Elguezabal A, Orrantia E, Bocanegra-Bernal MH. $\text{Al}_2\text{O}_3(\text{w})$ - $\text{Al}_2\text{O}_3(\text{n})$ - ZrO_2 (TZ-3Y)(n) multi-scale nanocomposite: an alternative for different dental applications? *Acta Biomater* [Internet]. 2010;6(2):563–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19560564> [cited 2016 Feb 1].
- [75] Kurtz SM, Kocagöz S, Arnholt C, Huet R, Ueno M, Walter WL. Advances in zirconia toughened alumina biomaterials for total joint replacement. *J Mech Behav Biomed Mater* [Internet]. 2014;31:107–16. Available from: <http://www.sciencedirect.com/science/article/pii/S1751616113001112> [cited 2016 Feb 1].
- [76] Magnani G, Brillante A. Effect of the composition and sintering process on mechanical properties and residual stresses in zirconia–alumina composites. *J Eur Ceram Soc* [Internet]. 2005;25(15):3383–92. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221904004224> [cited 2016 Feb 1].
- [77] Maccauro G, Bianchino G, Sangiorgi S, Magnani G, Marotta D, Manicone PF. Development of a new zirconia-toughened alumina: promising mechanical properties and absence of in vitro carcinogenicity. *Int J Immunopathol Pharmacol* [Internet]. 2009;22(3):773–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19822094> [cited 2016 Feb 1].
- [78] Spinelli MS, Maccauro G, Graci C, Cittadini A, Magnani G, Sangiorgi S. Zirconia toughened alumina (ZTA) powders: ultrastructural and histological analysis. *Int J Immunopathol Pharmacol* [Internet]. 2011;24(1 Suppl. 2):153–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21669156> [cited 2016 Feb 1].
- [79] Kohal R-J, Wolkewitz M, Mueller C. Alumina-reinforced zirconia implants: survival rate and fracture strength in a masticatory simulation trial. *Clin Oral Implants Res* [Internet]. 2010;21(12):1345–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20626420> [cited 2016 Feb 1].
- [80] Douillard T, Chevalier J, Descamps-Mandine A, Warner I, Galais Y, Whitaker P. Comparative ageing behaviour of commercial, unworn and worn 3Y-TZP and zirconia-toughened alumina hip joint heads. *J Eur Ceram Soc* [Internet]. 2012;32(8):1529–40. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221912000179> [cited 2016 Feb 1].
- [81] Vallée A, Faga MG, Mussano F, Catalano F, Tolosano E, Carossa S. Alumina-zirconia composites functionalized with laminin-1 and laminin-5 for dentistry: effect of protein adsorption on cellular response. *Colloids Surf B Biointerfaces.* 2014;114:284–93.
- [82] Schierano G, Mussano F, Faga MG, Menicucci G, Manzella C, Sabione C. An alumina toughened zirconia composite for dental implant application: in vivo animal results. *Biomed Res Int.* 2015;2015:157360.

- [83] Kohal RJ, Bächle M, Renz A, Butz F. Evaluation of alumina toughened zirconia implants with a sintered, moderately rough surface: An experiment in the rat. *Dent Mater* [Internet]. 2015;32(1):65–72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26621027> [cited 2015 Dec 15].
- [84] Spies BC, Balmer M, Patzelt SBM, Vach K, Kohal RJ. Clinical and patient-reported outcomes of a zirconia oral implant: three-year results of a prospective cohort investigation. *J Dent Res* [Internet]. 2015;94(10):1385–91. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26232388> [cited 2016 Feb 1].
- [85] Faga MG, Vallée A, Bellosi A, Mazzocchi M, Thinh NN, Martra G, . Chemical treatment on alumina–zirconia composites inducing apatite formation with maintained mechanical properties. *J Eur Ceram Soc*. 2012;32(10):2113–20.
- [86] Albrektsson T, Wennerberg A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *Int J Prosthodont* [Internet]. 2004;17(5):536–43. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15543910> [cited 2016 Feb 1].
- [87] Hoffmann O, Angelov N, Zafiropoulos G-G, Andreana S. Osseointegration of zirconia implants with different surface characteristics: an evaluation in rabbits. *Int J Oral Maxillofac Implants* [Internet]. 2012;27(2):352–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22442775> [cited 2016 Feb 1].
- [88] Lee J, Sieweke JHJH, Rodriguez NANA, Schüpbach P, Lindström H, Susin C, . Evaluation of nano-technology-modified zirconia oral implants: a study in rabbits. *J Clin Periodontol* [Internet]. 2009;36(7):610–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19538335> [cited 2016 Feb 1].
- [89] Bacchelli B, Giavaresi G, Franchi M, Martini D, De Pasquale V, Trirè A, . Influence of a zirconia sandblasting treated surface on peri-implant bone healing: an experimental study in sheep. *Acta Biomater* [Internet]. 2009;5(6):2246–57. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19233751> [cited 2016 Feb 1].
- [90] Ferguson SJ, Langhoff JD, Voelter K, von Rechenberg B, Scharnweber D, Bierbaum S, . Biomechanical comparison of different surface modifications for dental implants. *Int J Oral Maxillofac Implants* [Internet]. 2008;23(6):1037–46. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19216272> [cited 2016 Feb 1].
- [91] Spies BC, Sperlich M, Fleiner J, Stampf S, Kohal RJ. Alumina reinforced zirconia implants: 1-year results from a prospective cohort investigation. *Clin Oral Implants Res* 2016 Apr;27(4):481-90. doi: 10.1111/clr.12560. Epub 2015 Feb 11
- [92] Dohan Ehrenfest DM, Coelho PG, Kang B-S, Sul Y-T, Albrektsson T. Classification of osseointegrated implant surfaces: materials, chemistry and topography. *Trends Biotechnol* [Internet]. 2010;28(4):198–206. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20116873> [cited 2016 Feb 1].

