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Diatomite filler for resin composites application – A new approach for materials improvement

Cargas de diatomita para aplicações em resinas compostas – Uma nova estratégia para aprimoramento dos materiais

Relleno de diatomita para la aplicación de compuestos de resina: Un nuevo enfoque para la mejora de los materiales

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Abstract
The aim of this study was to evaluate physical-mechanical properties, degree of conversion, and chemical stability of a nanohybrid composite containing diatomite as filler. Degree of conversion (DC%) of diatomite-containing composite (Zirconfill®) was performed using FTIR immediately, and 1-, and 7-days post-curing. SEM was conducted to evaluate the surface of the resin after curing and measure particles size. Also, elemental characterization was performed to verify the major components of the composite through EDS. Mechanical characterization using 3-point bending test was performed prior and after thermo-cycling (10000 cycles) (n=10). Knoop microhardness (KHN) was used to characterize mechanical stability after chemical solutions aggression (water, juice, coffee, coke) up to 28 days (n=10/solution). After data normality evaluation using Shapiro-Wilk, One-way ANOVA and Tukey post hoc were conducted to verify differences between groups for DC% and mechanical properties. Split-plot ANOVA was used to compare groups for microhardness characterization (α=0.05). Immediate DC was 60% and significantly increased up to 80% at 7 days ($p<0.05$). Flexural strength of the diatomite-containing composite was 136.2 (23.7) MPa and significantly decreased to 75.1 (10.2) as a result of thermo-cycling. The flexural modulus was not significantly affected by the thermo-cycling ($p>0.05$). All the dietary solutions affected the KHN of the composite up to 21 days. For 28 days, the KHN evidenced and stabilization regarding all the solutions. Diatomite-containing composites present good degree of conversion and relevant mechanical properties and demonstrate time-dependent stability against chemical degradation.
Keywords: Diatomaceous earth; Composite resins; Strength.

Resumo

O objetivo deste estudo foi avaliar as propriedades físico-mecânicas, grau de conversão e estabilidade química de um compósito nano-híbrido contendo carga de diatomita. O grau de conversão (DC%) do compósito contendo diatomita (Zirconfill®) foi avaliado utilizando FTIR imediatamente, 1 dia e 7 dias após a polimerização. Microscopia Eletrônica de Varredura foi utilizada para avaliar a microestrutura após a polimerização e medir as partículas. A análise elementar dos componentes do compósito foi realizada por meio de EDS. A caracterização mecânica foi realizada utilizando flexão de três pontos (n=10) antes e após termo-ciclagem (10000 ciclos). Microdureza knoop (KHN) foi usada para caracterizar a estabilidade mecânica após agressão por agentes químicos da dieta (água, suco, café e refrigerante) até 28 dias (n=10/solução). A normalidade dos dados foi verificada pelo teste Shapiro-Wilk. Anova One-way e Tukey post hoc foram conduzidos para verificar diferenças para o grau de conversão e flexão. Anova de medidas repetidas foi usada para comparer os grupos para microdureza (α=0.05). O grau de conversão imediato foi de 60% e aumentou significativamente (80%) após 7 dias ($p<0.05$). A resistência à flexão foi de 136.2 (23.7) Mpa e não foi significativamente afetada após a ciclagem (75.1) ($p>0.05$). O módulo flexural também não foi afetado pela termociclagem. Todas as soluções testadas afetaram a KHN do compósito até 21 dias. Para 28 dias, os valores de KHN estabilizaram para todas as soluções. O compósito contendo diatomita demonstra bom grau de conversão e propriedades mecânicas relevantes além de uma estabilidade contra degradação química que é tempo-dependente.
Palavras-chave: Terra de diatomáceas; Resinas compostas; Resistência.

