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Biological and Structural Characterization of Y-TZP for Implant Abutments

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Abstract

The aim of this study was to performed to characterize three different cold isostatic pressed Y-TZP, one being controlled manufacturing, concerning its biocompatibility in contact with cultured human gingival fibroblasts and its crystalline structure by Micro-Raman Spectroscopy.

Keywords: Implant abutments; Y-TZP; Fibroblasts

Introduction

The development of high-strength ceramics such as Yttrium Tetragonal Zirconia Polycrystal (Y-TZP) for application in dental implants reconstructions including abutments and crows has emerged as a viable alternative by your biological and mechanical properties beyond better esthetic results [1]. It is a material that shows improvements in its properties until reaching a stability of the tetragonal phase at room temperature [2]. The Y-TZP chemistry stability ensures its biocompatibility and contributes to its optimal aesthetic and mechanical properties [3,4]. Know the biocompatibility and intrinsic structures of Y-TZPs can predict the clinical success of rehabilitation.

In cases in which the Y-TZP is used as abutments, the contacts with the gingival tissue occurs [5,6]. Fibroblasts are the most numerous resident cells in periodontium. However, they are needed for any implant or abutment materials that are not toxic and to be inert to them [7,8]. The formation of the epithelial attachment is influenced by the material composition of the transgingival implant components [8-10]. A previous study showed that Ti surfaces may play an important role in modulating the cytokine release in cultured Human Gingival Fibroblasts (HGF) [11]. In addition, studies have shown that zirconia bacterial adhesion, inflammatory infiltrate, micro vessel densities and vascular endothelial growth factor expressions are low compared to titanium, a material also used for abutments. This suggests that zirconia maintains a healthy periodontal tissue [12,13].

GROUPS	MATERIAL MANUFACTURER	COMPOSITION	
EP	Experimental	$Zr(hf)O_2$=94.7%	Zirconium Dioxide + Hafium
		Y_2O_3=5.25%	Itrium Trioxide
		SiO_2 = 0.020%	Silicon Dioxide
		Fe_2O_3 = 0.010%	Ferrum Trioxide
		Na_2O = 0.010%	Sodium Oxide
		CL = 0.020%	Clorium
		TiO_2 = 0.010%	Titanium Dioxide
ST	Schuetz – Schuetz (Germany)	$Zr(O_2)$ =< 96%	Zirconium Dioxide
		HfO_2 =>1%	Hafium Dioxide
		Y_2O_3 => 4%	Yttrium Trioxide
		Al_2O_3 =< 1%	Aluminium Trioxide
		SiO =< 0.02%	Silicon Oxide
ZK	Zirkonzahn – Zirkonzahn (Germany)	ZrO_2 = 91.5%	Zirconium Dioxide
		HfO_2 => 3.0%	Hafium Dioxide
		Y_2O_3 = 5%	Itrium Trioxide
		SiO_2 = 0.020%	Silicon Dioxide
		Fe_2O_3 = 0.0050%	Ferrum Trioxide
		Na_2O = 0.01%	Sodium Oxide

Table 1: Groups' names, materials, manufacturers and detailed compositions of the three Y-TZP discs studied. *Compositions according to the Manufacturer and Chemical Engineer information.

A highly localized stress on the zirconia surface results in a phase transformation from tetragonal into monoclinic or cubic forms [14,15]. The cracks dissipation is limited by this crystalline forms change, decreasing the risk of ions release from the bulk of the material. For periodontal tissue, the higher the strength of the material, the lesser damages can occur [16].

Materials and Methods

Y-TZP Blocks (Manufacturing controlled)

The blocks were obtained from isostatic pressing. This process consists of applying pressure in all directions during the pressing. It was used a flexible elastomeric membrane as a matrix. The elastomeric matrix cavity was filled by EP composition and sealed over with a metal plate (Table 1). The matrix outer surfaces were pressed by a fluid that transmits the pressures toward the matrix resulting in the powder compaction (Figure 1). The pressure was 120 MPa, for 5 seconds of each block at the temperature of 25°C of and the humidity of 60%.

Y-TZP discs

The Y-TZP discs EP, ST, ZK used in this study were machined from pre-sinterized zirconia blocks. The discs were prepared with a 14.4 mm diameter and 3.4 mm thickness before the final sintering. During the final sintering, the zirconia discs shrink approximately 20%. The final zirconia disks dimensions were 12 mm in diameter and 2.7 mm in thickness. Ten discs from each group were fabricated and tested (Table 1).

Cell culture

HGFs were primarily cultured as previously described [17,18].

