Effect of Surface Treatments on Zirconia Roughness and Wear of Opposing Artificial Enamel

by:

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Faculty of Dentistry
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Abstract:

Yttria-tetragonal zirconia polycrystal Y-TZP is used in dental restorations. Although occlusal adjustments are often necessary, their effect on Y-TZP surface roughness and tooth wear is unknown. This study investigated the effect of surface condition (Control polished–CPZ; ground–GRZ; repolished–RPZ; Glazed–GZ; Porcelain-veneered–PVZ) on zirconia roughness and wear of opposing enamel (steatite indenters) after chewing simulation (500,000 cycles, 80N, artificial saliva). A surface profilometer was used to evaluate zirconia roughness (μm) and steatite volume loss (mm$^3$). Representative specimens from each group were characterized by scanning electron microscopy (SEM). Three-way Analysis Variance (ANOVA) and Tukey HSD ($p\leq0.05$) showed that there was a significant interaction effect ($p<0.001$) between surface condition and CS. CS significantly increased roughness of GZ specimens. GRZ showed a smoother surface after CS. Two-way ANOVA and Tukey HSD indicated higher volume loss for indenters abraded directly against zirconia, as opposed to those abraded against porcelain and glaze material.
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Chapter 1. Introduction

Yttria-partially stabilized tetragonal zirconia polycrystal (Y-TZP) is frequently referred to as zirconia. Metal-free dental restorations made of zirconia are one of the most attractive treatment for teeth requiring indirect restorations, given zirconia’s biocompatibility, white color and high mechanical properties (Beuer et al., 2012). Zirconia has been the material of choice for frameworks in indirect restorations as opposed to metal, but because of its whitish and opaque color the coping needs to be veneered with porcelain, and these restorations are frequently referred to as bi-layer restorations (Tuncel et al., 2013). One of the most common clinical failures found in veneered zirconia (bi-layer) restorations is the cohesive fracture within the veneering porcelain (Guess et al., 2008).

An alternative way to avoid veneer fracture is to exclude the veneering material and to manufacture monolithic crowns entirely made from zirconia (Long, 2012). This is possible by using computer-aided-design/computer-aided-manufacture (CAD/CAM) - technology, where monolithic crowns can be milled in full anatomical contour. The milling of monolithic crowns has other advantages such as minimized manufacturing time and improved cost-effectiveness. Instead of building up the porcelain in several layers and firing in multiple firing cycles, the monolithic restoration can be personalized by using some staining techniques (Johansson et al., 2013).

Monolithic zirconia restorations have been gaining popularity as opposed to the use of bi-layer restorations (Raigrodski, 2013) because of the improved translucency of the CAD/CAM zirconia blocks. However, the outstanding mechanical properties of zirconia, such as high hardness and wear resistance, may have an effect on the opposing natural dentition, especially when the restoration’s surface is not perfectly smooth (Stawarczyk et al., 2013). Under clinical conditions,
monolithic zirconia crowns seem to cause more wear to the opposing enamel than the human enamel itself (Stober et al., 2014). This is even more problematic when occlusal adjustments of monolithic zirconia crowns are required after installation of the prosthesis. Grinding with a diamond bur is known to increase surface roughness of zirconia (Preis et al., 2015). While this roughness can be reduced depending on the type of material and technique applied for polishing (Lawson et al., 2014; Rashid, 2014), there is no standard method for polishing monolithic zirconia crowns yet (Lawson et al., 2014). A glaze layer applied on the surface of zirconia restorations is easily removed when occlusal adjustments are done or under physiological occlusal loading (Lameira et al., 2017).

Interestingly, a recent paper showed that the surface wear of artificial enamel was not affected by the roughness of different brands of zirconia after grinding and polishing (Preis et al., 2015). However, Amer et al. (2014) observed that material type and surface treatment are significant factors that affect wear of opposing human enamel. When different materials were compared, zirconia and feldspathic porcelain resulted in similar wear to the opposing enamel, which was significantly higher than the wear caused by lithium disilicate ceramic (Amer et al., 2014). A study performed by Lameira et al. (2017) showed that polished zirconia crowns cause significantly higher wear of artificial enamel than glazed zirconia crowns after 2.5 million cycles. If there is no esthetic demand, a highly polished zirconia is recommended over a glazed one because the glaze layer might wear away during function thus exposing the underlying unpolished surface to the opposing dentition. Nevertheless, if there is esthetic demand in the area, the zirconia should be highly polished and then glazed (Janyavula et al., 2013).

The applicability of the data available on recent wear and roughness studies is questionable, since low number of cycles (Jung et al., 2010) or low loading force (Stawarczyk et al., 2013) are
limitations of the methodologies used, compromising the relevance of the roughness data collected (Luangruangrong et al., 2014). Small sample size is also a compromising factor (Lameira et al., 2017). Lack of correlation between number of cycles and time of clinical use may also make the interpretation of the findings difficult. Therefore, the wear of the opposing enamel caused by monolithic zirconia restorations with or without occlusal adjustments still needs to be better investigated.
Chapter 2. Literature Review

The wear between human enamel and opposing restorative materials involves a very complex mechanism and the selection of a material for minimal wear of opposing enamel is not always easy. Wear and loss of enamel are unavoidable and may lead to the need for complex restorative procedures. In fact, the loss of enamel could cause instability of the occlusal balance as well as unfavorable effects on both natural and artificial dentition (Ramp et al., 1999). A smooth restoration surface is needed to avoid dental complications such as wear of the opposing dentition, plaque accumulation and other oral problems (Ghazal et al., 2009). It is also needed for the patient comfort (Ghazal et al., 2009). The two factors that play a role in the wear of the opposing dentition are roughness and hardness, the rougher the surface and the harder the material the greater will be the wear volume (Anusavice, 2003).

Generally speaking, ceramic materials are known to cause more abrasive wear of human enamel than other restorative materials, but little information is available concerning the effect of surface roughness of monolithic zirconia against the natural dentition (Ramp et al., 1999; Heintze et al., 2008). The use of different finishing techniques such as diamond burs and polishing kits may remove the glazing material of the restoration surface and lead to even more surface roughness (Rashid, 2014; Al-Wahadni & Muir Martin, 1998; Al-Hiyasat et al., 1997). It is considered important to re-establish the smooth or glazed-like zirconia surface using appropriate polishing instruments to minimize the effect of occlusal adjustments on the wear of the opposing dentition (Ramp et al., 1999; Heintze et al., 2008).

To quantify wear between ceramic materials and tooth structure, many materials have been used as test substrates. Human enamel may be considered the ideal antagonist to be used in terms
of achieving the real clinical conditions and that is due to its complexity in terms of morphological and structural properties. However, many other materials with similar composition have been widely used as experimental antagonists such as steatite styluses (Steatite: synthetic material mainly composed of Magnesium Silicate), ceramic styluses and bovine teeth, which makes it easier to standardize the shape of the antagonist and more precisely quantify wear. In regard to the recent approach of standardizing enamel cusps by grinding, studies do not mention changes in the wear volume of the standardized enamel as opposed to the unstandardized enamel antagonists (Krejci et al., 1999; Heintze et al., 2008) Even though steatite may not be considered the ideal alternative to human enamel in terms of its tribological and mechanical properties, the appropriateness of the steatite material as an antagonist for in vitro wear studies has been well documented (Wassell et al., 1994a; Wassell et al., 1994b; Hahnel et al., 2009). However, there is no agreement in the literature as to which material should be used as the antagonist in wear simulation tests (McCabe et al., 2002; Heintze et al., 2006).

Two-body and three-body wear tests may be used to investigate the wear characteristics of a given material. Two-body wear represents the attrition between two opposing surfaces, and the three-body wear methodology adds the third body, represented by food particles, which changes the distribution of the occlusal forces (Kwon et al., 2015). When results of two-body and three-body wear tests are compared, the three-body design results in lower wear in comparison to the two-body design (Amer et al., 2014). However, the direct comparison of the two methodologies in one experimental study (McCabe et al., 2002) showed that the wear pattern caused by the two designs is not comparable, and that restorative materials perform differently depending on the methodology applied (McCabe et al., 2002).
To be realistic, quantification of wear should be performed *in vivo*, but the lack of control over important variables such as chewing force, dietary intake, or environmental factors (humidity and temperature ranges) limit the contribution of *in vivo* studies to the evaluation of the wear of different materials (Esquivel-Upshaw *et al.*., 2012; Mundhe *et al.*, 2015). Additionally, *in vivo* studies are time-consuming, expensive, and very slow in generating precise results when compared to *in vitro* studies (Silva *et al.*, 2011). Therefore, the effect of different parameters and methodologies on the *in vitro* wear of ceramic materials will be discussed in this literature review.

### 2.1 Parameters and methodologies for wear evaluation

Tribology is the science or the study of the mechanisms of friction, lubrication and wear of interacting surfaces that are in relative motion. By definition, friction is the force resisting the relative motion between rubbing surfaces. Wear, however, is a process that occurs at any time when a surface is exposed to another surface or when a chemical reaction exists (Powers & Bayne, 1992; Heintze *et al.*, 2005; Zhou *et al.*, 2008), due to the gradual removal of the material from the surfaces through mechanical or chemical actions. Biomechanically, the oral function can result in some tribological movements that involve teeth and restorations (Mair *et al.*, 1992; Mair *et al.*, 1996; Oh *et al.*, 2002).