- [93] Bal BS, Rahaman MN. Orthopedic applications of silicon nitride ceramics. *Acta Biomater* [Internet]. 2012;8(8):2889–98. Available from: <http://www.sciencedirect.com/science/article/pii/S174270611200178X> [cited 2015 May 12].
- [94] Mazzocchi M, Bellosi A. On the possibility of silicon nitride as a ceramic for structural orthopaedic implants. Part I: processing, microstructure, mechanical properties, cytotoxicity. *J Mater Sci Mater Med* [Internet]. 2008;19(8):2881–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18347952> [cited 2015 Jul 31].
- [95] Mazzocchi M, Gardini D, Traverso PL, Faga MG, Bellosi A. On the possibility of silicon nitride as a ceramic for structural orthopaedic implants. Part II: chemical stability and wear resistance in body environment. *J Mater Sci Mater Med* [Internet]. 2008;19(8):2889–901. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18415002> [cited 2016 Feb 1].
- [96] Sorrell CC, Hardcastle PH, Druitt RK, Howlett CR, McCartney ER. Results of 15-Year Clinical Study of Reaction Bonded Silicon Nitride Intervertebral Spacers. 7th World Biomater Conf. 2004. p. 1872.
- [97] Taylor RM, Bernero JP, Patel AA, Brodke DS, Khandkar AC. Silicon nitride: a new material for spinal implants. *Orthop Proc* [Internet]. *Orthopaedic Proceedings*; 2010;92-B(SUPP I):133. Available from: http://www.bjjprocs.boneandjoint.org.uk/content/92-B/SUPP_I/133.1.abstract [cited 2016 Feb 1].
- [98] McEntire BJ, Bal BS, Rahaman MN, Chevalier J, Pezzotti G. Ceramics and ceramic coatings in orthopaedics. *J Eur Ceram Soc* [Internet]. 2015;35(16):4327–69. Available from: <http://www.sciencedirect.com/science/article/pii/S0955221915300790> [cited 2015 Oct 20].
- [99] Neumann A, Reske T, Held M, Jahnke K, Ragoss C, Maier HR. Comparative investigation of the biocompatibility of various silicon nitride ceramic qualities in vitro. *J Mater Sci Mater Med* [Internet]. 2004;15(10):1135–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15516875> [cited 2015 Jul 31].
- [100] Howlett CR, McCartney E, Ching W. The effect of silicon nitride ceramic on rabbit skeletal cells and tissue. An in vitro and in vivo investigation. *Clin Orthop Relat Res* [Internet]. 1989;(244):293–304. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2743672> [cited 2015 Sep 4].
- [101] Santos C, Ribeiro S, Daguano JKMF, Rogero SO, Strecker K, Silva CRM. Development and cytotoxicity evaluation of SiAlONs ceramics. *Mater Sci Eng C* [Internet]. 2007;27(1):148–53. Available from: <http://www.sciencedirect.com/science/article/pii/S0928493106000336> [cited 2016 Feb 1].
- [102] Cappi B, Neuss S, Salber J, Telle R, Knüchel R, Fischer H. Cytocompatibility of high strength non-oxide ceramics. *J Biomed Mater Res A* [Internet]. 2010;93(1):67–76. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19484770> [cited 2016 Feb 1].

