Zirconia-Reinforced Lithium Silicate Biocompatibility Polished in Different Stages–An In Vitro Study

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Abstract

The biocompatibility of zirconia-reinforced lithium silicate (ZLS) (Suprinity; Vita Zahnfabrik, Germany) for CAD/CAM was assessed *in vitro* with human gingival fibroblasts polished in different stages compared to yttria-stabilized tetragonal zirconia polycrystalline's (Y-TZP) (ProtMatMateriaisAvançados, SP, Brazil) performance. **Aim:**The study evaluated 72 test specimens measuring 14mm of diameter and 2mm of thickness, being 18 Y-TZP; 18 ZLS crystallized; 18 ZLS polished before and after crystallization; 18 ZLS polished after crystallization.

The specimens were tested for cell proliferation and viability using Trypan blue exclusion method.

The results showed that the crystallized ZLS surface with no polishing favored cell proliferation within 48 and 72 hours crystallization) showed no significant difference from Y-TZP in the same period.

Regarding cell viability, zirconia-reinforced lithium silicate crystallized only or polished before and after crystallization show results similar to those of Y-TZP, with a favorable biological profile for use in peri-implant regions.

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Introduction

The constant development of new technologies in dentistry has provided hybrid restoration materials capable of integrating, in a single material, functionality, aesthetics and biology. The material used in the prosthetic abutment should allow the formation of a gingival tissue barrier that will act as a protective sealing between the oral environment and the underlying bone.[1] The integrity of this region determines the long-term therapeutic results.[2] The junctional epithelium, the connective insertion and the peri-implant sulcus constitute the interface between gingival tissue and the contact material surface.[1,3-5]

Several *in vitro* and *in vivo* studies compared titanium and yttria-stabilized tetragonal zirconia polycrystalline. These materials provide adequate conditions for the peri-implant tissues healing and showed similar mechanical and biological performances.[6-10]

Lithium disilicate has been clinically proven and has showed favorable mechanical and aesthetic properties. On the other hand, its biological profile is unfavorable, showing cytotoxicity.[11,12] Its aging, however, reduces the surface cytotoxicity.[13]

New ceramic materials have been introduced into the dental market and are classified in several ways according to clinical use, composition, firing temperature, processing method, microstructure, translucence, abrasiveness, and resistance to fracture.[14-17] Hybrid ceramics, such as zirconia-reinforced lithium silicate (VITA-Suprinity) have showed compatible results to those of the well-established lithium disilicate.[18,19] However, there is a lack of reports on its surface's biological properties in peri-implant regions contacting the gingival tissue.

In this context, the present study aims to assess the *in vitro* influence of zirconia-reinforced lithium silicate (Suprinity - Vita Zahnfabrik) on the

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proliferation of human gingival fibroblast, and the effect of surface polishing in different stages.

Materials and methods

This study was approved by the Human Research Ethics Committee, CAAE: 1.795.630 and conducted at Faculdade São Leopoldo Mandic.

72 samples were fabricated measuring 14 mm of diameter and 2 mm of thickness. Of these, 54 were made of zirconia-reinforced lithium silicate (ZLS) (Suprinity-Vita Zahnfabrik, Germany) (18 crystallized ZLS; 18 polished before and after crystallization; 18 polished after crystallization); 18 test specimens made of yttria-stabilized tetragonal zirconia polycrystalline (Y-TZP) (Prot Mat Materiais Avançados, Brazil) (Table 1).

Yttria-stabilized tetragonal zirconia polycristalline	polished
Zirconia-reinforced lithium silicate	Polished before and after crystallization
Zirconia-reinforced lithium silicate	Polished after crystallization
Zirconia-reinforced lithium silicate	No polishing (only crystallization)

Table 1. Surface Description.

Zirconia-reinforced lithium silicate test specimens were obtained from CAD/CAM CEREC blocks, which were cut by a diamond disc in a Struers Minitom (BUEHLER Diamond Wafering Blade Series 15 High Concentration Arbor Size 1/2" 12.7mm, USA), under water cooling at 200rpm (Figure 1).

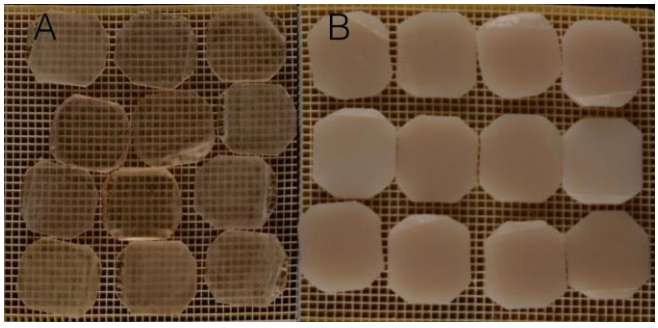

Figure 1. Test Specimens. A) Before Crystallization; B) After Crystallization.

