UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MARIA DO CARMO AGUIAR JORDÃO MAINARDI

INFLUENCE OF DIFFERENT SURFACE TREATMENTS ON BOND STRENGTH TO RESIN COMPOSITE, SURFACE CHARACTERISTICS AND CELL ADHESION OF POLYETHER-ETHER-KETONE

INFLUÊNCIA DE DIFERENTES TRATAMENTOS DE SUPERFÍCIE NA RESISTÊNCIA DE UNIÃO À RESINA COMPOSTA, CARACTERÍSTICAS SUPERFICIAIS E ADESÃO CELULAR DE POLIÉTER ÉTER CETONA

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Thesis presented to Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, in Restorative Dentistry Area.

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para o título de Doutora em Clínica Odontológica, na Área de Dentística.

Orientador: Prof. Dr. Flávio Henrique Baggio Aguiar

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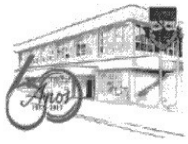

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PROF. DR. FLÁVIO HENRIQUE BAGGIO AGUIAR

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PROFª. DRª. DEBORA ALVES NUNES LEITE LIMA

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

DEDICATION

I dedicate this thesis to my parents Paulo and Bernadete for all love and understanding during my Post-Graduation period.

I also dedicate this thesis to my nephews Felipe and Rafael for all the love and happiness they always bring to my life that made much easier the journey during my Post-Graduation.

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ABSTRACT

The objective of the present study was to evaluate the influence of different surface treatments on bond strength, surface characteristics and cell adhesion of polyether ether ketone (PEEK). For this PEEK samples were polished with sandpapers (#600, #1200 and #2000). Chapter 1: PEEK samples were submitted to the following surface treatments: P- no functionalization; L- surface functionalization with laccase enzyme; CA-L- surface functionalization using Caffeic acid mediated by laccase ; LDO-L-surface functionalization using DOPA mediated by laccase; DO-L- surface functionalization using Dopamine mediated by laccase; AO- sandblasting with aluminum oxide; AO-L- sandblasting with aluminum oxide followed by functionalization with laccase. The surface characterization was carried out by the use of Contact Angle (n = 6), Surface Roughness (n = 7) and X-Ray Photoelectron Spectroscopy (XPS) (n = 1). The Microshear Bond Strenght (MSBS) (n = 7) was performed according to the groups. For the cell adhesion test, PEEK discs (2.0 cm diameter) were submitted to the following surface treatments: PBS (control); L; CA-L; DO-L; and LDO-L. After the treament the samples were incubated with HT22 cell line, for 24 hours under 37 °C and 5 % CO_2. After this period, the cells were stained with Brilliant Blue C stain and analysed under microscopy. The statistical analysis was made by Tukey test and one way-ANOVA ($\alpha = 0.05$). Chapter 2: The following surface treatments were performed on PEEK samples: SA- sulfuric acid; PS- piranha solution; SAO- aluminium oxide sandblasting. Untreated PEEK (P) was used as control. After the surface treatments, the surface roughness and the microshear bond strength (MSBS) to a resin composite, using two different adhesive systems (Single Bond and Visio.link) were measured. The results of surface roughness and MSBS were analysed by ANOVA and Tukey test ($\alpha = 0.05$). For the Chapter 1, the results obtained showed statistical differences among all the groups for contact angle ($p < 0.0001$). The group AO obtained the highest values (86.86°) while the group AO-L obtained the lowest values (20.66°). Both groups obtained the highest values for bond strength (12.40 and 12.15 MPa, respectively) and roughness (0.42 and 0.50 µm, respectively) which were statistical different from the other groups ($p < 0.0001$). There were no significant differences among the groups P, L, DO-L, CA-L and LDO-L for bond strength and roughness. The XPS analysis showed an increase in the

atomic concentration (%) of Nitrogen and Oxygen when PEEK was functionalized with Dopamine, DOPA or laccase, and a decrease in Carbon atomic concentration. The cell adhesion test showed different standard in cell adhesion among the groups. LDO-L and L groups showed the better cell adhesion standard while for CA-L there was no cell adhesion. For the Chapter 2, SAO had the highest surface roughness statistically different from the other groups, while P and PS had the lowest ones and were similar to each other. Regarding MSBS, there was no statistical difference among SA, SAO and PS, except when Single Bond was used with PS. In this case PS showed the lowest values. All samples from the groups P had pre-tests failures. It was concluded that PEEK can be treated by the methods proposed in both Chapters. The PEEK surface treatment which presented the best bond strength results was sandblasting with aluminium oxide, regardless the functionalization with laccase (Chapter 1) and sandblasting with aluminium oxide and sulfuric acid (Chapter 2). The surface treatment with DOPA with laccase, and just with laccase presented the best cell adhesion (Chapter 1).