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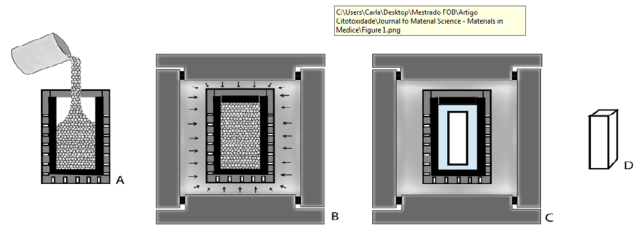

Figure 1: Sequence of Y-TZP EP Blocks Compression. A. The powder was put into the elastomeric matrix. B,C. The pressure was transferred towards the matrix resulting into a block. D. The block ready for use.

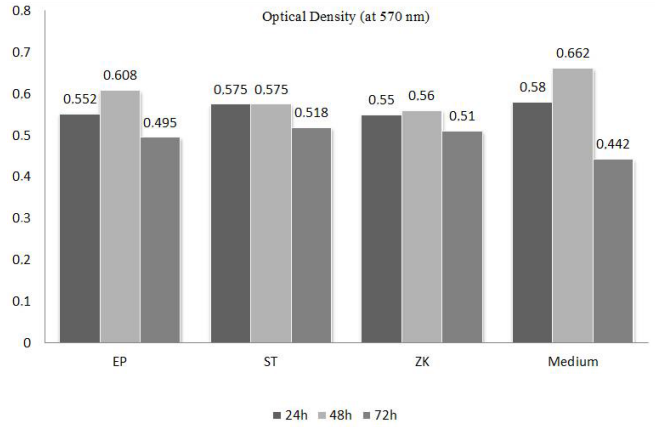

Figure 2: Direct Cytotoxic Evaluation of EP, ST (Shuetz), ZK (Zirkonzhan) Y-TZP discs and the Medium (control group). The data is expressed as the mean values at 24, 48 and 72 hours. No differences were shown for all Y-TZP discs and the control group.

Briefly, the fibroblasts were obtained from the gingival explants of clinically non-inflamed tissue, retrieved from the gingival margin of three volunteer donors after informed written consent in accordance with the Institutional Review Board of the Bauru School of Dentistry, University of São Paulo. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA), and antibiotics (600 µL/mL penicillin, 300 µL/mL gentamicin sulfate, and 100 µL/mL amphotericin B), incubated at 37ºC in a humidified atmosphere of 5% carbon dioxide until confluence and were used between the fourth and eighth passages for the experiments [17].

Cell viability assay

The cell viability was examined with 3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma Aldrich, St Louis, MO, USA).This test is based on the formation of a colored product (formazan) through the mitochondrial succinate dehydrogenase metabolism in cells. The test allows for the evaluation of viable, metabolic active cells. Briefly, the cells (in a density of 2×10^4) were incubated in 24-well plates on zirconia discs for 24, 48 or 72hrs (Directcytotoxic Evaluation-DCE). Concurrently, the cells were incubated with a medium that was previously in contact with the zirconia discs for 24, 48 or 72 hrs (Indirect Cytotoxic Evaluation - ICE). After discarding the supernatants, the adherent cells were treated with 5 mg/mL of tetrazolium salt (MTT; Sigma-Aldrich, St. Louis, MO) in a phosphate buffer and added with the DMEM medium. After incubation at 37°C for 4 hrs, the medium was discarded and Dimethyl sulfoxide (DMSO) was added to solubilize the formazan produced. Afterward, the plates were centrifuged (200g, 7 min). The absorbance of the formazan present in the supernatant was measured at a wavelength of 570 nm (Fluostar Optima, BMG Lab tech, Ortenberg, Germany). The mean absorbance values were corrected for a blank (medium only) and the results were reported as optical density.

Micro Raman Spectroscopy

Two discs from each group were evaluated. The Micro-Raman measurements were carried out at room temperature in backscattering geometry by means of a Jobin Yvon Micro Raman system, model T64000 (Groupe Horiba- Longjumeau/France) at the Physics Institute of São Paulo State University (UNESP, Bauru, SP, Brazil). The 514.5 nm (2.41 eV) radiation of an argon ion laser (Spectra Physic, Inc., California/USA) was used for the excitation. The beam was focused by means of a microscope of 500x magnification, the laser spot had a diameter of about 5 µm. No polarization analyzer was used for the scattered beam. In order to avoid any thermal damage, the laser power was kept as low as 10 mW. The Raman spectra was analyzed by means of a double subtractive monochromator, with a focal length of 0.64 m and equipped with a diffraction grating with 1800 grooves/mm. The slit width was set to 200 µm which provided us with a spectral resolution of about 2 cm^{-1}. The 5 spectra from each zirconia discs were recorded with a CCD (Spectra One -Group Horiba – Longjumeau/France) camera.

