Abstract: Alumina (Al₂O₃) ceramic implants do not stimulate osteoblasts in vivo. Surface alterations targeted at changing the chemistry or topography have been proposed to enhance the bioactivity of alumina. This surface modification is intended to improve oxide bioceramic’s ability to integrate with the biological environment and, in particular, to rapidly osteointegrate. In this study, the surface of zirconia-toughened alumina (ZTA) was functionalized using two methods: (i) Surface laser-patterning and successive filling of patterned wells with powder mixtures of bioglass and Si₃N₄; and, (ii) Si₃N₄ coating by pulse-laser sintering. Functionalized ZTA surfaces were characterized with vibrational spectroscopy, biological testing, and laser microscopy. Both enhancements resulted in osteoblast activation, a property that is relevant to osteosynthesis.

Keywords: zirconia-toughened alumina; silicon nitride; surface functionalization; pulsed laser technology; osteogenesis

1. Introduction

During bone formation, organic matrix (i.e., predominantly collagen type I) is synthesized by cells secreting a variety of glycoproteins, genetic markers, and bone sialoprotein [1]. A favorable environment for cellular apatite mineralization relies on local supersaturation of extra-cellular fluids with osteoblastic alkaline phosphatase (ALP), pyrophosphatase activity, and osteocalcin production. ALP increases phosphate concentration by cleaving phosphate groups; pyrophosphatase degrades pyrophosphate, which inhibits phosphate cleavage; and osteocalcin binds calcium [1,2]. The surface chemistry of orthopaedic implants designed to promote bone formation at tissue/implant interface should promote such chemical processes [3].
Although ceramic materials are considered to be bioinert [4,5], they can in fact stimulate or suppress osteoblastic activity [6,7]. For example, silicon nitride (Si$_3$N$_4$) ceramic implants are known to accelerate bone repair because of the surface chemistry of the material [8–11]. The release of silicic acid and reactive nitrogen species (RNS) on Si$_3$N$_4$ surfaces enhances osteogenic activity in osteosarcoma and mesenchymal cells [8–10]. Analyses of a retrieved Si$_3$N$_4$ human cervical fusion implant have confirmed the incorporation of silicon and nitrogen into the crystal lattice of native hydroxyapatite [11].

Data have also shown that hydrolysis at Si$_3$N$_4$ surfaces releases Si and RNS ions that favor bone formation, while Al$_2$O$_3$ surfaces lead to the elution of Al$^{3+}$ and reactive oxygen species (ROS) instead [12]. Al$^{3+}$ ions inhibit mineralization by slowing hydroxyapatite crystal formation and altering the influx and efflux of calcium from bone cells [13]. Moreover, the formation of ROS leads to oxidative stress in cells with significant perturbation of their normal anti-oxidative system [14]. In vivo and in vitro data have shown that Al$^{3+}$ and ROS-induced oxidative stress activates cell apoptotic signaling pathways [15–17]. ROS cause damage to cell proteins, lipids, and DNA; target important regulator pathways for calcium and its intracellular receptor calmodulin; and, ultimately, lead to osteoblast apoptosis [18–21]. Because of these properties, alumina orthopaedic implants are limited to low-wear applications, such as the bearings of prosthetic hip joints.

We sought to modulate the surface bioactivity of zirconia-toughened-alumina (ZTA), a material commonly used in prosthetic hip bearings using two approaches: (i) Surface laser patterning and successive filling of the patterned wells with powder mixtures of bioglass and Si$_3$N$_4$; and, (ii) Deposition of a micrometer thick Si$_3$N$_4$ layer using an automatic 3D-additive laser-sintering procedure. Our hypothesis was that these surface treatments would alter the Al$^{3+}$/ROS surface chemistry of the oxide ZTA ceramic into the Si$^{4+}$/RNS type, without affecting mechanical properties.

2. Experimental Procedures

2.1. Surface Functionalization Procedures

ZTA samples were machined from 36 mm diameter femoral heads (Biolox® delta, CeramTec, GmbH, Plochingen, Germany; year of production: 2014). Squared samples 10 mm $\times$ 10 mm with thickness of 3 mm were cut using a slow-speed diamond coated blade and polished to a roughness $0.22 \pm 0.1 \mu m$ as measured by laser microscope.