Key words: Polyether-ether-ketone. Enzyme. Catechol. Aluminium oxide. Acid. Adhesion.

RESUMO

O objetivo desse estudo foi avaliar a influência de diferentes tratamentos de superfície na resistência de união, características de superfície e adesão celular de poliéter éter cetona (PEEK). Capítulo 1: amostras de PEEK foram submetidas aos seguintes tratamentos de superfície: P - controle, sem modificação; L – funcionalização com a enzima lacase; CA-L - funcionalização com Ácido Caféico mediada por lacase; LDO-L - funcionalização com DOPA mediada por lacase; DO-L - funcionalização com Dopamina mediada por lacase; AO - jateamento com óxido de alumínio; AO-L- jateamento com óxido de alumínio seguida de funcionalização com lacase. A caracterização de superfície foi realizada pelas das técnicas de Ângulo de Contato (n = 6), Rugosidade Superficial (n = 7) e Espectroscopia de Fotoelétrons Excitados por Raios X (XPS) (n=1). Para o teste de Resistência de União ao Microcisalhamento (MSBS) (n = 7), PEEK foi tratado de acordo com os grupos e, em seguida, restaurados com a resina composta Filtek Z350 Flow, após o uso do sistema adesivo Adper Single Bond 2. Duas restaurações foram feitas por amostra. Após 24 horas, o teste de resistência de união foi realizado em uma máquina de ensaio universal à velocidade de 0,5 mm/min. Os valores de resistência de união foram calculados em MPa. Para o teste de adesão celular, discos de PEEK (2,0 cm de diâmetro) foram submetidos aos seguintes tratamentos: PBS (controle); L; CA-L; DO-L; e LDO-L. Em seguida, os discos foram incubados com a linhagem celular HT22, durante 24 h, à 37 °C e 5 % de CO_2. Após esse período, as células aderidas foram coradas com Briliant Blue C e analisadas em microscopia. Os valores de ângulo de contato, rugosidade e resistência de união foram analisados através do teste de Tukey e one-way ANOVA (α = 0,05). Capítulo 2: os seguintes tratamentos de superfície foram realizados nas amostras de PEEK: SA- ácido sulfúrico; PS- solução piranha; SAO- jateamento com óxido de alumínio. Amostras de PEEK (P) não tratadas superficialmente foram utilizadas como controle. Após os tratamentos de superfície, a rugosidade de superfície (μm) e resistência de união ao cisalhamento à uma resina composta, utilizando dois sistemas adesivos (Adper Single Bond e Visio.link), foram medidas. Os resultados foram submetidos à análise estatística, utilizando-se ANOVA e teste de TUKEY (α = 0,05). Para o capítulo 1, os resultados obtidos para ângulo de contato mostraram diferenças estatísticas entre

todos os grupos apresentados (p < 0,0001), sendo que dentre estes grupos, PEEK jateado com óxido de alumínio apresentou o maior valor (86,86°) enquanto PEEK jateado com óxido de alumínio e funcionalizado por lacase, apresentou o menor valor (20,66°). Esses mesmos grupos apresentaram os maiores valores de resistência de união (12,40 e 12,15 MPa, respectivamente), e maiores valores de rugosidade (0,42 e 0,50 µm, respectivamente), em ambos os casos com diferenças estatísticas em relação aos demais grupos (p < 0,0001), os quais não difereriram entre si. As análises de XPS mostraram um aumento na concentração atômica (%) de Nitrogênio e Oxigênio quando PEEK foi funcionalizado com Dopamina, DOPA ou lacase, em relação ao PEEK não funcionalizado. Do mesmo modo, houve uma redução na concentração atômica de Carbono para PEEK funcionalizado, em relação ao PEEK não funcionalizado. Diferentes padrões de adesão celular foram avaliados. Os grupos LDO-L e L obtiveram os melhores padrões de adesão celular, enquanto para o grupo CA-L não foi encontrada aderência de céuluas. Para o capítulo 2, o grupo SAO apresentou os maiores valores de rugosidade superficial, estatisticamente diferente dos outros grupos, enquanto P e PS apresentaram os menores valores, não diferindo entre si. Para MSBS, não houve diferenças estatísticas entre os grupos SA, SAO e PS, exceto quando Single Bond foi usado com PS. Neste caso, PS apresentou os menores valores. Todas as amostras do grupo P apresentaram falhas pré-testes. Conclui-se que PEEK pode ser superficialmente tratado com os métodos propostos nos capítulos 1 e 2. Os tratamentos de superfície que apresentaram os melhores valores de resistência de união foram jateamento com óxido de alumínio, independente da funcionalização com lacase (capítulo 1) e jateamento com óxido de alumínio e ácido sulfúrico (capítulo 2). Os tratamentos de superfície com DOPA com lacase, e somente com lacase, apresentaram os melhores padrões de adesão celular (capítulo 1).