Resumen

El objetivo de este estudio fue evaluar las propiedades físico-mecánicas, el grado de conversión y la estabilidad química de un compuesto nanohíbrido que contiene diatomita como relleno. El grado de conversión (DC%) del compuesto que contiene diatomeas (Zirconfill®) se realizó usando FTIR inmediatamente y 1 y 7 días después del curado. Se realizó SEM para evaluar la superficie después del curado y medir las partículas. Se realizó la caracterización elemental para verificar los componentes mayoritarios del compuesto a través de EDS. La caracterización mecánica mediante la prueba de flexión de 3 puntos se realizó antes y después del termociclado (10000 ciclos) (n = 10). Se utilizó la microdureza Knoop (KHN) para caracterizar la estabilidad mecánica después de la agresión de soluciones químicas (agua, jugo, café, coque) hasta 28 días (n=10/solución). Después de la evaluación de la normalidad de los datos usando Shapiro-Wilk, se realizaron ANOVA unidireccional y post hoc de Tukey para verificar las diferencias entre los grupos para el % de DC y las propiedades mecánicas. Se usó ANOVA de parcela dividida para comparar grupos para la caracterización de microdureza (α = 0.05). La DC inmediata fue del 60 % y aumentó significativamente hasta el 80 % a los 7 días ($p<0,05$). La resistencia a la flexión del material fue de 136,2 (23,7) MPa y disminuyó significativamente a 75,1 (10,2) como resultado del termociclado. El módulo de flexión no fue afectado significativamente por el termociclado ($p>0.05$). Todas las soluciones dietéticas afectaron el KHN del compuesto hasta 21 días. Durante 28 días, la KHN evidenció una estabilización respecto a todas las soluciones. Los compuestos que contienen diatomita presentan un buen grado de conversión y propiedades mecánicas relevantes y demuestran una estabilidad dependiente del tiempo frente a la degradación química.
Palabras clave: Tierra de diatomeas; Resinas compuestas; Resistencia.

1. Introduction

Resin composites are, currently, the most frequent material used in dental restorative process on clinical practice. The mechanical properties and esthetics support their use to anterior and posterior restorations (Ferracane, 2011). However, the composites are still susceptible to fractures, margin degradation and, secondary caries remains as the most common reason to replacement of restorations (Da Rosa Rodolpho et al., 2011; Mjör et al., 2000; Opdam et al., 2010). In order to improve the properties of the composites, many researchers have been developed with different alternatives to modify the material structure. Regarding composites matrix, some studies have focused on concerns about bisphenol A toxicity, or methacrylate's instability, by changing the functional groups (Fugolin et al., 2019), or developing antimicrobial monomers (de Souza Araújo et al., 2018; Imazato et al., 2014).

However, when looking for the filler particles, the main changes were more related to reducing size and modifying shape. Nowadays, most common particles used are barium glass and zirconium with spheric shape and nanometric or nano and micrometric sizes (nanohybrid composites) (Ferracane, 2011). The nanocomposites, which combine nanoparticles and their clusters, were an evident development of dental materials. It seems legitimate to affirm that these materials, due to their excellent polishing capacity, offer good strength and final aesthetics (Silikas et al., 2005). Moreover, it´s known that size, shape and

amount of composite inorganic particles influence its surface roughness after polishing (Ferracane, 2011; Stoddard & Johnson, 1991).

In demand for further improvements in mechanical properties and matrix/filler interactions, some porous fillers have been incorporated in dental composites (Wang et al., 2011). In this sense, a diatomite-containing composite is now available. Diatomite is a porous silicate derived from diatomaceous algae and it presents large surface area, low density, it has been used to increase mechanical properties in polymeric materials, and it is less expensive than others filler materials (Losic et al., 2009). Given the recent advances in nanotechnology research, it is possible to control diatomite pore size and morphology which can improve photonic, mechanical, absorptive and diffusive properties (Losic et al., 2009). In addition, this porous silica has been recently used as a drug delivery system for therapeutic and regenerative purposes (Maher et al., 2018; Ruggiero et al., 2014; Tamburaci & Tihminlioglu, 2017).