2.1.1. Laser-Patterning Procedure

Laser-patterning of ZTA samples was done with a Vision LWI V ERGO-Workstation equipped with an Nd:YAG laser (wavelength of 1064 nm). Focusing distance, nominal maximum power, and burst energy were set at 250 mm, 17 kW, and 70 J, respectively, while the applied potential and discharge time were regulated in the range 160–500 V and 1–20 ms, respectively. The laser-patterning workstation was equipped with a gas nozzle connected to an Argon source at 1.2 atm to locally limit the presence of oxygen at the location of laser impingement. A motorized x-y stage with a precision of 10 $\mu m$ was used to align the sample with the laser source and to produce a regular grid of equidistant wells 500 $\mu m$ in diameter with an interspace of 1.0 mm.

Standard 45S5 Bioglass® powder (Mo-Sci Corporation, Rolla, MO, USA) was used to fill the patterned wells. The composition in wt.% of the Bioglass® was, as follows: 45% SiO$_2$, 24.5% CaO, 24.5% Na$_2$O, 6% P$_2$O$_5$. The Bioglass® powder was mixed with 5 or 10 mol.% nm-sized $\alpha$-Si$_3$N$_4$ powder (SN-E10, Ube Co., Ube City, Japan). Powder mixtures of Bioglass® with 5 and 10 mol.% (corresponding to 5.51 and 10.69 wt.%) $\alpha$-Si$_3$N$_4$ were homogenized and melted in a platinum crucible in nitrogen gas atmosphere. After quenching, the melts were crushed into fine powder. Pure Bioglass® and Bioglass® mixtures with 5 and 10 wt.% Si$_3$N$_4$ were used to fill the wells of different ZTA samples ($n = 6$ for each type of filler). A schematic draft of the patterning and powder feeding procedures is shown in Figure 1a. The sample cross-section during excavation of a cylindrical well on the ZTA surface by the pulsed laser beam is depicted in Figure 1b.
2.1.2. Laser-Sintering Procedure

The starting micrometric powder used for laser-sintering the Si$_3$N$_4$ coating on the ZTA substrate consisted of β-Si$_3$N$_4$ (SINTX Co., Salt Lake City, UT, USA) mixed with 6 wt.% yttrium oxide (Y$_2$O$_3$, Grade C, H.C. Starck, Munich, Germany) and 4 wt.% aluminium oxide (Al$_2$O$_3$, SA8-DBM, Baikowski/Malakoff, Charlotte, NC, USA). The workstation and the Nd:YAG laser were the same as those described above. Successive layers of Si$_3$N$_4$ powder were placed on the water-wet surface of ZTA samples and the pulse laser repeatedly impinged to sinter/densify it.

The conditions applied to achieve Si$_3$N$_4$ coating densification were the following: laser wavelength 1064 nm, max pulse energy: 70 joule, peak power 17 kW, voltage range 400 V, pulse time 4 ms, and spot size 2 μm. The apparatus operated under a constant flux of nitrogen gas flow in order to limit Si$_3$N$_4$ decomposition and oxidation. The operation was repeated until obtaining a homogeneous Si$_3$N$_4$ layer with thickness, $t = 15 ± 5$ μm, over the entire surface of the ZTA substrate. A schematic draft of the overall laser-sintering procedure is schematically depicted in Figure 1c. The sample/laser interaction during sintering/densification of the Si$_3$N$_4$ coating from the powder bed is shown in cross section in Figure 1d.