Statistical analysis

The cellular viability data was analyzed by the two-way analysis of variance test followed by the Tukey post-test. Values of $p < 0.05$ were considered statistically significant.

The 5 spectra within each group had exactly the same spectral patterns and a mean was obtained from each group. The spectra among the groups were compared with regards to the differences among the wave numbers and the broadening bands.

Results

Cell viability

The optical densities of the formazan produced by the HGF in the EP, ST and ZK discs groups were measured after 24, 48 or 72 hrs via the MTT assay. For the Direct Cytotoxic Evaluation (DCE), no significant difference ($p>0.05$) was detected between the EP, ST and ZK zirconia discs regarding the cell proliferation, regardless of the time evaluated (Figure 2). Similarly, no significant difference ($p>0.05$) was observed between the EP, ST and ZK zirconia discs regarding the cell viability at 24 hrs for the Indirect Cytotoxic Evaluation (ICE). Conversely, a significant increase in the cellular viability was observed for the EP and ST discs at 72hrs when compared to 48hrs ($p<0.05$) (Figure 3).

Micro Raman Spectroscopy

The peaks used in this study corresponded to the main bands related to the zirconia component [14]. Strong peaks were found at 260, 320, 464 and 642 cm^{-1}. Additional weak features were observed for all groups at 147cm^{-1}. Those spectra features were very similar among the groups with no visible broadening of any band. However, a broadening band was observed only for the ZK at 403 cm^{-1}. The spectra of each group were composed of 924 data points in a spectral region between ~100 and 700 cm^{-1}. The mean spectra of the groups have the same baseline; however, they were dissociated to improve the visualization of each spectrum (Figure 4).

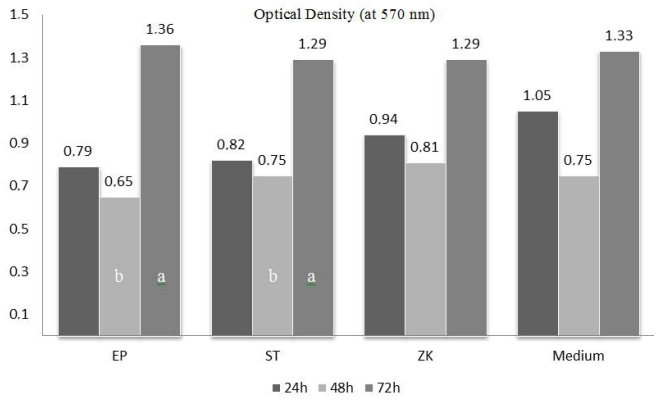

Figure 3: Indirect Cytotoxic Evaluation of EP, ST (Shuetz), ZK (Zirkonzhan) Y-TZP extracts and the Medium (control group). The data is expressed as the mean values at 24, 48 and 72 hours. A difference was shown between the cell viability at 72 hrs (letter a) compared to 48 hrs (letter b) for the EP and ST.

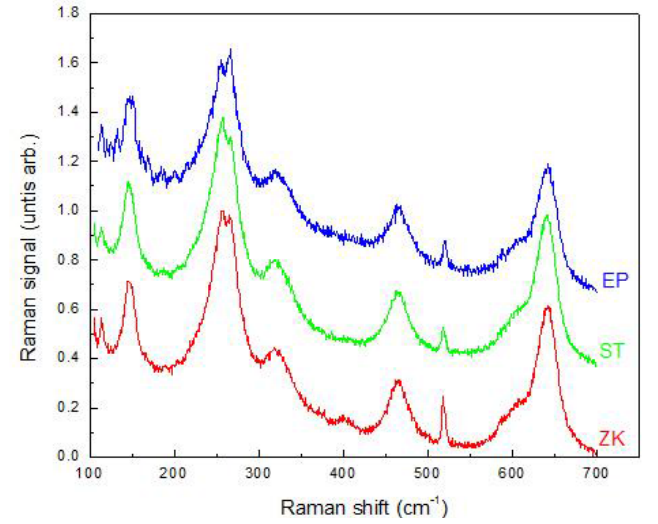

Figure 4: Raman spectra of the EP, ST and ZK groups. The peaks identified are related to Yttria tetragonal Y-TZP polycrystalline structures (~260, 320, 464, and 147cm^{-1}).