A wide range of devices for tribological testing of dental materials have been employed aiming to generate an automated simulation of the mastication movements in an automated motion (Heintze *et al.*, 2012). Even more sophisticated simulators have been developed to partially mimic clinical conditions as a way to predict the *in vivo* performance of restorative dental materials after mastication against natural enamel and other restorative materials (Sajewicz *et al.*, 2007; Heintze *et al.*, 2012).
2.1.1 The design of wear tests

The quantification of clinical wear is complicated, expensive, and time-consuming (Janyavula et al., 2013). Previous studies show relatively high standard deviations due to the biological spread between the studied individuals in terms of different factors that can affect the design of the test (Mitov et al., 2012; Janyavula et al., 2013).

Variations in the methodological design have also been observed. Some examples are: wide range of occlusal forces, from 25 N to 150 N (Heintze, 2006; Jung et al., 2010; Kim et al., 2012; Janyavula et al., 2013) number of cycles varying from 120,000 to 1,000,000 (Heintze, 2006; Jung et al., 2010; Kim et al., 2012; Janyavula et al., 2013) frequency of cycles (Heintze, 2006) low number of specimens (Lameira et al., 2017) and the antagonist materials (Wassell et al., 1994; Krejci et al., 1999; Heintze et al., 2008;). There is also discrepancy in estimating the correlation between number of cycles and clinical service. For example, Steiner et al. (2009) describe 1,200,000 cycles as being relative to 5 years of oral function, while some other authors estimate 200,000 cycles as corresponding to one year of service (Wiskott et al., 1995; Kelly, 1999).

It is believed that higher number of cycles can generate more wear (Chou & Lopez, 2006), but how this can correlate to the clinical service of a given material is not clear yet. Furthermore, most in vitro wear studies initially show a steep increase in wear prior to reaching a plateau phase (Heintze, 2006; Zhou et al., 2013; Lawson et al., 2014). Monasky & Taylor, (1971) and Mulhearn et al. (1962) explained that decrease in the wear by the dulling of the abrasive properties of the surface evaluated.

In terms of substrate, some studies use modified cusps of molars as antagonists (Clelland et al., 2001; Clelland et al., 2003; Amer, 2014). The cusps are modified aiming at standardizing the shape and the size of the antagonist specimens, allowing for similar distribution of forces to
the opposing surface. However, the use of un-modified human enamel would be the most realistic alternative as antagonist against crowns to evaluate the wear of tooth structure and ceramic materials (Al-Hiyasat et al., 1997), but high standard deviations and different wear patterns are associated with this type of antagonist (Sabrah, 2011).

2.1.2 The relevance of wear test parameters

Although the outcomes of in vitro wear studies are somewhat not directly correlated to the findings of the clinical data, well designed and controlled experiments are paramount to characterize the performance of materials under cyclic mastication forces (Kim et al., 2012). As human tooth’s and restorative material’s wear occur very slowly, it may need years to become measurable in vivo. Defining the main components of a wear simulator and the relevant outcome variables is important to understand the shortcomings of the current wear methodologies. Literature showed that the wear test set-up has to fulfill various requirements (Heintze et al., 2006), such as force, vertical displacement, contact time, horizontal sliding movement and antagonist surface.

2.1.2.1 Force

Wear testing simulators should generate clinically relevant forces, which are in the range of 20-120 N. Studies on human beings who chewed on different food items revealed that this is the vertical biting force range in the molar area (Schindler et al., 1998). There is considerable variation of the mastication load presented in the dental literature, and the difference between the average masticatory forces and maximum biting forces should be noted. The maximum biting force, which has been reported to be around 400–890 N in the molar area (Anusavice, 2003; Broadbent, 1999), is much higher than regular masticatory forces (Table 1), and posterior teeth
(molars and premolars) are exposed to higher masticatory forces than anterior teeth (Ehrlich & Taicher, 1981; Riise & Ericsson, 1983; Korioth et al., 1990; Korioth et al., 1997; Kumagai et al., 1999; Ciancaglini et al., 2002; Hattori et al., 2009). Ferracane et al. (2003) have shown that higher forces produce higher wear, but this increase in wear is not linear to the increase of loading (Ferracane et al., 2003). In order to best represent the worst case scenario for materials under investigation, wear simulators should reproduce masticatory forces of posterior teeth (Table 1). Forces have varied from 15 N (De Gee & Pallav, 1994) to 75 N (Leinfelder et al., 1989; Leinfelder et al., 1999) depending on the wear simulator used, and this can affect the outcomes of the wear studies. A review carried out by Lawson et al. (2013) analyzing the physiological parameters of clinical wear showed that forces varying between 20 N and 140 N are valid for the simulation of the masticatory forces.

Table 1. Physiologic forces of mastication. (extracted from Lawson et al., 2013)

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Measured Forces</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber sheet sensors with varying hardness (molar area)</td>
<td>100 N (hard), 126 N (harder), 140 N (hardest) [max]</td>
<td>28</td>
</tr>
<tr>
<td>Encapsulated load cell placed between teeth (molar area)</td>
<td>50-100 N (elastic), 20-50 N (plastic) and 30-60 N (brittle) [mean]</td>
<td>29</td>
</tr>
<tr>
<td>Strain gauge in denture tooth</td>
<td>3-17.5 N [mean] 49-78 N [max]</td>
<td>32</td>
</tr>
<tr>
<td>Strain gauge in bridge pontic area</td>
<td>20 N (potatoes) and 60 N (carrots) [mean]</td>
<td>33</td>
</tr>
<tr>
<td>Strain gauge in single prosthetic molar</td>
<td>92 N (meat), 126 N (carrots), 129 N (biscuit) [max]</td>
<td>34</td>
</tr>
<tr>
<td>Strain gauge in frame around natural molars</td>
<td>21.7 N (cookies) and 59.5 N (carrots)[mean]</td>
<td>36</td>
</tr>
</tbody>
</table>
2.1.2.2 Distance

An *in vivo* study of the mastication of solid food textures observed that the highest values of the vertical movements were measured to be between 16 and 20 mm, and these values decreased continuously in correlation to the number of chewing strokes and remained constant between 12 and 16 mm depending on the type of food (Schindler *et al.*, 1998). It is very unlikely that vertical dislodgement between upper and lower teeth would affect wear and this variable can indeed significantly increase the time needed for the application of masticatory cycles. Therefore, vertical dislodgement is often kept to a minimum.

2.1.2.3 Contact time

The contact time between stylus and material should be of a clinically relevant duration and should be stable during the simulation phase. Studies of human who chewed on different food showed that the actual contact time ranges between 400-600 ms per chewing cycle (Schindler *et al.*, 1998; Kohyama *et al.*, 2004; Heintze, 2006), but there has been no study so far indicating the effect of contact time on wear of restorative materials.

2.1.2.4 Sliding movements

The masticatory motion is a dynamic process that may affect the outcomes of the wear test and can be explained by two steps: attrition and abrasion. Attrition starts when two teeth are in contact with each other, and ends when the teeth are separated. Abrasion occurs when the upper and the lower teeth are in contact with a third body (food particles) and ends when the food particles are eliminated and the upper and lower teeth become in contact (DeLone, 2006). The average sliding movement *in vivo* was measured to be 0.3 mm in the first molar towards the
anterior and 0.18 mm towards the medial side (Gibbs et al., 1981; Kohyama et al., 2004). Some chewing simulators offer a range of sliding distances for in vitro testing, while some devices only include vertical forces, which are translated into impact, without any sliding movements. Yap et al (1997) observed that wear tests with vertical movements only cannot be on a par with wear methods combining vertical and horizontal sliding, mainly because the single vertical dislodgement of the antagonist does not reproduce either attrition or abrasion.

2.1.2.5 Antagonist surface

Many surfaces can act as antagonists in the in vivo condition, including metal, ceramic crowns, and human enamel, but enamel is considered as the ideal standard for both in vivo and in vitro wear studies (Lawson et al., 2013). Nonetheless, it has been reported that less dental wear is observed on natural enamel-to-enamel contacts than between enamel and any opposing restorative material (Etman et al., 2008). However, the use of natural enamel also has some limitations due to its inhomogeneity and the differences in morphology and size of teeth (Maas, 1991; Shortall et al., 2002). A pointed stylus, for example, produces more wear than a ball-shaped stylus, as the latter has a greater contact area between stylus and material than a sharp one (Preis et al., 2012; D’Arcangelo et al., 2016).

The results of previous in vitro studies in which a specific material as an antagonist of the human enamel were examined have been questioned, due to the fact that the test parameters differed widely between different tests (Kim et al., 2012). Most studies use flat polished ceramics and prepared enamel cusp specimens from extracted molars as their antagonists (Hacker et al., 1996; Ramp et al., 1997; Ramp et al., 1999). The test chambers are filled with water and sliding movement is integrated in the wear generating processes (Jagger & Harrison, 1994; Jagger & Harrison, 1995; Ramp et al., 1997; Ramp et al., 1999).
Previous studies showed that feldspathic porcelain (low-fusing and conventional) and natural enamel cause similar wear to the opposing enamel (Ratledge et al., 1994; Clelland et al., 2001). But when porcelain type was compared, studies demonstrated that low-fusing porcelain results in less wear to the opposing enamel than conventional porcelain (Derand & Vereby, 1999; Metzler et al., 1999). Data variability is similar when antagonists of either ceramic or steatite (MgOSiO₂) are compared (Shortall et al., 2002). Steatite has been frequently used as antagonist alternative to human enamel since many of its mechanical and physical properties are similar to those of human enamel (Wassell et al., 1994; Mehl et al., 2007).