Apart from that, there is evidence that diatomite incorporation results in higher mechanical properties into polymeric materials for different applications (Cacciotti et al., 2019; Liang, 2009; Wang et al., 2011). By this way, the effects of diatomite incorporation in dental composites could be an interesting alternative regarding the monomer interpenetration trough diatomite pores during polymerization and better entanglement between matrix and filler (Wang et al., 2011) which can result in higher degree of conversion and good mechanical properties. However, from the best of our knowledge, there are feel studies regarding chemical and mechanical properties of diatomite-containing dental composites. Thus, the aim of this study is to perform the physicochemical characterization of a novel nanohybrid composite with diatomite filler content Zirconfill® and its resistance to degradative conditions *in vitro*.

2. Methodology

Experimental design

All the experiments in this study were performed *in vitro*. In order to characterize Zirconfill® (BM4, Maringá, PR, BRA), a single nanohybrid resin composite with the presence of diatomite in the composition. Thus, we performed the following analyses: degree of conversion (DC), polymerization shrinkage, surface and filler characterization, flexural strength, and Knoop Microhardness (KHN).

Degree of conversion

The degree of conversion (DC) of the resin was measured using an FTIR Vertex 70 (Bruker Corporation – Billerica, MA - USA). All the measurements were made using absorbance in near-IR spectra with a 4 cm^{-1} resolution, 32 scans with a range of 4000 to 1000 cm^{-1} and 10kHz scanner velocity. Resin samples were inserted into a silicon matrix (5x1mm), then transferred to the FTIR device before polymerization to quantify the carbon double bonds peak. After the initial measurement, the samples were photocured with a photocuring unit Valo Cordless (Ultradent Products Inc., South Jordan, UT, USA) 1200 mW/cm^2 for 40 seconds and the absorbances were measured again to verify the variations in the peak of the double bond. The DC was also measured at 24h and 7 days after polymerization. The following formula was used to quantify the DC of the samples and the values were expressed in percentage: $DC(\%) = [1 - (R_{cured}/R_{uncured})]$.

Surface and filler characterization

Surface and filler characterization were performed using Scanning Electron Microscopy (SEM). Two samples of the diatomite-containing composite were prepared (2x2mm), using a silicon mold, and photocured using Valo cordless (Ultradent) for 40 seconds. The samples were mounted in metallic stubs and sputtered with gold and analyzed in SEM. Besides, one sample

with the same dimensions was mounted in a stub and sputtered with carbon in order to analyze the elements present in the composite structure by EDS. Regarding filler characterization, 2 g of the composite were placed in a vial and immersed in organic solvent (acetone) and centrifuged until complete removal of the matrix preserving only the inorganic part as described before by Fronza et al. 2015 (Fronza et al., 2015) and Ruivo et al., 2019 (Ruivo et al., 2019).The SEM and EDS analyses were made in microscope Tescan Mira 3 (Tescan Orsay Holding, Kohoutovice, CZ).

Flexural strength and flexural modulus

In order to evaluate the flexural strength, the ISO 4049 (International Organization for Standardization, 2009) protocol was followed. Briefly, 20 specimens 25 x 2 x 2 mm of the composite were prepared using a metallic matrix and photocured with Valo Cordless (Ultradent) for 20 seconds in three different regions of the sample. After photocuring, the samples were immersed in distilled water for 24h at 37 °C. Then, 10 samples were submitted to a 3-point bending test and the other 10 were submitted to 10000 thermal cycles (5°C – 55°C) prior to mechanical evaluation. To perform the 3-point bending test, the specimens were put in a universal testing machine (INSTRON, Northwood, MA, USA) with 1mm/min of crosshead speed. After the specimen fracture, flexural strength (MPa) and the flexural modulus were recorded for comparisons between groups with and with no thermal cycles.

Knoop Microhardness (KHN)

Forty discs (4 x 2 mm) of the diatomite-containing resin were prepared using a silicon matrix on a glass plate. The resin was inserted into the matrix, covered with a polyester strip and photocured for 20 seconds using the Valo Cordless photocuring unit (Ultradent) with 1200mW/cm² of irradiance. Then, the discs were embedded in PVC tubes using acrylic resin. After acrylic resin set reaction, the samples were polished with silicon carbide paper (#400, #600, #1200, and #2000), felt disc and abrasive paste.