Palavras chave: Poliéter eter cetona. Enzima. Catecol. Óxido de Alumínio. Ácido. Adesão.

SUMMARY

1 INTRODUCTION

Polyether ether ketone (PEEK) is a synthetic semicrystalline polymeric material (Rae et al., 2007, Heimer et al., 2016) used as a substitute to metallic devices due to its high performance and low elastic modulus. For this reason, since 1998 PEEK has been commercialized as an implant material replacing titanium alloys, stainless steel and Cr-Co alloys. Furthermore, PEEK has been regarded as a potential substitute for the traditional alloplastic materials used in dental prosthesis (Schwitalla et al., 2013, Schwitalla et al., 2015).

The main reason to use PEEK as implants is due to the low elastic modulus of this polymer presents be similar to the bone decreasing the stress propagation when a load is applied on the implant (Deng et al., 2015; Zhao et al., 2016). Other advantages of PEEK includes stability at high temperatures (Schmidlin et al., 2010), proper mechanical properties and dimensional stability, biocompatibility, radiolucence and compatibility with imaging techiniques (Schiwitalla et al., 2015; Silthampitag et al., 2016). In dentistry, PEEK has been studied as copings for metal free prosthesis, healing abutments, partial removable prosthesis and implants (Hallmann et al., 2012).

However, despite the characteristics that make the material attractive, PEEK has the disadvantage to be chemically inert and highly hydrophobic jeopardizing the osteointegration when it is used in implants (Evans et al., 2015) and also the adhesion to resin compounds (Stawarczyk et al., 2015).

Several attempts have been made with the purpose to improve the surface characteristics of PEEK making this material proper to be used in the medical and dental field. Nanohydroxyapatite-based, TIO_2 nanoparticles, BMP-2 growing factor (Najeeb et al., 2015; Yang et al., 2015; Shimizu et al., 2016) or plasma treatment are among the methods applied when the target is the use of PEEK as implants (Briem et al., 2005).

The hydrophobic behaviour of PEEK is also the main factor related to the difficult adhesion of it to resin materials used in dentistry. Many methods have been applied to modify PEEK surfaces in order to improve the resin adhesion. These methods aim the anchorage of reactive groups on PEEK surface through plasma treatment, UV light and laser, surface sandblasting with abrasive particles such as aluminium oxide or further the etching with sulfuric acid 98%, *piranha* solution

(mixture of hydrogen peroxide and sulfuric acid) or the combination of both. Besides the anchorage of reactive groups, such as -OH and sulfonic groups, these methods increase the surface roughness of PEEK as well favouring the adhesion with resin compounds (Hallmann et al., 2012). However, the hazardous potential of sulfuric acid and piranha solution makes their handle difficult, dangerous and harmful to health and environment (Stawarczyk et al., 2014). In this context, an environment friendly methodology without damages to health and environment is of great interest and advantageous in comparison to the methods already tested.