Study designs where ceramic-to-ceramic surfaces are evaluated have also been used in in vitro wear testing of dental ceramics. The use of more standardized samples result in decreased variability of the data when compared to opposing natural enamel, not only due to a better standardization of the antagonists’ shapes but also because knowing the mechanical properties of both indenters and surface allow researchers to better correlate the effect of each material on the wear of the antagonist (Heintze et al., 2008). However, this strategy does not reproduce the clinical scenario most frequently observed. While a variety of materials have been used as the antagonist in wear simulator tests, there is no agreement in the literature about which of these materials should be considered as the ideal and standard choice for wear simulation tests (Heintze, 2006).
2.1.3 Two-body and three-body wear test

The main advantage of an *in vitro* wear design is to identify the basic mechanisms leading to the undesirable and uncontrolled wear of either tooth structure or restorative material (Amer *et al.*, 2014). The *in vivo* dynamics of wear oscillates between three-body and two-body wear patterns, due to the breaking and dissolution of food particles with the action of cyclic masticatory loads and saliva. When analyzing the effect of different food particles on wear features on the occlusal surface of teeth, Maas (1994) carried out compression tests using abrasive grit particles. The results of this study showed that larger particles produce more wear than small particles which may be related to the better distribution of forces when small particles are used (Maas, 1994).

Eisenburger & Addy, (2002) found that the amount of load significantly influenced enamel wear by attrition both in acidic and neutral conditions, but Zhou *et al.* (2013) stated that wear seemed to be independent of the loading forces. Although such studies have taken the effects of food particles and normal load on tooth wear, their focus was on the volume loss, rather than investigating the wear mechanism in details (DeGee *et al.*, 1986; Eisenburger & Addy, 2002).

Two-body and three-body wear tests seem to be related solely to different methodologies to evaluate wear, but in fact there is no clear correlation between the results of three-body and two-body *in vitro* studies (Condon & Ferracane, 1997). Eight wear testing methods have been described by the International Standards Organization (ISO - 14569-2), and among these only three wear testing methods contain a third body (ISO, *SO/TS 14569-2:2001*).
2.1.3.1 Two-body wear

During masticatory function, abrasion results from the sliding forces of one tooth against another, and the teeth involved are known as first and second bodies. This tooth-to-tooth contact without the existence of a third body is localized and restricted to one occlusal contact area between the two teeth (Pallav, 1996; Lambrechts et al., 2006). Several two-body wear simulators have been designed and used with various degrees of success and limitations in comparison to clinical trials. Examples of those machines are: capsule, compule concept, two-body abrasion single-pass sliding, two-body wear rotating counter sample, pin-on-disk tribometer, abrasive disk, etc. (Zhou et al., 2013).

A two-body wear study performed by Albashaireh et al. (2010) showed that zirconia samples worn significantly less than other ceramics used in the study in terms of both vertical and volumetric loss. Stawarczyk et al. (2013) showed that among the zirconia groups used in the study, glazed zirconia caused the most wear to the antagonist enamel while polished zirconia caused the least wear, but interestingly developed higher incidence of enamel cracks. The two-body wear results from Bai et al. (2016) also demonstrated that highly polished zirconia causes the least wear of the antagonist enamel when compared to glazed zirconia. The authors also reported that the glazed zirconia surface presented chipping fracture and cracks, which indicated a combination of fatigue and/or abrasive wear.

2.1.3.2 Three-body wear

In the three-body wear test, abrasion is caused by forceful sliding of one tooth on another with the existence of food particles or other medium acting as the third body. When the occlusal surfaces are separated with a third body, the abrasive particles act as a slurry abrading the whole contact surface (Krejci et al., 1990; Satou et al., 1992). Three-body wear simulators are designed
to get as close as possible to the clinical wear that occurs between surface of materials and teeth, when bodies slide against each other with food particles between them (Kadokawa et al., 2006; Kwon et al., 2015).

Three-body wear design has been used to evaluate the effect of ceramic restorations on natural tooth structure. Sorensen et al. (2011) evaluated different full ceramic crowns against natural enamel using poppy seeds as the abrasive medium. Feldspathic porcelain caused the most wear to the opposing enamel, while lithium disilicate caused the least, which was similar to gold. Amer et al. (2014) investigated the effect of different ceramic surface finishing techniques on the wear of the opposing standardized enamel using a food slurry in between. Results demonstrated that polished zirconia caused the least wear to the opposing enamel while glazed zirconia caused the most.

2.1.3.3 Three-body wear machines

Three-body wear simulators often have a chamber where a third body exists. This third body acts as a layer between the two surfaces in contact. The food particles, for example, are restrained during the whole masticatory movements. Many three-body wear simulators have been widely used in previous studies, with some variation in terms of stylus, medium, type of movements, number of cycles, and set up of the samples. The most common systems with their available parameters are shown in Table 2 (De Gee et al., 1986; Krejci et al., 1990; Satou et al., 1992; Pallav, 1996).
Table 2. Examples of equipment used for three-body wear simulation, and parameters available.

<table>
<thead>
<tr>
<th>Wear machine</th>
<th>Stylus</th>
<th>Medium</th>
<th>Movement</th>
<th>Force</th>
<th>Frequency</th>
<th>Cycles</th>
<th>Set-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTA wear machine</td>
<td>A textured and hardened steel counter-wheel</td>
<td>Rice/millet seed shells suspension</td>
<td>Sliding</td>
<td>15-20 N adjustable load 0-50 N</td>
<td>1 Hz</td>
<td>100,000-200,000 cycles</td>
<td>Sample chamber holding up to 12 samples</td>
</tr>
<tr>
<td>OHSU: Oregon Health Sciences University Oral Wear Simulator</td>
<td>Enamel-conical</td>
<td>Poppy seeds+PMMA beads</td>
<td>Impact + sliding</td>
<td>Abrasion load 20 N and attrition load 70 N</td>
<td>1.2 Hz</td>
<td>50,000–100,000 cycles</td>
<td>Multi-mode simulator</td>
</tr>
<tr>
<td>University of Alabama Wear Simulator</td>
<td>Polymetal-conical</td>
<td>PMMA beads</td>
<td>Impact + sliding</td>
<td>75.6 N vertical</td>
<td>1.2 Hz</td>
<td>100,000–200,000–400,000 cycles</td>
<td>Four stations device</td>
</tr>
<tr>
<td>Zurich computer-controlled masticator</td>
<td>Enamel</td>
<td>water +alcohol + tooth brushing</td>
<td>Impact+sliding</td>
<td>49 N</td>
<td>1.7 Hz</td>
<td>120,000, 240,000, 640,000 and 1,200,000 load cycles</td>
<td>Masticator</td>
</tr>
</tbody>
</table>

Many substrates are employed to simulate the food bolus or slurry during the reproduction of the masticatory movement in three-body wear tests. Examples of simulated-food mediums are: slurry of water and unplasticized polymethylmethacrylate beads, polymethylmethacrylate powder, hydroxyapatite slurry, green carborundum slurry, soft (CaCO₃) abrasive, hard (SiC) silicon carbide abrasive, millet-seed/PMMA-beads (De Gee et al., 1986; Krejci et al., 1990; Satou et al., 1992; Pallav, 1996). The large variety among the studies using slurry as a third body in terms of materials, cycles, and forces used, however, compromise the reproducibility and comparison of the reported results.
2.1.4 Latest technologies employed in wear measurements

In recent years, the number of studies involving dental wear quantification has increased and includes optical imaging (Amer et al., 2014), laser scanning (Albashaireh et al., 2010; Ghazal et al., 2009), surface mapping and profilometry technique.

Profilometry analysis may involve contact and non-contact measurements, where a laser or mechanical stylus is used to trace the surface and record a three dimensional and/or two dimensional data (DeLong, 2006). Three-dimensional data leads to quantification of wear by volume (Magne et al., 1999; Albashaireh et al., 2010), while two-dimensional data collection leads to assessment of wear by vertical loss (Sabrah, 2011; Stober et al., 2016). Burgess et al. (2014) used a non-contact light profilometer and observed that the main advantage of using non-contact profilometry is the shorter time needed for the reading (DeLong, 2006; Heintz, 2016). Surface scanning with a laser in the non-contact profilometry technique requires an opaque surface that minimizes reflection of the laser shots. Additionally, some non-contact and laser scanning techniques require a plastic replica or an impression of the worn surface for the scanner to be able to assess wear (Heintze et al., 2012; Janyavula et al., 2013). Contact profilometers, on the other hand, present more accurate results, and there is no need for coating, but damaging of the surface, especially if it is not hard enough, may occur (DeLong, 2006; Stawarczyk et al., 2013).