After polishing procedures, the Knoop microhardness (KHN) of all samples was evaluated in a micro indenter HMV-2 (Shimadzu – Japan). Three measurements were made on each sample using a load/speed ratio of 50g/5s. The global mean of the samples was obtained, and these data recorded as the baseline. Subsequently, the samples were divided into 4 groups (10 discs/group) and immersed in different solutions (water, coffee, coke, orange juice) and left under 37 °C for 28 days. The solutions were changed every day. Every 7 days, the samples were removed from the solutions, cleaned with distilled water and the microhardness measurements retaken for intragroup comparisons.

Statistical analysis

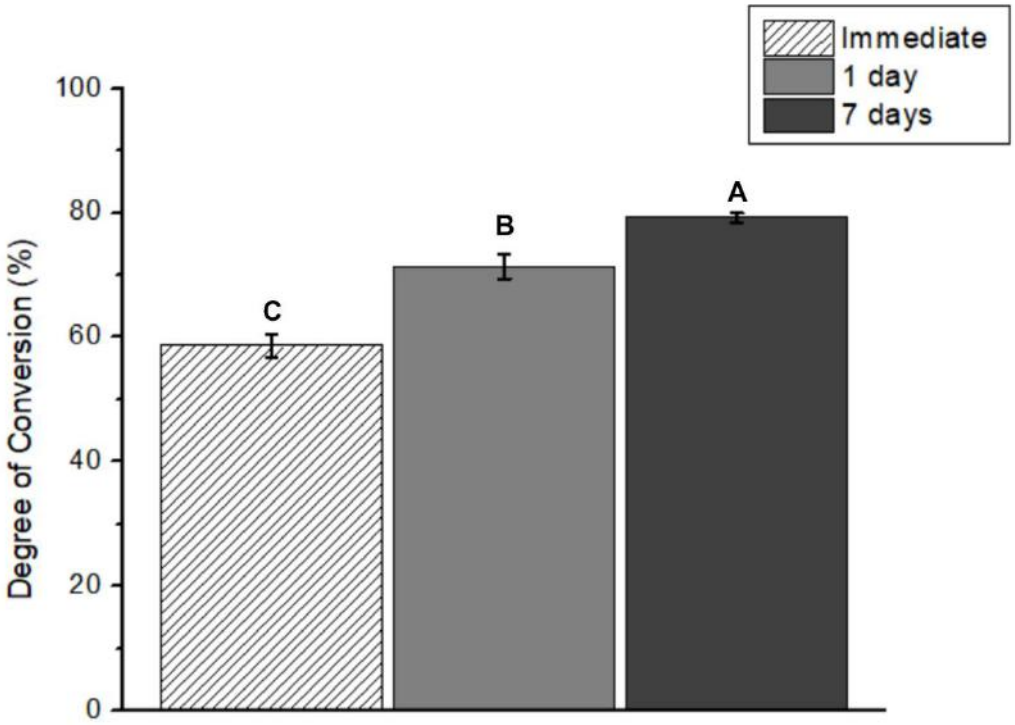
Data homogeneity for all tests was evaluated using Shapiro-Wilk. Degree of Conversion and Flexural Strength were analyzed using One-Way ANOVA. Knoop Microhardness data were compared using split-plot ANOVA. The comparisons between groups were made with Tukey test considering $\alpha=0.05$. All the analyses were made using software SPSS Statistic 21 (IBM statistics corp., Armonk, NY, USA).

3. Results

Degree of conversion

The immediate, 1 days and 7 days degree of conversion (DC) for diatomite-containing composite (Zirconfill®) is exhibited in Figure 1. The immediate conversion it was about 60% and it presented a significant increase ($p<0.05$) for different time points reaching ~80% of conversion after 7 days.