Discussion

The great advantage of controlling the production of blocks of Y-TZP is able to dominate the processing method used and the source and amount of raw material used. EP were designed to obtain a material with controlled composition by cold isostatic pressing and pre-sintering in order to obtain a Y-TZP inert abutments since it is need to be nontoxic in order to help the attachment and growth of the surrounding gingiva [8]. The composition of EP differs from the others by the presence of titanium oxide (TiO_2), chlorine (Cl), iron oxide (Fe_2O_3) and sodium oxide (Na_2O_3). The amount of these elements varied from 0.010 to 0.020% by volume and little representation did not affect negatively the results of biocompatibility.

The direct cytotoxic evaluation simulated the direct contact between the abutment's Y-TZP and oral gingival fibroblasts at 24, 48 and 72 hrs. The EP biological behavior was similar to the two other commercially available materials, regardless of the periods of evaluation, suggesting that the cellular viability is not affected by this material (Figure 2). In a previous study, $Zr0_2$ abutments showed higher amounts of collagen deposition as well as larger fractions of fibroblasts and leukocytes adhered to the surface than the connective tissue interface of the Ti abutments, suggesting that the soft tissue healing to zirconia's abutments is better than alloy abutments [10]. When comparing the zirconia to the feldspatic porcelain, the adhesion capacity and the cellular growth rate of the fibroblasts were strongly increased [7].

The indirect cytotoxic evaluation was done to simulate the indirect contact of the gingival fibroblasts from the surrounding gingival tissues with the zirconia's abutment at 24, 48 and 72 hrs. After 72 hrs, there was a proliferation of cultured HGFs compared to 48 hrs in the EP and ST disc groups (Figure 3). It seems that the direct contact of the cells with the discs had no impact in the HGF metabolism, a later exposition of the cells that are far from the EP and ST discs (indirect contact) could benefit the proliferation rate by increasing the cell viability. EP has TiO_2 and Cl. These components are sediments not possible to separate during zirconium dioxide extraction, however, Zirconia containing TiO_2 in larger percentages improved fibroblast cells adhesion on their surfaces [18].

Chemically, the phase transformation caused changes in the Zr-O bonds detected by the Raman spectroscopy [19,20]. Raman scattering is best suited for systems in which the electron cloud may be deformed [21]. More specifically, during the Raman measurements, when a laser light strikes a sample, it acts by deforming the electron cloud, which will cause scattering and elements detection [21].

The bands are a set of wave numbers which contain a main wave number related to the peak of the band. The current study identified five wave numbers of the peaks (~260, 320, 464, 642 and 147 cm^{-1}) similar to the frequencies of vibration corresponded to the six Raman active bands of the tetragonal Y-TZP [15] (Figure 4). According to some studies it is possible to identify the three main phases (monoclinic, tetragonal and cubic) because they exhibits changes in the zirconium oxide bond angles and lengths, indicating the phase transformation [15,19,22]. The main broad bands shown in our study were at ~260, 464, and 642 cm^{-1} (Figure 4). They are due to tetragonal or cubic phases [15]. Different chemical stabilizers and crystal size probably caused few spectral variations at the tetragonal/cubic phases of this study (~260, 464, and 642 cm^{-1}) [22,23] (Figure 4). The chemical stabilizers are almost the same for the three studied groups, except for some above described tracer elements for the EP (titanium dioxide and Clorium) and for the ST (aluminium trioxide). The EP Y-TZP was pressed by isostatic pressure, which is a powder compression inside a flexible mold under a pressurized fluid action. This procedure ensures a homogenous load distribution under the mold surface. This hindrance that the pressure applied is uniformly distributed to all the regions, causing density gradients upon the formed Y-TZP blocks [24]. In contrast, the uniaxial pressure causes the friction among the powder particles and also the friction between them and the mold surfaces [24,25].

These differences mainly compared to the ST and ZK, can be maintained for the EP, since the pressing of the chemical components ensures the mechanical stability, avoiding any premature phase transformations identified by the bands (Figure 4). The higher the quality in the pressing step, the lesser the intrinsic defects are produced, which can influence the grain growth and density at the grain boundaries during the elements compaction. Commercially Y-TZPs phase transformations were identified by Raman after fatigue, which is expected for that moment, not before clinical use [20]. Therefore, we suppose that the Y-TZPs tetragonal phase shown in the current study can indicate that the intrinsic stability can preserve the fibroblast cells viability in contact with them.

Conclusion

Within the limitation of this study, all the Y-TZP showed the biocompatibility and crystalline structures expected for Y-ZTP and recommended for use as implants abutments.

Acknowledgment

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