A possibility to assess in vivo wear is to use an intra-oral optical scanner and automated 3D computer vision (Meireles et al., 2016). Different times of application of phosphoric acid on the surface of natural enamel and wear analysis by an intra-oral scanner showed the technique to be accurate, generating precise data without the need for replication of the samples (teeth) to be evaluated. Authors noted that the approach may also be used to assess wear in vitro.
2.2 Surface properties and wear

2.2.1 The correlation between roughness, hardness and wear

Selective restorative materials are known to induce tooth wear. Moreover, surface roughness and damage of the surface of restorative materials have been known to play an important role in the wear process. Rough ceramic surfaces can cause more opposing tooth wear than smooth ceramics (Ghazal et al., 2009). Therefore, occlusal adjustments significantly affect the wear behavior of ceramic restorations (Güngör et al., 2015). As a result, abrasion between ceramics and natural teeth remains a cause of concern and the resultant wear may be influenced by both, surface roughness and the hardness of the restorative material (Tang et al., 2015). Zirconia is a material with high hardness values, estimated to be about four times higher than that of human enamel (Chun & Lee, 2014) and steatite, since steatite has mechanical and physical properties that are similar to those of human enamel (Wassell et al., 1994; Mehl et al., 2007). Literature has shown that surface roughness has more effect on opposing tooth wear than superficial hardness (Olivera et al., 2006). At the same time, the wear process may be more closely related to the ceramic microstructure than to the surface roughness due to the possible early exposure of the material’s microstructure after the removal of the glaze layer (Elmaria et al., 2006). However, there are no systematic studies evaluating the effects of the surface roughness on the wear behavior of the antagonist materials yet.

In the case of zirconia restorations, the surface of aged or transformed monoclinic zirconia presents higher roughness that may also affect the wear of the opposing surface (Ghazal et al., 2009), since higher zirconia surface roughness has been shown to increase wear rates of opposing enamel (Ghazal et al., 2009). Additionally, monoclinic transformation reduces surface hardness.
because of microstructural changes and microcracking, leading to material loss on the surface of zirconia (Aldegheishem et al., 2014). Grain size, for example, is an important microstructural parameter that may affect the mechanical and tribological properties of zirconia. A decrease in the ceramic’s grain size may increase its wear resistance (Lucas et al., 2015) and decrease the monoclinic phase transformation rate (Denry & Kelly, 2008).

2.2.2 The correlation of aging and wear

Many previous studies have investigated the effect of aging on the performance of dental restorative materials, and the existence of a positive and/or a negative correlation between aging, wear, roughness, and hardness (Burgess et al., 2014). The main issue with zirconia-based materials is their hydrothermal instability (Flinn et al., 2012; Tang et al., 2015). At low temperatures (<500°C) and in the presence of water, the tetragonal (t) phase of zirconia changes to monoclinic (m). This transformation is known as low temperature degradation (LTD). The t-m transformation proceeds gradually from the surface into the bulk of the ceramic, and is associated with surface roughening, micro-cracking and other issues (Burgess et al., 2014).

Considering that monolithic zirconia restorations have only been available for a short period of time comparing with other dental materials, the aging of full contoured zirconia crowns can be a concern due to the possible surface phase transformation when they are in a direct contact with saliva. Studies show different susceptibility of dental zirconia to hydrothermal degradation from one brand to another (Kohorst et al., 2012). Yttrium oxide content, grain size, density and presence of a cubic phase are factors that can affect the ceramics’ resistance towards aging (Lucas et al., 2015).

The most widely accepted zirconia aging method is cited in the ISO standard, which states
that autoclave aging under 2 bar of pressure for five hours producing less than 25% of zirconia monoclinic phase can be considered suitable for clinical use (Lugi & Sergo, 2010; Lucas et al., 2015). While autoclaving is considered the standard method, other methods such as chewing simulation (Stawarczyk et al., 2012) and boiling in water (Papanagiotou et al., 2006) have also been investigated. One of the ways researchers try to study the long-term effects of zirconia LTD is by correlating artificial aging with in vivo aging (Lucas, 2015). Correlations between in vivo LTD and artificial LTD are undergoing scrutiny to find the actual likeness of events in which they occur. For example, correlation of 3-4 years in clinical performance for every 1 hour of autoclave aging has been established for an orthopedic zirconia (Chevalier et al., 2007), but it seems like the aging of translucent dental zirconia happens at a much faster rate (Cattani-Lorente et al., 2016). Based on the material’s properties, both aging and surface treatments on zirconia can influence phase stability and the mechanical strength of the material (Kvam & Karlsson, 2013; Tang et al., 2015).
2.3 Study of wear in dentistry

2.3.1 Systematic reviews on wear evaluation

As previously mentioned, the in vivo analysis of dental materials is time-consuming and complicated, and wear is generally assessed in wear simulators in vitro. Various devices with different force parameters and wear mechanisms are available. Heintze et al. (2008) in a systematic review have shown that the wear data from different wear simulators is not comparable, because the methods follow different wear testing concepts. For example, systematic reviews on the in vitro wear testing of ceramic materials were performed previous to the design of a laboratory study, so that the outcomes could be compared to the existing literature (Heintze et al., 2008; Muts et al., 2014; Passos et al., 2014). While one systematic review took a more inclusive approach, considering all in vitro studies performed up to that date (Heintze et al., 2008), the two others (Muts et al., 2014; Passos et al., 2014) included only in vitro studies with detailed descriptions of the test methods which were conducted within a specific time period, and concluded that no efforts have been made so far to systematically assess the possible influencing factors of a laboratory test on material and the opposing antagonist wear.

2.3.2 Effect of occlusal adjustments

Chair-side adjustment of ceramic restorations is usually required to achieve ideal occlusal contacts. Such surface adjustments include grinding with a diamond bur. The surface treatments that follow the adjustments may alter the wear performance of ceramic restorations (Au & Klineberg, 1994). Grinding per se results in the loss of the glaze layer and compromises surface smoothness (Janyavula et al., 2013). Intra-oral polishing is a well-established method to re-establish the glaze-like surface, but this procedure is not employed by all clinicians (Preis et al., 2015). Even without the application of any adjustment procedure, the thin glaze layer over a
zirconia crown is known to be worn within the first six months after the insertion of the restoration (Preis et al., 2012).

Few available data in the dental literature indicate that occlusal adjustments may cause several issues to the restoration and to the opposing dentition (Ramfjord et al., 1983; Kawai et al., 2000). The adjusted rough surface may lead to more abrasive wear of the opposing dentition and/or increase the rate of plaque accumulation (Monasky & Taylor, 1971; Anusavice, 1996) and it may also lead to the irritation of the tissues in contact (Swartz & Phillips, 1957; Sarikaya et al., 2011). Inappropriate occlusal adjustments may even lead to the fracture and clinical failure of the restoration as it has been addressed in many studies (Kelly et al., 1989; Anusavice et al., 1992; Harvey et al., 1996; Rekow et al., 2007; Preis et al., 2012).

2.3.3 In vivo zirconia wear

The oral tooth wear is multifactorial with physical and chemical processes interacting (Zhou et al., 2013). However, it has been accepted that the main advantage of in vivo studies is to examine the tribological behavior of teeth and restorative materials in the real oral environment (Mundhe et al., 2015).

Silva et al. (2011) carried out a comparison between zirconia and lithium disilicate glass-ceramics in vivo and in vitro. The results of this randomized and controlled in vivo trial showed that the performance of lithium disilicate glass-ceramic crowns after four years is comparable to the performance of zirconia crowns after seven years in terms of replacement rate due to either maximized or catastrophic failures. Authors also observed that lithium disilicate glass-ceramic is wear resistant and wear friendly to the opposing enamel in a manner similar to that of veneering ceramics.
Unfortunately, very little information is available in the literature about the mechanisms that occur as a consequence of different wear patterns among individuals, since most clinical in vivo studies analyze survival rates and fracture rates of the restorative material rather than evaluation of wear (Esquivel-Upshaw et al., 2006; Silva et al., 2011; Esquivel-Upshaw et al., 2012). Therefore, limited clinical data is available regarding the in vivo wear of zirconia and its effect on the opposing enamel (Etman et al., 2008; Esquivel-Upshaw et al., 2012).

2.3.4 In vitro zirconia wear

The performance of zirconia has been widely investigated in vitro, and that is due to several factors including the lower cost, the shorter time needed and the larger flexibility in controlling and changing the variables in an in vitro study design when compared to the clinical investigation. In the recent years, most of the studies have concentrated on the effect of surface treatments such as polishing and glazing of zirconia and/or other materials on the wear of the opposing human enamel or other materials (Beuer et al., 2012; Kim et al., 2012; Janyavula et al., 2013; Kontos et al., 2013; Preis et al., 2013; Stawarczyk et al., 2013; Amer et al., 2014; Luangruangrong et al., 2014).

Kim et al. (2012) examined the wear of human enamel and feldspathic porcelain after chewing simulation against zirconia materials, heat-pressed ceramic, and feldspathic porcelain. The results of their study demonstrated that zirconia caused the least wear on the opposing enamel while no significant difference was observed among the three zirconia substrates used in the study. The authors also reported that the feldspathic porcelain caused highest level of wear on the opposing enamel while as antagonists more wear resistance than human enamel.