Figure 1 - Mean and standard deviation of diatomite-containing composite's degree of conversion (%) after different time post light curing (Immediate, 1 day and 7 days). Different capital letters indicated statistical differences between groups ($p<0.05$).



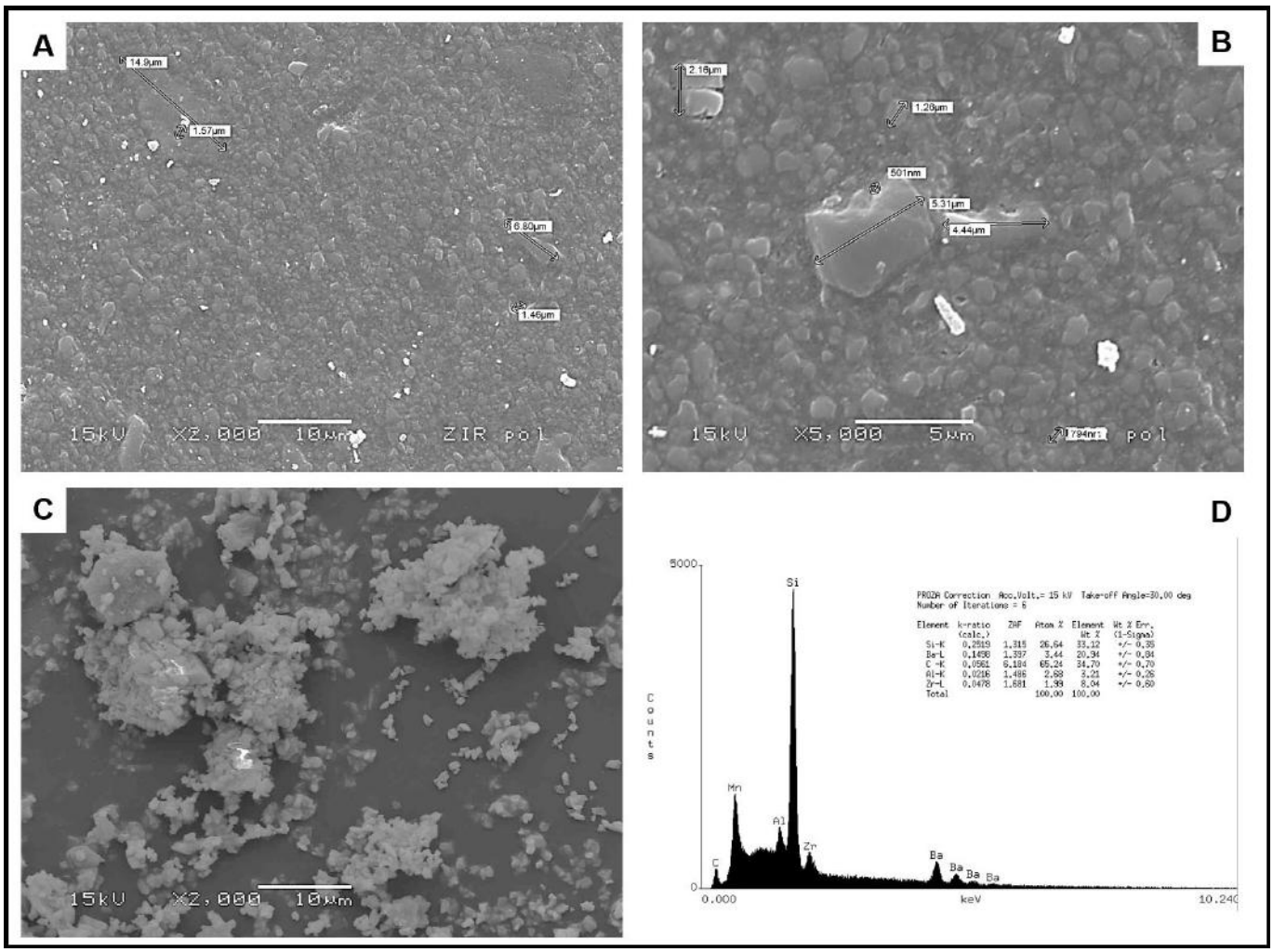
The overall degree of conversion increased as the time passed.