Yttria-stabilized tetragonal zirconia test specimens were obtained from Yttria-stabilized tetragonal ZrO_2blocks (3% mol).

Polishing was done using a basic sequence of two polishing rubbers Pink (EVE Diatwist medium, Germany) and Grey (EVE Diatwist fine, Germany) with W&H Perfecta 300 electric motor (W&H Dentalwerk, Brumoos, Austria).

The discs were cleaned and disinfected according to Wataha& Lockwood's[20] (1998) method. Prior to the cell culture, the discs were washed with water, rinsed three times with deionized water in ultrasonic tank for five minutes, wrapped and sterilized in autoclave.

Cell lines

The human gingival fibroblast cell lines were obtained from fragments of human gingiva stored at the Cell Bank of the Cell Culture Laboratory of the Institute and Research Center São Leopoldo Mandic (Campinas-SP, Brazil) retrieved from patients submitted to periodontal surgery. The cells were isolated with primary culture from three different patients (individual genotypic and phenotypic variation) using the Explant technique. These were done in biological triplicate for all the following experiments.

Cell culture assay

Cells were culturedat the Cell Culture Laboratory of the Buccal Pathology Course of the Institute and Research Center São Leopoldo Mandic (Campinas, São Paulo) in Dulbecco-modified Eagle medium (DMEM, Sigma Chemical Co, USA), supplemented with 10% bovine fetal serum (Cultilab, Campinas) and 0.1% antibiotic-antimycotic solution (Sigma, USA). The cultures were kept in a CO_2incubator (Thermo Scientific Forma, Series II, Water Jacket, USA), in humid atmosphere composed of 95% of air and 5% of carbon dioxide.

To maintain cell viability, all procedures were conducted in a biosafety cabinet to keep all materials and substances used in the cell culture sterile.

When achieving sub confluency, the cells were plated in 24-wells plates at 20,000 cells/well (~110 cells/mm^2) on the zirconia-reinforced lithium silicate and on yttria-stabilized zirconia test specimens for different times (24.48 and 72 hours) and used in the experiments described in the following.

All experiments were done in biological triplicates to assure reliability considering individual genotypic and phenotypic variations.

Experimental groups

For each biological analysis, four experimental groups with 18 test specimens each were defined, as shown in Table 1.

Cell viability was given by Trypan blue exclusion method.

Cell proliferation was determined by a single tester, blind for experimental group, who counted the number of stained and non-stained cells with reverse phase microscopy (Nikon Eclipse TS100, New York, USA).

Cell precipitates were suspended in 1 ml PBSA. 10µl of this solution was then dispensed in a test tube added of 10 µl Trypan blue (Sigma, USA). Following that, 10µl of this new solution was transferred to a hemocytometer or Neubauer Chamber (Boeco, Hamburg, Germany) to perform cell count in a reverse-phase microscope (Nikon Eclipse TS100, New York, USA). Cell counts were given by:

$$\text{Total number of cells} = \frac{\text{Number of cells x Initial Vol. x Dilution X } 10^4}{\text{Number of fields used in the count}}$$

Statistical analyses

Variance analysis with two criteria was applied to compare proliferation and viability of human gingival fibroblasts on different surfaces and plating times. Tukey's test was used for multiple comparisons. Statistical analyses were performed on SPSS 23 (SPSS Inc., Chicago, USA), with 5% of significance.

Results

The variance analysis with two criteria applied to the gingival fibroblasts proliferation data showed a significant effect of surface-time interaction (P<0.001, with a test's power of 93.2%).

Tukey's test showed no significant difference between contact surfaces regarding fibroblasts proliferation in the first 24 hours of plating. At 48 hours, however, the number of fibroblasts per area was significantly higher on the crystallized zirconia-reinforced lithium silicate in comparison to all other surfaces. At 72 hours, there was no significant difference between crystallized zirconia-reinforced lithium silicate polished before and after crystallization and the surface polished only after crystallization regarding gingival fibroblasts proliferation (Figure 2 and 3). Only crystallized zirconia-reinforced lithium silicate showed a proliferation rate significantly higher than that of yttria-stabilized tetragonal zirconia polycrystalline.