Another study performed by Beuer et al. (2012) investigated the performance of polished monolithic zirconia, glazed zirconia, and porcelain veneered zirconia against stainless steel
antagonists. This study reported higher wear of the opposing antagonist caused by the polished monolithic zirconia when compared to the glazed and porcelain veneered zirconia. However, there was higher wear of the antagonist caused by lithium disilicate when compared to zirconia substrates in another study of different monolithic dental ceramics (Preis et al., 2013). Polished, ground, and repolished zirconia samples showed no wear of the zirconia surface, while glazed zirconia showed removal of the glaze layer by the steatite antagonist. Kontos et al. (2013) investigated the effect of surface treatment (only fired, sandblasted, ground, ground then polished, and ground then glazed) on the wear of zirconia and opposing steatite antagonist. The authors reported similar results of the antagonist wear for sandblasted, ground, and ground then glazed zirconia. Ground then polished samples showed significantly lower wear value than all of the other groups.

The in vitro evaluation of wear cannot reproduce all the variables that are involved in the clinical wear of the restoration. The existing in vitro wear simulator devices duplicate only one or two of the wear mechanisms that are actually present in the mouth, and most of the wear machines use test samples with a flat surface, while natural teeth and restorations have more complicated shapes, resulting in complex stress distribution at various sites on the surface (Zhou et al., 2013). However, the results of in vitro studies are to be considered because they provide simulation of the most influential parameters for the dynamics of wear under controlled conditions.

Therefore, this thesis experiment will focus on evaluating wear of artificial enamel opposing zirconia after simulation of occlusal adjustments and other surface conditions by replicating in vivo conditions, such as high mastication loads, high number of cycles, mouth temperature and saliva, aiming to design a clinically-realistic experiment with parameters in an in vitro design that are as close as possible to the clinical scenario.
Chapter 3. Objectives and Hypotheses

3.1 Objectives

The present study aims to investigate:

- The effect of surface condition on zirconia surface roughness.
- The effect of surface condition on the wear of opposing artificial enamel.

3.2 Hypotheses

The null hypotheses are:

- The surface condition has no effect on the roughness of zirconia.
- The surface condition of zirconia does not affect the wear of opposing enamel.
Chapter 4. Materials and Methods

4.1 Materials

Yttria-partially stabilized tetragonal zirconia polycrystal (Y-TZP) pre-sintered CAD/CAM materials from two commercial brands (BruxZir, Lot number B 0633325; Expiry date: 10-2017; Glidewell Laboratories, Newport Beach, CA, USA; Lava Plus, Lot number 520217; Expiry date: 9-2016; 3M ESPE, Seefeld, Germany) were used. BruxZir Standard (OSZ) Milling Blanks were provided in discs of 98.5 mm diameter and 15 mm height and manufactured with straight edges. Lava Plus Frame was provided in blocks of 42 mm length, 24 mm width, and 17 mm height.

4.2 Sample preparation

Pre-sintered zirconia materials (BruxZir discs and Lava blocks) were carefully cut with a diamond-embedded blade (Buehler-Series 15LC Diamond; Buehler, Lake Bluff, IL, USA) (Figure 1) under water cooling in a high speed saw (600 rpm) (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain 6 x 6 mm slices with 2.0 mm thickness. The specimens were sintered following manufacturers’ instructions (Table 3 and Table 4). The final dimension of the samples was 5 x 5 x 1.8 mm due to 20 to 25% sintering shrinkage. Samples were cleaned ultrasonically in isopropanol solution for 5 minutes and air-dried (Ebeid et al., 2014).
Table 3. Sintering cycle employed for BruxZir (Furnace GL-2239-1110, Glidewell Laboratories).

<table>
<thead>
<tr>
<th>Ramp up cycle</th>
<th>Ramp down cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start temperature:</strong> 25°C</td>
<td><strong>Start temperature:</strong> 1530°C</td>
</tr>
<tr>
<td><strong>Heat rate:</strong> 10°C/min</td>
<td><strong>Ramp time:</strong> 180 minutes</td>
</tr>
<tr>
<td><strong>Ramp time:</strong> 151 minutes</td>
<td><strong>Final temperature:</strong> 70°C</td>
</tr>
<tr>
<td><strong>Final temperature:</strong> 1530°C</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hold time:</strong> 120 minutes</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. Sintering cycle employed for Lava (Lava Furnace, 3M ESPE).

<table>
<thead>
<tr>
<th>Cycle stage</th>
<th>Temperature Start</th>
<th>Temperature End</th>
<th>Heating rate</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying</td>
<td>Room temperature</td>
<td>Room temperature</td>
<td>-</td>
<td>2 hr.</td>
</tr>
<tr>
<td>Heating</td>
<td>Room temperature</td>
<td>800°C</td>
<td>20°C</td>
<td>39 min.</td>
</tr>
<tr>
<td>Heating:</td>
<td>800°C</td>
<td>1450°C</td>
<td>10°C</td>
<td>65 min.</td>
</tr>
<tr>
<td>Dwell time</td>
<td>1450°C</td>
<td>1450°C</td>
<td>-</td>
<td>120 min.</td>
</tr>
<tr>
<td>Cooling</td>
<td>1450°C</td>
<td>800°C</td>
<td>15°C</td>
<td>43 min.</td>
</tr>
<tr>
<td>Cooling</td>
<td>800°C</td>
<td>250°C</td>
<td>20°C</td>
<td>28 min.</td>
</tr>
</tbody>
</table>

Figure 1. Cutting the samples in the pre-sintered stage with a diamond embedded blade in the Isomet 1000 saw.
4.3 Experimental groups

The sample size calculation was performed based on data (Standard deviation and mean) available from previous studies and also based on the ability of these studies to statistically discriminate between groups with a similar protocol (Janyavula et al., 2013; Burgess et al., 2014). The power analysis indicated that n=5 in a study design of five experimental groups per material would be sufficient to identify the effect of treatment. Experimental groups were defined as follows (Figure 2):

Figure 2. Diagram defining the experimental groups according to their surface treatment.
4.4 Surface treatment

Samples from both materials were randomly distributed among the groups and treatments were applied as detailed below (n=5, per zirconia brand).

1. Control polished zirconia (CPZ):

After sintering, samples were polished using the dental laboratory technique according to instructions from the zirconia’s manufacturers. Polishing was performed with an extra-oral polishing kit (K0238 Dialite® ZR Extra-Oral Zirconia Polishing System, Brasseler USA®), which was composed of epoxy-based diamond impregnated polishers with medium and fine grits for initial polishing of the samples using the dental laboratory technique (Figure 3). The extra-oral polishing technique was performed as follows: pre-polishing with a medium grit disc-shaped tip (1/10 mm 2 mm) (H8MZR.HP) applying regular manual pressure always in a parallel direction until the entire surface presented similar surface finishing; then, final polishing was performed by using the fine grit disc-shaped tip (H8FZR.HP) with increased manual pressure in a parallel direction until a smooth or glossy surface could be observed by visual inspection.
This procedure was repeated for all samples from groups CPZ, GRZ, and RPZ. Five samples from this group were kept as control, and the remaining 10 samples were randomly distributed in the following groups.

2. Ground zirconia (GRZ):

Monolithic zirconia samples polished with the above dental laboratory technique were subsequently ground with a diamond bur to simulate occlusal adjustments (n=10), as follows: a diamond bur (Meisinger, 837LF FG 014, 27–76 μm, Neuss, G) (Figure 4) in a high-speed handpiece and under water cooling (Preis et al., 2012) was applied to the surface of zirconia in parallel direction for 10 seconds (two strokes, 5 seconds each) (İşeri et al., 2012). A new diamond bur was used after the preparation of five samples. This procedure was repeated for all samples from groups GRZ and RPZ. For GRZ group, no further polishing was done. The remaining five samples received further surface treatment (RPZ).
3. Repolished zirconia (RPZ):

Monolithic zirconia samples polished with the above dental laboratory technique and adjusted with the above diamond bur were repolished using an intra-oral polishing system (eZr™ intra-oral Adjustment Finishing & Polishing System, Garrison Dental solution – Figure 5), which is a diamond-based polishing system with medium and fine grits used in procedures of repolishing. The repolishing of the zirconia samples was performed as follows: a medium grit flame-shaped tip (4 x 10 mm) (FPZM020) in a high-speed handpiece was applied in a parallel direction and under regular manual pressure to the entire zirconia surface; final high-gloss polishing was performed by using the fine grit flame-shaped tip with increased manual pressure in a parallel direction until a smooth surface could be observed by visual inspection.

Figure 4. Photomicrographs of the surface of the diamond bur (Meisinger, 837LF FG 014, 27–76μm, Neuss, G) used to simulate occlusal adjustments (GRZ, RPZ). A. overview; B. closer view showing evidence of the diamond-impregnated surface.
4. Glazed zirconia (GZ):

For this group, glazing was applied with the Zenostar glaze system on top of unpolished zirconia. A thin layer of the spray glaze was applied on the surface, and a firing cycle was applied according to the manufacturer’s instructions (Table 5).

Table 5. Glazing cycle employed to the glaze group (GZ).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Firing parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start temperature</td>
<td>480 °C</td>
</tr>
<tr>
<td>Heat rate</td>
<td>80°C/min</td>
</tr>
<tr>
<td>High temperature</td>
<td>790°C</td>
</tr>
<tr>
<td>Drying time</td>
<td>2 minutes</td>
</tr>
</tbody>
</table>
5. Porcelain veneered zirconia (PVZ):

In this group, the fully sintered unpolished zirconia slices were veneered by using the powder build-up technique with IPS e.max Ceram veneer material (Ivovlar Vivadent) following the manufacturer’s instructions.