Catechols are small molecules found in vegetal and animal kingdom, e.g. different types of tea, vegetables and small organisms like squids and mussels. The strong adhesion of mussels to several kinds of substrates in moisture environment has inspired the studies in bio-inspired adhesives, polymers and coatings based in catechol chemistry (Faure et al., 2013). It has been proven that catechol can modify different types of substrates, such as metallic, inorganic or organic substrates by electrostatic interactions or covalent bonding. Furthermore, it can be used as linkers for the immobilization of biomacromolecules and particles on inert surfaces. As it was mentioned, the catechols have different mechanism to adhere to surfaces and react with other molecules (Figure 1), and these mechanisms include the oxidation of catechols, which happens with the use of molecular Oxygen, named autoxidation, oxidation agents like sodium periodate or hydrogen peroxide (Yu et al., 1999) or it can be catalysed by enzymes or metallic ions (Yang at al., 2014). For the reaction with other molecules (crosslinking), the oxidation step is the initial reaction (Burzio et al., 2000) (**a)**). The obtained quinone is highly reactive towards nucleophiles like thiols (**b)**) and amines (**c)**) in a 1,4-Michael addition (Liu et al., 2006). In the presence of primary amines Schiff Base formation occurs (**d)**). After the initial oxidation step a stable radical can be formed (**e)**) which become dimerized (Burzio et al., 2000) (**f)**). Alternatively, crosslinking can be induced by the addition of Fe(III) ions (Sever et al., 2004).

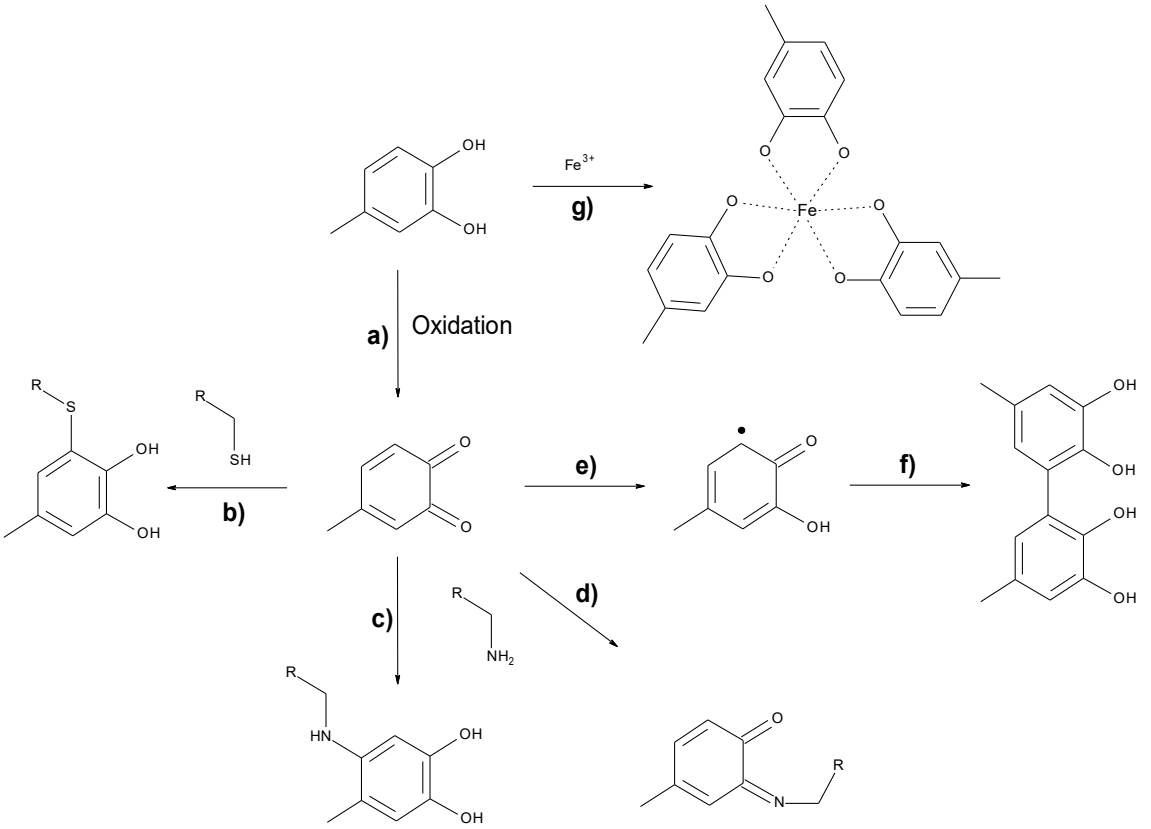

Figure 1: Oxidation (a) and crosslink of catechol initia. Reaction of reactive quinone with thiol (b) and amines in Michael-addition reaction (c). Formation of Schiff-Base in the presence of primary amines (d). An unstable radical formed (e) and the dimer (f) formed from that.