2.2. Surface Characterization

The surface morphology of the textured ZTA surface before and after filling with Bioglass$^®$/Si$_3$N$_4$ powder mixtures was characterized with a confocal scanning laser microscope (Laser Microscope 3D and Profile measurements, Keyence, VKx200 Series, Osaka, Japan) capable of high-resolution optical images with depth selectivity. All images were collected using 20x magnification. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) (JSM-700 1F, JEOL, Tokyo, Japan) were used to acquire high-resolution images and chemical composition maps of Si$_3$N$_4$-coated substrates.
FTIR spectra were obtained using a high sensitivity spectroscope (Spectrum 100FT-IR Spotlight400; Perkin-Elmer Inc., Waltham, MA, USA). The spectral resolution of this equipment was 0.4 cm$^{-1}$. Average FTIR spectra were computed for each substrate from eight independent measurements. Pre-processing of raw data, which included baseline subtraction, smoothing, normalization, and fitting of the raw spectra, were performed using “R” [22].

2.3. Cell Culture and Biological Tests

SaOS-2 human osteosarcoma cells were cultured and incubated in 4.5 g/L glucose DMEM (D-glucose, L-Glutamine, Phenol Red, and Sodium Pyruvate; Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum. The cells were then kept proliferating in petri dishes for 24 h at 37 °C. After adjusting the final cell concentration at $5 \times 10^5$ cell/mL, the cultured cells were deposited on the surface of Si$_3$N$_4$-coated and uncoated ZTA substrates ($n = 3$ each type) previously sterilized by exposure to UV light. Osteoconductivity tests were conducted with seeding the cells in an osteogenic medium (DMEM supplemented with 50 µg/mL ascorbic acid, 10 mM β-glycerol phosphate, 100 mM hydrocortisone, and ~10% fetal bovine calf serum), and then incubating the samples for 7 days at 37 °C. The medium was changed twice during the week of incubation.

Immunocytostaining was performed with fixing the SaOS-2 cells with 4% paraformaldehyde for 15 min and then incubating the samples for 30 min at room temperature with the following primary antibodies: mouse anti-human Gla osteocalcin (TakaraBio, Kusatsu-shi, Japan) and rabbit anti-human osteopontin (dilution = 1:500) (IBL, Maebashi-Shi, Gunma, Japan). The cells were incubated with fluorescence conjugated and secondary antibodies Goat anti-Mouse Antibody FITC Conjugated (Bethyl Laboratories, Montgomery, TX, USA) and Goat anti-Rabbit Antibody PE Conjugated and (1:200) (Thermo Fisher Scientific, Waltham, MA, USA). Cell nuclei were stained with stained with Hoechst33342 (1:100) (Dojindo, Kumamoto-Shi, Japan). The stained samples were observed under a fluorescence microscope (BZX710; Keyence, Osaka, Japan). Confocal scanning laser microscopy images were obtained with a 3D laser confocal microscope (OLS4000-SAT; Olympus Co., Tokyo, Japan) in order to assess the volumetric amounts per unit area of the bone tissue produced by the SaOS-2 cells after one-week exposure to the substrates. The amounts of osteocalcin and osteopontin in the deposited bony tissue were also estimated by direct pixel counting on fluorescence micrographs using automatic software.

2.4. Statistical Analyses

Data relating to osteogenesis were analyzed by calculating their mean value ± one standard deviation. The Student’s $t$-test was used to detect statistically significant differences between data, $p$ values < 0.01 being considered statistically significant and labeled with an asterisk in the figures.

3. Experimental Results

3.1. Laser Patterned and Bioglass®/Si$_3$N$_4$-Filled ZTA Surfaces

Figure 2a,b show high-magnification images of a laser-drilled well on the ZTA surface as obtained by optical microscopy and laser topography, respectively. Similar images for the well after filling with Bioglass®/Si$_3$N$_4$ powder are shown in Figure 2c,d, respectively. Preliminary calibrations, which were shown in a previous paper [23], located the optimal conditions for patterning the ZTA surface.