For this purpose, zirconia samples were cleaned ultrasonically in deionized water for 10 minutes to remove residues and minimize contamination. Veneering material was mixed with IPS e.max Ceram build up liquid by using a glass spatula to achieve a creamy consistency (Figure 6). The mixing proportions were 2.5 g powder to 1g liquid. Incremental layers of IPS e.max Ceram veneering material were added and fired (Figure 7) until the final thickness of 2.5 mm was achieved (1.8 mm zirconia and 0.7 mm porcelain veneer) (Lameira et al., 2017). After the last firing cycle, samples did not receive any surface treatment or glaze application, so that this group could be different from the glazed group.

Figure 6. Mixing of the IPS e.max Ceram veneer material (Ivovlar Vivadent).
4.5 Roughness measurement

After preparation, all specimens were cleaned ultrasonically in deionized water for 5 minutes and air-dried (Ebeid et al., 2014). Initial zirconia surface roughness assessment was performed using a surface profilometer (Figure 8) (Alpha-Step D-600, KLA Tencor Corp) with a 3D scan recipe shown in Table 6, and a dedicated software (KLA Tencor Apex 3D Mountains) (Figure 9) (Burgess et al., 2014). An area of 2.1×2.1 mm corresponding to the center of the specimen was considered for the baseline roughness values. Mean roughness (Ra) of each zirconia sample was measured in µm prior to the application of chewing simulation (Subași & İnan, 2012). Samples were stored in deionized water in plastic containers at room temperature until chewing simulation was applied.

After the application of chewing simulation, samples were cleaned ultrasonically and the area that presented the most visual signs of abrasion of the indenter was considered for 2.1×2.1
mm roughness evaluation. The mean roughness (Ra) of each zirconia sample was compared before and after the application of chewing simulation.

![Figure 8. Profilometer (Alpha-Step D-600, KLA Tencor Corp) used for the quantification of the roughness of zirconia samples and the wear of steatite indenters.](image)

Table 6. 3D scan recipe employed for the use of the surface profilometer (Alpha-Step D-600, KLA Tencor Corp).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Scan Size (μm)</td>
<td>2100.000</td>
</tr>
<tr>
<td>Y Scan Size (μm)</td>
<td>2100.000</td>
</tr>
<tr>
<td>Scan Speed (μm/s)</td>
<td>20</td>
</tr>
<tr>
<td>Traces</td>
<td>10</td>
</tr>
<tr>
<td>Sampling Rate (Hz)</td>
<td>200</td>
</tr>
<tr>
<td>Y Spacing (μm)</td>
<td>24.000</td>
</tr>
</tbody>
</table>
4.6 Chewing Simulation (artificial aging)

Occlusal wear was induced in a chewing simulator (CS-4.4, SD Mechatronik GMBH, Feldkirchen-Westerham, Germany). The bottom of the zirconia specimens was embedded in polymethylmethacrylate (PMMA) and inserted into metallic rings connected to the base of the equipment. The chewing simulation compartments were filled with artificial saliva (Table 7) (Ablal et al., 2009) and the temperature was kept constant at 37° Celsius. The volume of saliva and temperature in the chambers were monitored by the operator twice a day. Spherical steatite indenters (6 mm diameter – SD Mechatronik GMBH, Feldkirchen-Westerham, Germany) were used to simulate the antagonist surface and 80 N load was applied at a speed of 60 mm/sec in a 1 mm horizontal motion (Figure 11). Four specimens were cycled simultaneously and 500,000 cycles were performed (Stawarczyk et al., 2013). After 500,000 cycles, the specimens were evaluated in an optical microscope with 60 X magnification for the presence of any fracture or cracks. After aging the samples, they were cleaned ultrasonically in alcohol, air-dried and roughness and wear were analyzed (Stawarczyk et al., 2013).
Table 7. Composition for preparing 5 Liters of artificial saliva.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Chemical component</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 g</td>
<td>CMC Carboxymethylcellulose</td>
</tr>
<tr>
<td>10 g</td>
<td>Methyl-4-hydroxybenzoate</td>
</tr>
<tr>
<td>0.3 g</td>
<td>MgCl2-6H2O</td>
</tr>
<tr>
<td>0.83 g</td>
<td>CaCl2-2H2O</td>
</tr>
<tr>
<td>0.49 g</td>
<td>K2HPO4</td>
</tr>
<tr>
<td>3.12 g</td>
<td>KCL</td>
</tr>
<tr>
<td>0.02 g</td>
<td>F</td>
</tr>
<tr>
<td>5 L</td>
<td>Water</td>
</tr>
</tbody>
</table>

Figure 11. Chewing simulation parameters employed.
4.7 Evaluation of antagonist wear

One new steatite indenter was scanned using the surface profilometer (Alpha-Step D-600, KLA Tencor Corp) (Figure 12) with the same recipe showed in Table 6 and with a dedicated software (KLA Tencor Apex 3D Mountains) (Figure 13) to register the baseline dimensions of the antagonist. Following chewing simulation, the wear of the opposing steatite indenters was then evaluated by calculating the volume of material loss (mm$^3$) based on the diameter and the height of the worn surface compared to the baseline (unaged) indenter (Lameira et al., 2017).

4.8 Statistical analysis

Roughness data was analyzed by Three-way Analysis Variance (ANOVA) and Tukey HSD ($p = 0.05$) with the independent variables being material brand, surface condition and chewing simulation. Comparisons among groups were performed with Tukey Honest Significance Difference (HSD) test at a significance level of 5%. Wear data were analyzed by Two-way Analysis Variance (ANOVA) and Tukey HSD ($p = 0.05$) with the independent variables being zirconia brand and surface condition. Tukey HSD test was also used to compare the volume loss among surface treatment groups.

4.9 Scanning Electron Microscopy (SEM)

For surface characterization, one zirconia substrate and steatite indenter from each experimental group were cleaned in acetone in an ultrasound bath, and mounted on stubs with carbon adhesive tape and colloidal silver paint. The specimens were gold sputtered and observed under Scanning Electron Microscopy (SEM) (JEOL 6610LV) with high vacuum mode under 40, 500 and 1000 X magnifications.
Figure 12. Left: Steatite indenter on the base of the profilometer (Alpha-Step D-600, KLA Tencor Corp). Right: A. a contact reading needle while reading one of the zirconia’s samples, B. the needle’s shadow.

Figure 13. Profile of a control steatite indenter (Alpha-Step D-600, KLA Tencor Corp).
Chapter 5. Results

5.1. Roughness Evaluation

5.1.1 Analysis of results

Data was analyzed by Three-way Analysis Variance (ANOVA) and Tukey HSD (p = 0.05). The independent factors were material (two levels), aging (two levels) and surface condition (five levels). There was no effect of brand on zirconia surface roughness (p = 0.216). However, ANOVA indicated that there was a significant surface treatment*chewing simulation interaction effect (p < 0.001). Mean and standard deviation for zirconia surface roughness following the five surface treatments were calculated before and after the application of chewing simulation for each zirconia brand (Table 8 and Table 9), and the results were then pooled due to the lack of effect of brand the surface roughness (Table 10 and Figure 14). Comparisons among groups were performed with Tukey Honest Significance Difference (HSD) test at a significance level of 5%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before CS</th>
<th>After CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcelain veneered</td>
<td>15.3 (4.2)</td>
<td>16.4 (2.7)</td>
</tr>
<tr>
<td>Ground</td>
<td>4.1 (1.3)</td>
<td>4.6 (2.5)</td>
</tr>
<tr>
<td>Glazed</td>
<td>4.4 (1.5)</td>
<td>11.96 (1.4)</td>
</tr>
<tr>
<td>Repolished</td>
<td>3.9 (1.5)</td>
<td>4.2 (1.9)</td>
</tr>
<tr>
<td>Control Polished</td>
<td>1.6 (0.47)</td>
<td>1.6 (0.4)</td>
</tr>
</tbody>
</table>

Table 8. Roughness values (µm) - Means (and standard deviations) for Lava groups before and after chewing simulation (CS).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before CS</th>
<th>After CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcelain veneered</td>
<td>12.6 (2.0)</td>
<td>14.0 (2.2)</td>
</tr>
<tr>
<td>Ground</td>
<td>4.0 (1.7)</td>
<td>3.4 (1.3)</td>
</tr>
<tr>
<td>Glazed</td>
<td>3.0 (1.5)</td>
<td>15.2 (3.1)</td>
</tr>
<tr>
<td>Repolished</td>
<td>2.8 (1.4)</td>
<td>4.1 (1.6)</td>
</tr>
<tr>
<td>Control Polished</td>
<td>1.0 (0.3)</td>
<td>1.2 (0.3)</td>
</tr>
</tbody>
</table>

Table 9. Roughness values (µm) - Means (and standard deviations) for BruxZir groups before and after chewing simulation (CS).
Table 10. Roughness values (µm) - Means (and standard deviations) for pooled Lava and BruxZir groups before and after chewing simulation (CS)*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before CS</th>
<th>After CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcelain veneered</td>
<td>13.43 (3.64) Aa</td>
<td>15.22 (2.90) Aa</td>
</tr>
<tr>
<td>Ground</td>
<td>4.04 (1.64) Bb</td>
<td>4.02 (2.20) Bb</td>
</tr>
<tr>
<td>Glazed</td>
<td>3.70 (1.80) Bbc</td>
<td>13.54 (3.11) Aa</td>
</tr>
<tr>
<td>Repolished</td>
<td>3.37 (1.63) Bbc</td>
<td>4.14 (1.85) Bb</td>
</tr>
<tr>
<td>Control Polished</td>
<td>1.30 (0.51) Bc</td>
<td>1.43 (0.41) Bb</td>
</tr>
</tbody>
</table>

*Dissimilar lowercase letters within the same column and uppercase letters within the same row indicate significant difference (Tukey HSD, p < 0.05).