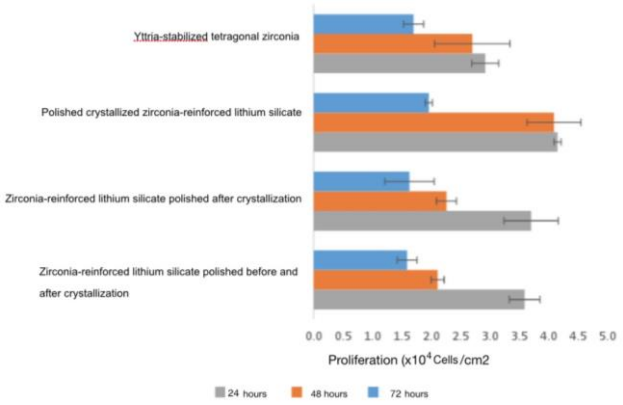

Figure 2. Average Values and Standard Deviation of Cell Proliferation (number of cells/cm^2) according to Surface and Plating Time.

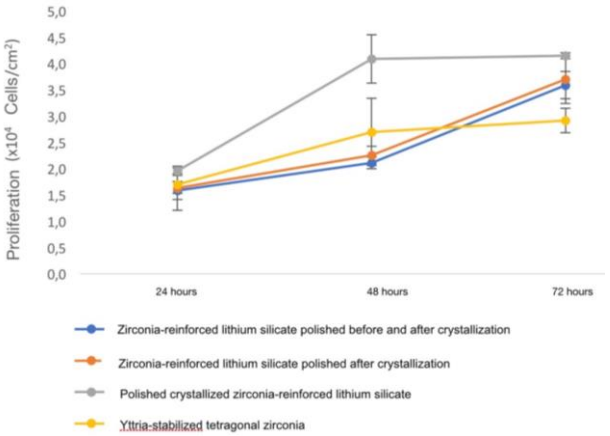

Figure 3. Cell Proliferation according to Surface and Plating Time.

For both zirconia-reinforced lithium silicate surfaces polished before and after crystallization or polished only after crystallization, proliferation was peaked on the third day of plating, with no significant differences between first and second days. On the other hand,

crystallized zirconia-reinforced lithium silicate and yttria-stabilized tetragonal zirconia polycrystalline proliferation peaked after 48 hours, with no significant difference between 48 and 72 hours of plating (Figure 3 and 4).

Regarding cell viability, the variance analysis with two criteria identified a significant effect of surface-time interaction (P<0.001, with test's power of 91.1%).

Tukey's test revealed that, at 24 hours of plating, crystallized zirconia-reinforced lithium silicate and yttria-stabilized tetragonal zirconia polycrystalline surfaces favored fibroblasts viability in comparison to the samples of post-crystallization polished zirconia-reinforced lithium silicate. When the zirconia-reinforced lithium silicate surface was polished before and after crystallization, viability was intermediary since it did not significantly differ from the other groups. At 48 hours, crystallized zirconia-reinforced lithium silicate and yttria-stabilized tetragonal zirconia polycrystalline showed no difference in viability results from the other surfaces (Figure 4 and 5).

For zirconia-reinforced lithium silicate, whether polished or not, viability at 48 and 72 hours of plating was significantly higher than that at 24 hours (Figure 4 and 5). On yttria-stabilized tetragonal zirconia polycrystalline surface, viability after 72 hours did not significantly differ from that observed in previous times.

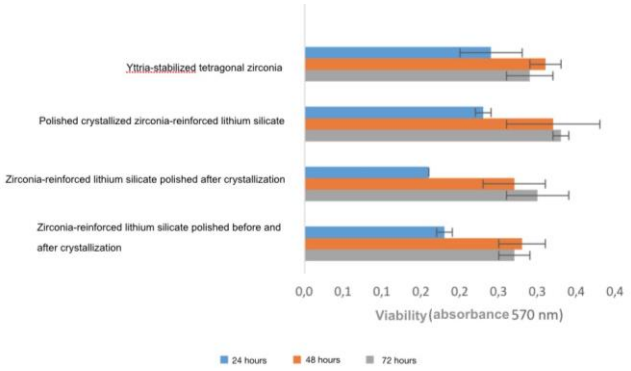

Figure 4. Average Values and Standard Deviation for Cell Viability as Given by Absorbance, According to Surface and Plating Time.