As shown in Figure 2, pulsed-laser drilling produced wells of a regular circular shape and with relatively sharp contours. Of note, ZTA partially melted under the laser beam; the molten material was ejected from the well producing some roughening in the neighborhood of the well border. Filling of the laser-patterned wells was done by first pressing the Bioglass®/Si$_3$N$_4$ powder mixtures onto the ZTA surface, followed by manual removal of excess materials with a sharp blade. The powder mixtures adhered to the internal walls of the wells. In this study, we selected a diameter of 500 µm for the patterned wells, consistent with pore sizes used in bone fusion implants clinically [24–27]. Figure 3a,b
show a 4 × 4 grid of wells 500 μm in diameter patterned on the ZTA surface as imaged by optical and laser scanning microscopies, respectively. An in-depth characterization of the morphology of the wells is shown in Figure 3c. This latter characterization revealed a regular cylindrical shape for the wells with an irregular morphology for the bottom surface at depths comparable with the well diameter. Results of optical and laser microscopy showing BioGlass®/Si₃N₄ powder filling of the wells are given in Figure 3d,f, respectively. These latter observations showed that the wells were completely filled with the powder mixture.

Figure 2. High-magnification images of a laser-drilled well on the ZTA surface: (a) optical microscopy and (b) laser topography; optical and laser images of the well after filling with BioGlass®/Si₃N₄ powder in (c,d), respectively.

Figure 3. (a) optical and (b) laser micrographs of a 4 × 4 grid of wells 500 μm in diameter patterned on the ZTA surface; (c) three-dimensional laser microscopy characterization of the morphology of the wells; optical and laser images of the well grid after filling with BioGlass®/Si₃N₄ powder in (d,e), respectively.

Figure 4 shows the results of fluorescence microscopy obtained on (a) pristine ZTA surface, (b) patterned ZTA surface, (c) patterned ZTA surface filled with BioGlass® powder only, (d) patterned ZTA surface filled with BioGlass®/5 wt.% Si₃N₄ powder mixture, and (e) patterned ZTA surface filled
with Bioglass®/10 wt.% Si₃N₄ powder mixture, after exposure to SaOS-2 cells for 1 week. Nuclei (blue), osteopontin (red), and osteocalcin (green) are tagged by fluorescence microscopy in the upper, middle, and bottom images of each section of Figure 4. After exposure for 1 week to SaOS-2 cells, the pristine ZTA substrate (Figure 4a) showed no cell proliferation and weak signals of osteocalcin and osteopontin, indicating a nearly absent bioactive response to osteoblasts. Similarly, the patterned and unfilled ZTA sample (Figure 4b) showed no signals and very weak osteocalcin/osteopontin signals within the wells and on flat regions, respectively.

Cell proliferation and osteocalcin/osteopontin synthesis by osteoblasts were improved in patterned ZTA surfaces filled with Bioglass® only (Figure 4c). Cell proliferation (blue signal) and osteopontin (red signal) expression were enhanced on patterned ZTA surfaces filled with Bioglass®/Si₃N₄ mixtures, with higher Si₃N₄ fractions showing stronger the blue and red fluorescence signals (cf. Figure 4d,e). Green osteocalcin fluorescence, localizing mineralized tissue, was detected not only in the filled wells, but also in the un-patterned regions of the ZTA surface when the wells were filled with Bioglass®/Si₃N₄ mixtures.

Figure 5 summarizes quantitative laser microscopy data in terms of area coverage and specific volume of osteoblastic tissue expression on different substrates. Both data sets were computed by excluding well areas to improve accuracy in comparing different samples, because of difficulty in evaluating strongly fluorescent areas. Area coverage and specific volume of biological tissue on ZTA patterned with Bioglass®/Si₃N₄ mixtures were ~200% and ~250% higher than that of unpatterned ZTA. There was no statistically significant difference among patterned ZTA samples filled with only Bioglass® and different Bioglass®/Si₃N₄ mixtures outside the well areas. Figure 4 shows a strong increase in green fluorescence signals from the patterned ZTA surfaces when the powder mixture filling the wells included a fraction of Si₃N₄ powder. While Bioglass® was stimulatory, the addition of Si₃N₄ further enhanced osteoblast proliferation (cf. Figure 4c,d). Figure 6a shows the results of FTIR charactrizations on the full set of unpatterned, patterned, and patterned/filled ZTA samples. Therefore, the addition of Bioglass® led to a significant increase in signal intensity in the spectral region at around 1000 cm⁻¹, which represents mineral hydroxyapatite, and the further addition of Si₃N₄ to Bioglass® led to an increase in relative intensity of signals at ~1500 cm⁻¹, which are associated with amides, and represent the biological matrix of bone tissue.