Source: Own Authors.

SEM and EDS

Figure 2 presents the structure of the diatomite-based composite after polymerization, with particle measurements (A-B) at two different magnifications (2000X and 5000X), the isolated particles (C), and elemental analysis distribution from EDS (D). The particles measurements evidenced particles ranging from ~500 nm to up to ~20 µm. It was possible to visualize small particle clusters, and mostly regular particles distribution after matrix removal. The elemental characterization confirmed the silicon-zirconium mixed oxide and identified the presence of silicon (~33 wt%), barium (20.9 wt%), and zirconium (8 wt%). Also, about 3 wt% of aluminum was also evidenced in the EDS analysis.

Figure 2 - SEM images of the composite surface and the particles size (A-B) at different magnification (2000X and 5000X) and distribution of the particles after organic matrix removal (2000X) (C). EDS elemental characterization is presented in (D). The particles sizes ranged from ~500 nm to up to 30 µm and the elemental distribution evidenced the presence of barium, silicon, zirconium and aluminum.



Source: Own Authors.

Flexural strength

Data from flexural strength (Table 1) demonstrated the diatomite-containing resin presented high flexural strength and flexural modulus prior to thermo-cycling. Flexural strength significantly decreased ($p<0.05$), but the modulus was not significantly imparted after 10.000 thermo-cycles.

Table 1 - Mean and standard deviation (SD) of flexural strength and flexural modulus for Zirconfill® before and after 10000 thermo-cycling. Different lower letters in column represent statistical difference ($p<0.05$).

Cycling condition	Flexural strength (MPa)	Flexural Modulus (GPa)
Before thermo-cycling	136.2 (23.7) a	12.6 (1.9) a
After thermo-cycling	75.1 (10.2) b	8.2 (0.7) a

Source: Own Authors.

Microhardness

KHN values for Zirconfill® before and after storage in different solutions are exhibited in Table 2. All the solutions affected KHN of Zironfill® at 7, 14 and 21 days. There were no differences between 21 and 28 days of storage. Also, no differences between solutions were evidenced for the evaluated time-points.

Table 2 - Mean and standard deviation of the Knoop microhardness (KHN) for the diatomite-containing composite after different periods of immersion in dietary solutions. Different capital letters represent statistical difference ($p<0.05$) in the same row (time points) while lower letters represent statistical difference in the same column (solution at the same time point).

SOLUTION	TIME				
	Baseline	7 days	14 days	21 days	28 days
Water	129 (16.3) Aa	116.3 (9.8) Ba	108.4 (3.3) Ca	102.8 (8.4) Da	103.3 (3.2) Da
Coke	135.5 (9.7) Aa	120.3 (5.6) Ba	110 (7.2) Ca	105.7 (8.5) Da	103.2 (6.4) Da
Coffee	136.3 (6.2) Aa	121.5 (7.2) Ba	113 (5.9) Ca	107.1 (5.8) Da	105.6 (6.9) Da
Orange Juice	130.4 (9.4) Aa	119.4 (9.6) Ba	117.8 (9.1) Ca	108.8 (6.0) Da	105.8 (3.6) Da

Source: Own Authors.

4. Discussion

Diatomite has been introduced as a biomedical adjuvant for different purposes (Maher et al., 2018; Tamburaci & Tihminlioglu, 2017) due to its high surface area, high porosity, mechanical properties, and thermal stability (Jing et al., 2013; Losic et al., 2009). The incorporation of this material in composites was tested before (Wang et al., 2011), but neither for commercial material nor involving chemical and mechanical properties association. In this study, we bring evidence that commercial composite with diatomite as a filler can reach a high degree of conversion and mechanical properties while has considerable chemical stability.