- [103] Neumann A, Kramps M, Ragoß C, Maier HR, Jahnke K. Histological and microradiographic appearances of Silicon Nitride and Aluminum Oxide in a rabbit femur implantation model. *Materwiss Werksttech* [Internet]. 2004;35(9):569–73. Available from: <http://doi.wiley.com/10.1002/mawe.200400778> [cited 2016 Feb 1].
- [104] Neumann A, Unkel C, Werry C, Herborn CU, Maier HR, Ragoss C. Prototype of a silicon nitride ceramic-based miniplate osteofixation system for the midface. *Otolaryngol Head Neck Surg* [Internet]. 2006;134(6):923–30. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16730531> [cited 2016 Feb 1].
- [105] Webster TJ, Patel AA, Rahaman MN, Sonny Bal B. Anti-infective and osteointegration properties of silicon nitride, poly(ether ether ketone), and titanium implants. *Acta Biomater* [Internet]. 2012;8(12):4447–54. Available from: <http://www.sciencedirect.com/science/article/pii/S1742706112003571> [cited 2015 Sep 4].
- [106] Gorth DJ, Puckett S, Ercan B, Webster TJ, Rahaman M, Bal BS. Decreased bacteria activity on Si₃N₄ surfaces compared with PEEK or titanium. *Int J Nanomed* [Internet]. 2012;7:4829–40. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3439860&tool=pmcentrez&rendertype=abstract> [cited 2015 Sep 4].
- [107] Guedes e Silva CC, König B, Carbonari MJ, Yoshimoto M, Allegrini S, Bressiani JC. Tissue response around silicon nitride implants in rabbits. *J Biomed Mater Res A* [Internet]. 2008;84(2):337–43. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17607762> [cited 2016 Feb 1].
- [108] Guedes e Silva CC, König B, Carbonari MJ, Yoshimoto M, Allegrini S, Bressiani JC. Bone growth around silicon nitride implants—an evaluation by scanning electron microscopy. *Mater Charact* [Internet]. 2008;59(9):1339–41. Available from: <http://www.sciencedirect.com/science/article/pii/S1044580307003968> [cited 2016 Feb 1].
- [109] Anderson MC, Olsen R. Bone ingrowth into porous silicon nitride. *J Biomed Mater Res A* [Internet]. 2010;92(4):1598–605. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19437439> [cited 2016 Feb 1].
- [110] Roebben G, Sarbu C, Lube T, Van der Biest O. Quantitative determination of the volume fraction of intergranular amorphous phase in sintered silicon nitride. *Mater Sci Eng A* [Internet]. 2004;370(1–2):453–8. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0921509303009419> [cited 2016 Feb 1].
- [111] Becher PF. Microstructural design of toughened ceramics. *J Am Ceram Soc* [Internet]. 1991;74(2):255–69. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1991.tb06872.x> [cited 2016 Feb 1].
- [112] Sun EY, Becher PF, Plucknett KP, Hsueh C-H, Alexander KB, Waters SB, . Microstructural design of silicon nitride with improved fracture toughness: II, effects of yttria and alumina additives. *J Am Ceram Soc* [Internet]. 2005;81(11):2831–40. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1998.tb02703.x> [cited 2016 Feb 1].

- [113] Becher PF, Sun EY, Plucknett KP, Alexander KB, Hsueh C-H, Lin H-T,. Microstructural design of silicon nitride with improved fracture toughness: I, effects of grain shape and size. *J Am Ceram Soc* [Internet]. 2005;81(11):2821–30. Available from: <http://doi.wiley.com/10.1111/j.1151-2916.1998.tb02702.x> [cited 2016 Feb 1].
- [114] ISO 26602:2009 - Fine ceramics (advanced ceramics, advanced technical ceramics) -- Silicon nitride materials for rolling bearing balls - 2009-02-01
- [115] ASTM-F2094/F2094M-11 - Standard Specification for Silicon Nitride Bearing Balls - May 1, 2011
- [116] Bucciotti F, Mazzocchi M, Bellosi A. Perspectives of the Si₃N₄-TiN ceramic composite as a biomaterial and manufacturing of complex-shaped implantable devices by electrical discharge machining (EDM). *J Appl Biomater Biomech* [Internet]. 2010;8(1): 28–32. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20740419> [cited 2015 Sep 5].
- [117] Passeri G, Cacchioli A, Ravanetti F, Galli C, Elezi E, Macaluso GM. Adhesion pattern and growth of primary human osteoblastic cells on five commercially available titanium surfaces. *Clin Oral Implants Res* [Internet]. 2010;21(7):756–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20636730> [cited 2015 Jul 22].
- [118] Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J,. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). *J Biomed Mater Res* [Internet]. 1995;29(3):389–401. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7542245> [cited 2015 Sep 5].
- [119] Guedes e Silva CC, Higa OZ, Bressiani JC. Cytotoxic evaluation of silicon nitride-based ceramics. *Mater Sci Eng C* [Internet]. 2004;24(5):643–6. Available from: <http://www.sciencedirect.com/science/article/pii/S0928493104000736> [cited 2015 Sep 4].

IntechOpen