Consistent with fluorescence data in Figure 4 and their quantification in Figure 5, no mineralized tissue was detected on either pristine or patterned ZTA surfaces. Figure 6b gives a plot of intensity ratios between apatite and organic matrix signals in the FTIR spectra as a function of the type of ZTA substrate. Addition of Bioglass® in the patterned wells led to an increase in the intensity of mineral

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**Figure 4.** Fluorescence microscopy results on (a) unpatterned ZTA, (b) patterned ZTA, (c) patterned ZTA filled with Bioglass®/5 wt.% Si₃N₄ powder mixture, and (d) patterned ZTA filled with Bioglass®/10 wt.% Si₃N₄ powder mixture. The samples were exposed to SaOS-2 cells for 1 week.
hydroxyapatite bands. Further addition of 10 wt.% fraction of Si₃N₄ in the filler mixture brought the FTIR inorganic/organic band ratio of ZTA in the range recorded for healthy bone tissue [28].

![Graph showing quantitative laser microscopy data of area coverage and specific volume of biological tissue grown by osteoblasts on different substrates.]

**Figure 5.** Quantitative laser microscopy data of area coverage and specific volume of biological tissue grown by osteoblasts on different substrates.

![FTIR spectra showing hydroxyapatite bands and amide I.](image)

**Figure 6.** (a) Results of FTIR characterizations on unpatterned, patterned, and patterned/filled ZTA samples (sample specifications in labels); and, (b) plot of intensity ratios between apatite and organic matrix signals from FTIR spectra as a function of the type of ZTA substrate (cf. labels).

### 3.2. Laser-Sintering of Si₃N₄-Coated ZTA Surfaces

Figure 7a,b show optical and laser micrographs, respectively, taken on the surface of a partly Si₃N₄-coated ZTA surface across the interface between coated and uncoated regions (left and right sides of the micrographs, respectively). Figure 7b shows that the average thickness of the Si₃N₄ coating was in the order of \( t = 15 \pm 5 \text{ µm} \). The Raman spectra in the sections (c) and (d) of Figure 7 were taken on the ZTA uncoated and Si₃N₄-coated sides, respectively. In (c), the ZTA spectrum is composed of several bands belonging to the tetragonal and the monoclinic zirconia polymorphs (labeled as T and M, respectively) and one band from the alumina phase (at \( \approx 397 \text{ cm}^{-1} \); labeled A) [29]. Conversely, in the Si₃N₄-coated area, the Raman spectrum only presents the Si-N skeletal triplet at low frequencies and the Si-Si vibrational band at \( \approx 510 \text{ cm}^{-1} \). This latter Raman band testifies the presence of free silicon clusters as a product of partial decomposition of the Si₃N₄ structure during laser sintering [30].

Figure 8 shows SEM/EDS analyses performed on the same region in Figure 7 after 1-week in vitro exposure to SaOS-2 osteoblasts in biological environment. The main feature in the SEM micrograph (a) is the presence of a thick coverage of bony apatite only on the left side of the micrographs (i.e., the part coated with Si₃N₄). EDS elemental analyses of Si, Al, and Ca (in (b), (c), and (d), respectively) revealed Si-rich areas in coating area and no diffusional transport of the Al element into the coating during laser sintering (the very weak Al contamination in the coating area near the coated/uncoated interface,
as seen in (c), is believed to arise from melting residues created during laser processing. The presence of Si-rich areas conspicuously corresponded with the presence of Ca from bony hydroxyapatite.

![Figure 7](image)

**Figure 7.** (a) Optical and (b) laser micrographs taken on the top surface of a partly Si$_3$N$_4$-coated BIOLOX®*delta* ZTA surface across the interface between coated and uncoated regions (cf. labels); in (c,d), Raman spectra collected on the ZTA uncoated and Si$_3$N$_4$-coated sides, respectively (labels explained in text).

![Figure 8](image)

**Figure 8.** (a) SEM picture and EDS (Energy Dispersive X-ray Spectroscopy) maps of (b) Si, (c) Al, and (d) Ca collected in the same region shown in Figure 7 after 1-week in vitro exposure to SaOS-2 osteoblasts in biological environment.