Each particle of diatomite presents a well-arranged porosity structure (Jing et al., 2013) ranging from few nanometers to micrometers. Besides, it is purposed that the nano-porous organization of diatomite could allow monomer penetration and cross-reaction through the interior of the particles. It is important to note that some limitations in the degree of conversion are associated with the reduction in the polymer network mobility by its surroundings and structure (I. Sideridou et al., 2002). Thus, this complementary polymerization, through the pores of diatomite, could justify our degree of conversion findings. Moreover, that residual polymerization reaching 80% of conversion also contributes to reduce water sorption and solubility that cause hydrolytic degradation (Ferracane, 2006; I. D. Sideridou & Achilias, 2005) and composite discoloration (I. Sideridou et al.,

2002). Meanwhile, the long polymerization could affect polymerization ratio and chain relaxation, but those aspects were not investigated in this study.

Apart from that, the degree of conversion is also a determinant factor to the mechanical strength of the composites where the higher the conversion the better the mechanical properties (Ferracane, 2011; I. Sideridou et al., 2002). By this way, the interlocking and the polymerization of the monomers through diatomite pores also increases the mechanical properties. Considering the high values of initial flexural strength, the high flexural modulus, and the Knoop microhardness, we confirm that diatomite is acting reinforcing the mechanical properties. In this sense, diatomite-containing composite presented a high flexural strength (~136MPa) which allows its recommendation as a material for occlusal surfaces following the International Standards (International Organization for Standardization, 2009). These findings could be related to the fact that diatomite particles deflect cracks and create frictional forces which increases the strength of the material (Wang et al., 2011). In contrast, the significant decrease in the mean value after thermocycling, below 80 MPa, is probably associated with mechanical deformation in the chemical bonds after extreme temperatures variations (Pieniak et al., 2019), which is a common concern for composite restorations. However, more than five specimens presented mechanical strength higher than that, which is also approved according to ISO 4049 (International Organization for Standardization, 2009) requirements. Furthermore, there was no difference in the flexural modulus before and after thermocycling.

The type of filler influences the handling, esthetics, and mechanical properties of dental composites (Pala et al., 2016). Here, the elemental characterization confirmed the presence of zirconium, barium and silicon as the manufacturer describes as a mixed particles of silicon and zirconium. The content of barium and silicon indicate that barium glass and diatomite are the major components of the filler. Moreover, the filler type and content influence the microhardness of the composites (Hahnel et al., 2010), and the presence of nanohybrid particles of diatomite and zirconia in this composite contributed to the high initial microhardness values. Nevertheless, the new composite with diatomite exhibited some susceptibility to solvent degradation up to 21 days and after that there was a stabilization. This situation is probably occurred due to the plasticizing effect, that separate the molecules in the polymer network and induce reduction in the microhardness (Ferracane, 2006). It is well understood that dental restorations are affected by saliva and chemical beverages in the oral cavity. Besides, pH and temperature variations in that beverages create negative effects to the matrix filler interface in the composite structure (Pala et al., 2016). On the other hand, the microhardness remained above 100 KHN. The high degree of conversion and the mechanical stability created by diatomite interaction with the matrix possibly inhibits a catastrophic decrease in the microhardness induced by different solutions' pH.

Although this is an *in vitro* study focused on basic characterization of the composite, the outcomes presented here indicate that diatomite is an interesting particle to be used as filler in dental composites, that improves degree of conversion, mechanical properties, and reduces chemical degradability to dietary solutions. On the other hand, surface properties such as color stability, surface roughness and loss of gloss of these composites are still unexplored. Therefore, further studies can be directed to evaluate these properties and compare diatomite-containing composites to the ones containing other types of filler to stablish their differences and predict its clinical success.

5. Conclusion

The physicochemical characterization of a novel nanohybrid composite Zirconfill®, with diatomite content, showed that the porous nature of diatomite promoted excellent physical and mechanical properties, high degree of conversion and low susceptibility to dietary solutions degradation conditions *in vitro*, constituting a promising alternative as filler for nanohybrid dental composites.

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