Figure 14. Mean and standard deviation for pooled zirconia surface roughness before and after chewing simulation.
5.1.2 Effect of aging and surface treatment on the roughness of zirconia surface

Before the application of chewing simulation, the highest surface roughness was presented by the porcelain veneered samples (13.43 \( \mu m \pm 3.64 \)) which was significantly higher than all of the other four groups (\( p < 0.0001 \)). Surface grinding significantly increased roughness (4.04 \( \mu m \pm 1.64 \)) when compared to the control group (1.30 \( \mu m \pm 0.51 \)) and surface repolishing after grinding resulted in intermediate values (3.37 \( \mu m \pm 1.63 \)), which were similar to the ground group and to the control group. Intermediate values were also observed when a glaze layer was applied to the surface of zirconia (3.70 \( \mu m \pm 1.80 \)).

After chewing simulation, Tukey HSD showed that the surface roughness of the porcelain veneered zirconia samples was significantly higher (\( p < 0.0001 \)) (15.22 \( \mu m \pm 2.90 \)) when compared to the control (1.43 \( \mu m \pm 0.41 \)), repolished (4.14 \( \mu m \pm 1.85 \)) and ground samples (4.02 \( \mu m \pm 2.20 \)). The glazed zirconia samples presented significantly higher surface roughness values (13.54 \( \mu m \pm 3.11 \)) after chewing simulation. The application of chewing simulation to the glazed zirconia resulted in a surface roughness that was similar to the porcelain veneered samples and significantly higher than that of the control, ground, and repolished samples (\( p < 0.0001 \)). After chewing simulation, the roughness of the control, ground and repolished samples did not change significantly.

Representative SEM images for the five groups before and after chewing simulation are shown in Figure 15, Figure 16, Figure 17, Figure 18 and Figure 19.
Figure 15. SEM at different magnifications for the control zirconia: A. before chewing simulation; B. after chewing simulation, showing even and parallel grazing marks compatible with the abrasion against the indenter; and C. overview of the surface after chewing simulation, showing a smoother surface outside the abrasion area.
Figure 16. SEM micrographs at different magnifications for the ground zirconia. A. before chewing simulation; B. after chewing simulation showing a smoother surface due to the partial removal of the peaks by the abrasion against the indenter; C. overview of the surface after chewing simulation showing a rougher area outside of the abrasion area.
Figure 17. SEM micrographs at different magnifications for the repolished zirconia. A. before chewing simulation; B. after chewing simulation showing a smoother surface due to the partial removal of the peaks by the abrasion against the indenter; C. overview of the surface after chewing simulation showing a rougher surface outside of the abrasion area.
Figure 18. SEM micrographs at different magnifications for the glazed zirconia: A. before chewing simulation; B. after chewing simulation indicating removal of the superficial layer and exposure of the material's structure; and C. an overview of the surface after chewing simulation, showing a smoother surface outside of the abrasion area.
Figure 19. SEM micrographs at different magnifications for the porcelain veneered zirconia. A. before chewing simulation; B. after chewing simulation showing the removal of the superficial glass layer with exposure of fluorapatite glass-ceramic material; C. overview of the surface after chewing simulation showing a smoother surface outside of the abrasion area.
5.2 Wear Evaluation

5.2.1 Analysis of results

Data were analyzed by Two-way Analysis Variance (ANOVA) and Tukey HSD (p = 0.05). The independent factors were material (two levels) and surface treatment (five levels). ANOVA showed no effect of material on the steatite volume loss (p = 0.064). However there was a significant effect of surface treatments on the wear of the steatite indenters (p = 0.025). Tukey HSD test was used to compare the volume loss among surface treatment groups. Due to the absence of effect of zirconia brand on wear, results for samples abraded against Lava and BruxZir were pooled according to the surface treatment. Mean and standard deviation for steatite volume loss are presented in Table 11.

5.2.2 Effect of zirconia surface treatments on the wear of opposing artificial enamel

The analysis of volume loss of the steatite indenters showed similar wear values for all the samples abraded directly against monolithic zirconia, irrespective of the surface finishing (Table 11). The highest wear of the indenters was caused by the control samples (0.09 mm³ ± 0.07), while the glazed group (0.02 mm³ ± 0.03) and the porcelain veneered group (0.02 mm³ ± 0.02) caused the least volume loss of the opposing indenter (p = 0.008). Abrasion against ground (0.07 mm³ ± 0.06) and repolished zirconia (0.06 mm³ ± 0.07) resulted in intermediate volume loss, statistically similar to control, glazed, and porcelain veneered zirconia samples. Representative SEM images for the steatite indenters at baseline and abraded against the five groups with different surface treatments after chewing simulation are shown in Figure 20.
Table 11. Mean and standard deviation (SD) for steatite volume loss after chewing simulation (in mm³)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.09^a</td>
<td>0.07</td>
</tr>
<tr>
<td>Ground</td>
<td>0.07^{ab}</td>
<td>0.06</td>
</tr>
<tr>
<td>Repolished</td>
<td>0.06^{ab}</td>
<td>0.07</td>
</tr>
<tr>
<td>Glazed</td>
<td>0.02^b</td>
<td>0.03</td>
</tr>
<tr>
<td>Porcelain veneered</td>
<td>0.02^b</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Dissimilar letters indicate statistically significant differences (p < 0.05).*
Figure 20. SEM micrographs for the steatite indenters: A. baseline; B. abraded against the control zirconia; C. abraded against ground zirconia; D. abraded against repolished zirconia; E. abraded against glazed zirconia; F. abraded against porcelain veneered zirconia. Larger abrasion areas are shown in B, C and D.
Chapter 6. Discussion

The use of monolithic zirconia has become a topic of interest for the restoration of severely damaged teeth, mainly because the high incidence of chipping or cracks within the veneer layer can compromise the longevity of the bi-layer restorations (Saito et al., 2010). The application of zirconia in monolithic configuration is possible because of the high surface hardness of the material combined with improved translucency (Tischert et al., 2000).

However, occlusal adjustment of zirconia crowns is required in most cases after installation of the prosthesis, which will result in a rougher surface and/or removal of the glaze layer. This surface roughness may be reduced depending on the type of material and the technique applied for polishing (Lawson et al., 2014; Rashid, 2014), but there is no standard method for polishing monolithic zirconia crowns yet (Lawson et al., 2014). In the case of glazed restorations, this layer is easily removed when occlusal adjustments are necessary and also under physiological occlusal loading (Lameira et al., 2017). As a result, early researchers advised that re-glazing a restoration after clinical adjustment may be necessary (Barghi et al., 1976).

The relevance of the information available in the literature on wear and roughness is questionable because of either low number of cycles (Jung et al., 2010; Kontos et al., 2013) or low loading (Kontos et al., 2013; Stawarczyk et al., 2013). Lack of correlation between the number of cycles and time of clinical use may also make the interpretation of the findings difficult (Luangruangrong et al., 2014). In the same way, very low load forces when compared to the mastication forces also may be another reason that compromises the significance of the findings (Kontos et al., 2013).
Five hundred thousand cycles and 80 N load were applied in the current study, considering that 500,000 cycles correspond to between 2 and 5 years of clinical service (Wiskott et al., 1995; Kelly, 1999; Steiner et al., 2009) which can be considered more realistic than the number of cycles used in previous studies (Jung et al., 2010; Kontos et al., 2013). Similarly, in terms of load, 80 N load was applied because, based on previous studies, this is considered a high chewing load that is still within the daily average for an adult without parafunctional habits (Tortopidis et al., 1998). We hypothesize that a loading that more closely duplicates the forces applied during oral function will challenge the material in a different way, having an effect on the readings of wear and roughness as opposed to the low values presented in previous studies (5 N-49 N) (Jung et al., 2010; Kim et al., 2012; Stawarczyk et al., 2013; Janyavula et al., 2013).

The results of the present study indicate that the first null hypothesis, which stated that the surface treatments have no effect on the roughness of zirconia substrates, is rejected. The second null hypothesis, which stated that the surface treatments applied to zirconia surface do not affect the wear of opposing enamel, is also rejected.