Optical and laser micrographs of an island of bone tissue expressed by osteoblasts on the Si$_3$N$_4$-coated side of the ZTA surface are shown in Figure 9a,b respectively. The three-dimensional laser microscopy view in section (c) of the same figure reveals the details of the bone structure elevation reaching ~100 μm above the plane of the coated structure. Average FTIR spectra collected in the neighborhood of the interface between Si$_3$N$_4$-coated and ZTA uncoated zones (Figure 9d) revealed a four-fold higher intensity of the (PO$_4$)$^{3-}$ band of mineral apatite in the former zone, as compared to the
latter. In sum, the consistent findings using EDS, laser microscopy, and FTIR support the hypothesis that Si$_3$N$_4$ enhanced the osteogenic properties of ZTA ceramic.

![Figure 9](image_url)

**Figure 9.** (a) Optical and (b) laser micrographs of a large island of bone tissue grown by osteoblasts on the Si$_3$N$_4$-coated side of the BIOLOX® delta ZTA surface; (c) three-dimensional laser microscopy view of the same island of bone tissue; and, (d) average FTIR spectra collected in the neighborhood of the interface between Si$_3$N$_4$-coated and ZTA uncoated zones including the (PO$_4$)$^{3-}$ band of mineral apatite.

### 4. Discussion

Aluminum ions released by implant biomaterials may be toxic to bone [31]. Alumina coatings were designed to promote the osteointegration of prosthetic hip femoral stems, but this strategy created mineralization defects akin to those encountered in dialysis-induced osteomalacia, and related to Al$^{3+}$ ionic accumulation in bone [32]. Histological analyses detected Al$^{3+}$ ions released from the alumina coating, while X-ray diffractometry clarified the related structural modifications of periprosthetic bone tissue [32]. Follow up data at 2–5 years after implantation surgery showed that bone adjacent to alumina-coated stems had demineralized lamellar and Haversian structures because of Al$^{3+}$ ion release from the alumina coating [32]. An in vivo animal study examined Al$_2$O$_3$-coated cylinders implanted in rabbit femoral condyles for one to six months [33]. Post-mortem analyses identified Al$^{3+}$ ions at the interface between non-mineralized and mineralized bone; these ions reduced osteoblastic activity, induced apoptosis, and discouraged bone remodeling [17,34]. We recently compared in vitro osteoblastogenesis of mesenchymal cells cultured on Al$_2$O$_3$ versus other biomaterial substrates [35]. Raman spectral changes suggested a common apoptotic mechanism in cells exposed to Ti6Al4V alloy and Al$_2$O$_3$ substrates with intracellular biochemical changes consistent with toxicity [36]. These observations were consistent with the data of Brauchle et al. who monitored the Raman spectra of apoptotic osteosarcoma and chondrosarcoma cell lines in situ [37].

Aluminum interrupts osteoid calcification by preventing the growth of calcium phosphate crystals [38,39]. The material localizes at the bone mineralization front, where Al$^{3+}$ ions inhibit calcification. Al$^{3+}$ cations can substitute for Ca$^{2+}$ in the structure of apatite [40], thereby locking the structure and inhibiting further mineralization [13].

Figure 10 describes the interaction between ZTA (which contains Al$_2$O$_3$) and apoptotic osteoblasts. The off-stoichiometry equations governing the cell/ZTA-substrate interactions in aqueous environments...
support Al$^{3+}$ ion elution, and the formation of hydroxyl aluminols [41]. Other than incorporation in the apatite structure, Al$^{3+}$ ions also undergo dissociative adsorption entailing the transfer of charge from the Al$_2$O$_3$ cluster to hydroxyl, oxygen, and proton radicals [12,42]. The consequent (exogenous) development of ROS at the biomolecular cell/substrate interface probably irreversibly damages proteins and nucleic acids, and causes peroxidation of membrane lipids, ultimately leading to cell apoptosis [16]. This hypothesized mechanism of action is consistent with previously published data showing no cell proliferation and osteogenesis inhibition on Al$_2$O$_3$ surfaces [35].