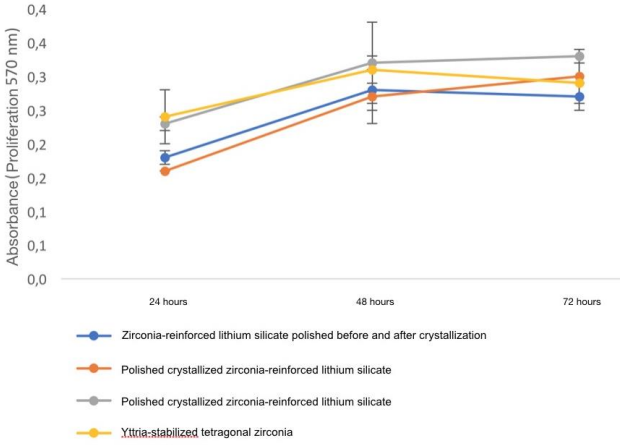

Figure 5. Cell Viability according to Surface and Plating Time.

Discussion

The integration of CAD/CAM with implantodontics has opened new avenues for rehabilitation planning. Associated to that, a growing and diversified market of ceramic materials and its convenient milling mode makes these materials increasingly attractive to professionals. The evolution of materials has opened a range of metal-free options, such as the new categories of hybrid materials like resin-infiltrated ceramics networks, resin nanoceramics and zirconia-reinforced lithium silicate that present mechanical properties similar to those of lithium disilicate.[18] However, there is little information reporting the cytotoxic effects of zirconia-reinforced lithium silicate for use in peri-implant regions, which justifies the present study

. Zirconia-reinforced lithium silicate blocks used here are industrially manufactured, are homogeneous, have no defects and are clinically accepted.[21,22]

Understanding peri-implant biology described by Waerhaug[3] (1978), Berglundh et al.[1] (1991), Berglundh & Lindhe[4] (1996) and Abrahamsson et al.[5] is critical for planning and performing complex cases.[23]

Y-TZP was chosen as control since several works have shown its high degree of biocompatibility[7,8,24-27] and because it has been successfully used in biomedicine and dentistry, mainly in peri-implant areas as prosthetic abutments.[7,8]

Titanium and zirconium oxide abutments offer the adequate conditions for soft tissues healing. However, given the weak adhesion between zirconia abutments and coating ceramics, zirconia implant-supported restorations

exert a purely mechanical retention where the ceramics were stratified over the abutment.[7]

Cytotoxicity of lithium disilicate diminishes with the material's aging in a biological environment, possibly improving cell responses. However, most ceramic materials do not present high biological risks.[11,13] These findings disagree with Tetè et al.,[28] that showed that lithium disilicate presents cytotoxicity and poor biological behavior, indicating a need for more studies that focus on the reaction of biological tissues to this material. The ceramics presentation, be it applied, injected or machined and its polishing influences results as described by Brackett et al.[12]
New ceramics compounds can be a biological and aesthetical solution for rehabilitations, joining together the best features of each material.

The in vitro proliferation and cell viability results showed in the present study indicate that zirconia-reinforced lithium silicate provide similar, if not better, conditions than Y-TZP in the first 48 and 72 hours, which are important for the stabilization of soft tissues in restorations over implants[2]. No toxicity has been observed on the studied surfaces, no lise nor cell death, and cell proliferation have been observed in all time intervals - 24, 48 and 72 hours. Cell proliferation at 48 and 72 hours was significantly higher for ZLS (without polishing/ only crystallized) compared to Y-TZP, while the other groups showed no significant difference. Samples of polished ZLS showed cell proliferation after 72 hours, indicating a possible negative effect of polishing, such as the release of material or surface modification. Brackett et al.[12] highlighted the possibility of a cytotoxic potential in the case of repolishing or even brushing.

After 24 hours, cell viability of crystallized-only ZLS and Y-TZP were significantly higher than ZLS polished after crystallization. On the other hand, ZLS samples polished before and after crystallization were in an intermediate position relative to the other groups, with no significant differences. Initial phase quality influences directly on the cells' ability to proliferate and differentiate when in contact with the surface.[29] Thus, this result may indicate that polishing the material before crystallization might improve the quality of the interaction of the biomaterial and repolishing might be deleterious during the first hours of interaction.

The results shown here indicate that zirconia-reinforced lithium silicate crystallized-only or polished before and after crystallization can be used in peri-implant regions, with cell growth similar to that presented by Y-TZP. Further studies are needed to verify ZLS efficacy as a peri-implant restoration material. The promotion of adhesion, the good biological and mechanical properties, and the aesthetics of this hybrid material can represent another paradigm shift in implant-supported aesthetical restorations.

Conclusions

The characteristics presented in this *in vitro* study regarding proliferation and viability of human gingival fibroblasts on zirconia-reinforced lithium silicate crystallized or polished before and after crystallization are similar to those of yttria-stabilized tetragonal zirconia polycrystalline, with a favorable biological profile for use in peri-implant regions.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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