Before chewing simulation, the highest surface roughness was presented by the porcelain veneered samples (Table 7) which was significantly higher than all of the other four groups (p < 0.0001). This result can be due to several reasons. First, the porcelain veneered surface did not receive a final coverage with glaze, polishing or any additional surface treatment. This procedure was carried out to avoid having the same surface material on the porcelain veneered and glazed zirconia specimens, as well as to investigate the effect of the porcelain on the opposing enamel, since the removal of the glaze material after 2.5 million chewing cycles has been demonstrated (Lameira et al., 2017). Therefore, the veneer layer investigated in this study was more porous than in the clinical scenario, when the porcelain would be covered with the glaze layer. Second, the
Veneering material is a fluorapatite veneering ceramic that is composed of glass powder, fused silica dioxide (SiO$_2$) (60%) and alumina trioxide (Al$_2$O$_3$) (40%) crystals (IPS e.max® Ceram, 2005; Roselino et al., 2013). The presence of small particles like nano-fluorapatite (300 to 500 nm) extruding from the glassy matrix (Figure 19A) led to greater roughness in comparison to the glazed one, since the glaze material is composed of a glass matrix with no filler particles (Stawarczyk et al., 2016). Finally, the smoothness of the glazed surface was confirmed by the similar roughness values of glazed zirconia and polished (control) zirconia. This finding is in agreement with previous studies (Bottino et al., 2006; Tholt et al., 2006; Wang et al., 2009; Yuzugullu et al., 2009).

After the application of chewing simulation, the surface roughness of the porcelain veneered zirconia samples was still high (Table 10), but interestingly, glazed zirconia samples presented roughness values that were similar to the porcelain veneered samples. The surface roughness of the glazed zirconia was then significantly higher (p < 0.0001) than the control, repolished and ground samples. This increase in surface roughness was due to the removal of the most superficial and smooth surface, which exposed the inner structure of the glaze layer, with voids, bubbles and irregularities (Figures 18B and 18C). The visual inspection of specimens indicated that this phenomenon started around 250,000 cycles. A clinical study has previously shown that the glaze layer can be removed around the first six months after the installation of the restoration (Etman, 2009).

The similar baseline roughness values between ground and repolished groups can be explained by the simplicity of the polishing procedure in an in vitro condition. Samples were flat, fully accessible, and the operator had the possibility of controlling the pressure applied. This may not be the case in an intra-oral occlusal adjustment, due to all the limitations associated with an in vitro condition.
*vivo* procedure on a surface with an intricate anatomy such as the occlusal surface of teeth. Therefore, one should not assume that the intra-oral polishing would be able to generate a surface polishing similar to the polishing provided by the laboratory after occlusal adjustments in the clinical scenario.

The comparison of the roughness values before and after the application of chewing simulation for the ground (Figure 16) and repolished (Figure 17) groups should be combined with the SEM images of the surface. Even though the statistical analysis showed similar roughness before and after the chewing simulation (Figure 14), SEM indicated the smoothening of the abraded area after chewing simulation for both ground (Figure 16C) and repolished (Figure 17C) specimens. These conflicting results are a consequence of the limitations of the surface profilometry technique. While opposing steatite indenter was abraded on the zirconia specimen in an extension of approximately 1 mm, while the profilometer roughness reading was pre-set to an area of 2.1×2.1 mm². Therefore, the roughness reading incorporated both abraded and non-abraded areas. Only a technique sensitive enough to particularly scan the abraded surface would be able to characterize changes in surface topography of this magnitude. In the absence of such technique, it is recommended to combine quantitative analyses with the qualitative assessment of the surface through higher magnification imaging techniques.

The volumetric loss measurement is considered the most effective way to assess wear of the opposing surface (DeLong *et al*., 1986; Magne *et al*., 1999), but many previous studies have measured the vertical loss instead of measuring the total volume loss (Sabrah, 2011; Stober *et al*., 2016). Volume loss obtained by surface profilometer was used in the present study to quantify the wear of the opposing artificial enamel (steatite indenters). There was a significant effect of surface treatment on the wear of the steatite indenters (*p* = 0.025) and analysis of results show that the
wear of the steatite indenters was rather affected by the surface material - zirconia, glaze or porcelain – than by the surface finishing technique – grinding or polishing. The highest volume loss was caused by the control (polished) zirconia specimens (Table 11) which was similar to ground and repolished specimens but significantly higher than the wear caused by glazed and porcelain veneered specimens ($p = 0.008$). These results are in agreement with the study of Beuer et al., (2012) which reported higher wear for polished zirconia when compared to the glazed zirconia. However, zirconia has been considered “wear-friendly” due to results that showed evidence of significantly lower wear when human enamel is abraded against zirconia as opposed to glazed and porcelain-veneer materials (Janayula et al., 2013). These contradictory results may be explained by the methods employed. While Janayula et al. used only 10 N to simulate masticatory forces and a mix of glycerin/distilled water for humidifying the surfaces, the present study used a considerably higher load (80 N) and artificial saliva to simulate the oral environment. It is possible that the higher loading forces between zirconia and steatite maximized the damaging effect of the significantly harder zirconia (Candido et al., 2014; Aydin et al., 2015) on the artificial enamel substrate. This, combined with the fact that the present study did not have the glycerin to act as a lubricating agent during the chewing cycles may have maximized the wear caused by zirconia. Therefore, the present study indicates that the use of zirconia as a monolithic material under clinically-relevant masticatory conditions may maximize the wear of the antagonist tooth.

The effect of the polishing technique applied to the antagonist surface and consequent surface roughness on enamel wear has been previously demonstrated (Albashaireh et al., 2010; Preis et al., 2011; Kim et al., 2012; Janyavula et al., 2013; Kontos et al., 2013). Nonetheless, this study showed absence of effect of zirconia surface finishing technique on the volume loss of artificial enamel, as demonstrated by Preis et al. (Preis et al., 2012; Preis et al., 2013). The initial
zirconia roughness values indicated that only ground zirconia was significantly rougher than the control polished zirconia (Table 10), but it can be hypothesized that the roughness variation was not suffice to affect the wear of the opposing surface during the application of the chewing cycles, as indicated by the visual comparison of Figure 15A (control) and Figure 16A (ground zirconia).

The analysis of results showed that glazed and porcelain veneered groups caused the least volume loss to the opposing indenters, and these findings were corroborated by the SEM findings, which show a significantly smaller abrasion area for steatite indenters abraded against glazed (Figure 20E) and porcelain veneered (Figure 20F) zirconia. The SEM of the opposing surfaces (Figures 18C and 19C), however, show that the material on the surface of the zirconia substrate was partially removed by the abrasion against the indenter. This was a consequence of the lower surface hardness of both, veneering porcelain and glaze, when compared to the zirconia substrate and to the steatite indenter (Shortall et al., 2002; Ghazal et al., 2008). It is possible that longer chewing cycles (e.g. 2x10⁶ cycles) would result in the total removal of the surface material, with exposure of the underneath unpolished zirconia, which might have an impact on the dynamic wear process of the opposing enamel. But this hypothesis is far beyond the scope of the current study and should be further investigated in a near future as an attempt to establish a correlation between longer clinical surface and preservation of the opposing tooth structure. The results of the present study showed that there was no effect of material (zirconia brand) either on surface roughness (p = 0.216) or on the steatite volume loss (p = 0.064). This can be explained by the similarities in the chemical composition between the two brands, which resulted in similar flexural and compressive strength, for example (Alghazzawi & Janowski, 2015). Additionally, in a study with experimental zirconia materials, Tong et al. (2016) observed that irrespective of grain size, Y-TZP with similar chemical composition present similar surface hardness (Tong et al., 2016).
As above mentioned, one of the limitations of the current study was the number of cycles applied (500,000). It is possible that a higher number of chewing cycles would have an impact on the significance of the findings, especially for the glazed and the porcelain veneered groups, since the total removal of the surface material is expected and may change the wear pattern of the opposing surface. However, increasing the number of cycles implies in a longer study, which may compromise the number of groups to be evaluated. Another factor is the technique used for roughness evaluation and quantification of wear. Re-setting the profilometer reading area to a smaller surface that would only include the abraded area on the surface might result in a more accurate reading for the least abraded specimens. On the other hand, this would implicate that samples with significantly larger abraded surfaces would have some of the abraded area left out of the reading surface, making it difficult to find a balance between all the groups’ specificities. Therefore, the evaluation of other techniques for quantification of wear and roughness is encouraged. Furthermore, even though precise dimensions are critical for quantification of wear, this study findings demonstrate the importance of the surface characterization under SEM for understanding the roughness and wear pattern of a given substrate.
Chapter 7. Conclusion

Within the limitations of this *in vitro* study, the following conclusions can be drawn:

1. There is no significant effect of material (zirconia brand) on the surface roughness of zirconia or on the wear of opposing artificial enamel.
2. Surface finishing technique has a significant effect on roughness of monolithic zirconia.
3. Surface finishing technique applied to monolithic zirconia surface does not have a significant effect on the wear of opposing artificial enamel (steatite indenter).
4. The material composition applied on top of zirconia significantly affects the volume loss of opposing artificial enamel.
5. The interaction of surface condition and chewing simulation significantly affects the roughness of zirconia.

7.1 Future directions

1. To investigate the effect of other technologies (e.g. 3D Scan) for the quantification of wear and roughness between monolithic zirconia and other ceramics materials and dental enamel.
2. To compare the outcomes of two-body and three-body wear tests of monolithic zirconia opposing natural or artificial enamel.
3. To evaluate the effect of finishing techniques on roughness and wear when other ceramic materials are used (e.g. lithium disilicate, zirconia-toughened alumina).
4. To evaluate the wear and phase transformation on zirconia-based restorations under extreme masticatory conditions (e.g. higher loads combined with longer chewing cycles).
5. Correlate wear and other mechanical properties such as fatigue and survival rates.
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