![Figure 10](image)

**Figure 10.** Schematic drafts of RNS- and silanol-based chemical interaction between Si$_3$N$_4$ substrate and proliferating human osteoblasts (left side) and ROS- and aluminol-based chemical interaction between BIOLOX$^\text{®}$$^{\text{delta}}$ ZTA and apoptotic osteoblasts (right side). The chemical reactions involved with the different substrates in aqueous solution are described on top of each side of the figure. The square box in the chemical formula of hydroxyapatite modified by Al$^{3+}$ ions represents Ca vacancies.

Laser patterning and laser coating of ZTA with Bioglass$^\text{®}$ and/or Si$_3$N$_4$ represent two approaches toward overcoming the above limitations. Bioglass$^\text{®}$/Si$_3$N$_4$ mixtures stimulated osteoblast synthesis of bone tissue, the former favoring bone matrix mineralization and the latter enhancing cell proliferation and formation of bone matrix. The benefits of Bioglass$^\text{®}$ in surface mineralization [43]), and the favorable osteogenic chemistry of Si$_3$N$_4$ [8–11] are already known. Figure 10 shows a schematic of the interaction between Si$_3$N$_4$ and highly active osteoblasts, specifically the cascade of off-stoichiometric equations governing the cell/Si$_3$N$_4$-substrate interactions in aqueous environment. The surface kinetics following Si$_3$N$_4$ hydrolysis leads to elution of NH$_4^+$/NH$_3$ ions and the formation of hydroxyl silanols [44–46]. The availability of free electrons leads to decomposition of ammonia molecules with the formation of exogenous NO (used by the cells to signal further osteoblastogenesis) together with other RNS, while NH$_4^+$ enters the cytoplasmic space through specific channels as a nutrient. Note that exogenous RNS are also capable to cleave phosphate groups, thus supporting endogenous ALP in increasing phosphate concentration [47]. Concurrently, the presence of silicic acid promotes osteogenesis [10,11].
Recent work has suggested the application of $\text{Al}_2\text{O}_3$ and ZTA in spine fusion and dental implants [48–52]. Biomaterial surface chemistry, and prior clinical experience with $\text{Al}_2\text{O}_3$-coated hip implants suggests caution, however. To combine the long-term structural reliability of ZTA with enhanced bone healing, surface functionalization with highly osteogenic materials like Bioglass®/$\text{Si}_3\text{N}_4$ may be a strategy to protect host tissues from aluminum ions, while promoting osteogenesis.

5. Conclusions

Commercially available ZTA surfaces were functionalized to improve their otherwise poor osteogenic properties. The response of SaOS-2 cells was monitored on: (i) ZTA ceramic surfaces patterned with cylindrical wells before and after filling in with Bioglass®/$\text{Si}_3\text{N}_4$ powder mixtures; and, (ii) ZTA surfaces coated with laser-sintered $\text{Si}_3\text{N}_4$. Results showed that:

a. Osteoblasts cultured on ZTA underwent apoptosis from the surface chemistry of ZTA, such that no osteogenic properties were manifest on ZTA.

b. Surface patterning of ZTA surface with a cylindrical well grid (500 µm in diameter and depth) failed to improve cell proliferation and osteogenesis.

c. Filling the patterned wells with Bioglass® alone increased the amount of mineralized apatite on the ZTA surface, with no impact on cell proliferation.

d. Enhanced osteoblast proliferation and a favorable bone mineral/matrix ratio could be obtained by adding a 10 wt.% fraction $\text{Si}_3\text{N}_4$ to the Bioglass® mixture.

e. Coating ZTA with a few µm thick sintered $\text{Si}_3\text{N}_4$ layer enhanced osteogenesis by imparting the osteogenic properties of $\text{Si}_3\text{N}_4$ onto ZTA.

In conclusion, with both techniques we successfully obtained an improvement of the osteogenic properties of ZTA. This application can be of use in load-bearing applications where a good integration with the human body is required, as in spinal fusion cages, teeth implants and other applications that require contact with the human bone.


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