In this context, the proposal of this study was to evaluate the influence of different surface treatments on the bond strength to a resin composite, surface characteristics and cell adhesion of PEEK. The surface treaments were carried out using different sources of catechol (Dopamine, DOPA and Caffeic Acid), laccase enzyme and aluminium oxide abrasive particles.

2 CHAPTERS

2.1 Chapter 1: “Influence of enzymatic-based surface treatments on bond strength to resin composite, surface characteristics and cell adhesion of Polyether ether ketone”.

ABSTRACT

The objective of the present study was to evaluate the influence of different surface treatments on bond strength, surface characteristics and cell adhesion of polyether ether ketone (PEEK). For this PEEK samples were polished with sandpapers (#600, #1200 and #2000) under water cooling and cleaned with dodecyl sodium phosphate in ultrasound bath, followed by isopropanol and distilled water. Afterwards PEEK samples were submitted to the following surface treatments: P- no functionalization; L- surface functionalization with laccase enzyme; CA-L- surface functionalization using Caffeic acid mediated by laccase ; LDO-L-surface functionalization using DOPA mediated by laccase; DO-L- surface functionalization using Dopamine mediated by laccase; AO- sandblasting with aluminum oxide; AO-L- sandblasting with aluminum oxide followed by functionalization with laccase. The surface characterization was carried out by the use of Contact Angle (n = 6), Surface Roughness (n = 7) and X-Ray Photoelectron Spectroscopy (XPS) (n = 1). The Microshear Bond Strenght (MSBS) (n = 7) was performed according to the groups described above. Two restorations with the composite Filtek Z350 Flowable were made in each sample. Prior the restoration the adhesive Adper Single Bond 2 was applied. The bond strength test was performed in universal test machine (Ez-Test) under the speed 0.5 mm/min and given in MPa. For the cell adhesion test, PEEK discs (2.0 cm diameter) were submitted to the following surface treatments: PBS (control); L; CA-L; DO-L; and LDO-L. After the treament the samples were incubated with HT22 cell line, for 24 hours under 37 °C and 5 % CO_2. After this period, the cells were stained with Brilliant Blue C stain and analysed under microscopy. The statistical analysis was made by Tukey test and one way-ANOVA ($\alpha = 0.05$). The results obtained showed statistical differences among all the groups for contact angle ($p < 0.0001$). The group AO obtained the highest values (86.86°) while the group AO-L obtained the lowest values (20.66°). Both groups obtained the highest values for

bond strength (12.40 and 12.15 MPa, respectively) and roughness (0.42 and 0.50 µm, respectively) which were statistical different from the other groups ($p < 0.0001$). There were no significant differences among the groups P, L, DO-L, CA-L and LDO-L for bond strength and roughness. The XPS analysis showed an increase in the atomic concentration (%) of Nitrogen and Oxygen when PEEK was functionalized with Dopamine, DOPA or laccase, and a decrease in Carbon atomic concentration. The cell adhesion test showed different standard in cell adhesion among the groups. LDO-L and L groups showed the better cell adhesion standard while for CA-L there was no cell adhesion. It was concluded that PEEK can be functionalized by laccase-mediated oxidation of catechols or just with laccase. The PEEK modification procedure which presented the best bond strength results was sandblasting with aluminum oxide, regardless the functionalization with laccase. The modification with DOPA with laccase, and just with laccase had the best cell adhesion.

Key words: Polyether-ether-ketone. Enzyme. Catechol. Aluminum oxide. Sandblasting. Cell.

INTRODUCTION

Polyether ether ketone (PEEK) (figure 1) is a semicrystalline and biocompatible polymer that presents higher mechanical properties than other biocompatible polymers which make this material an interesting focus of study in the biomedical and dental field (Montero et al., 2017; Sampaio et al., 2016). Other characteristics that make PEEK so attractive are the low elastics modulus, around 3.6 GPa, which can better match to bone and dentin in comparison to metallic devices (Yang et al., 2015).

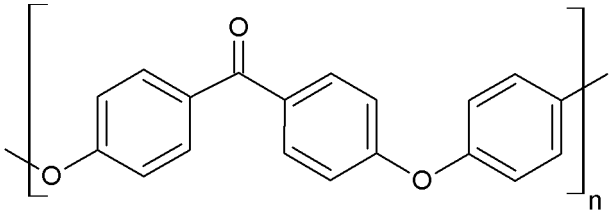

Figure 1: PEEK structure.

Despite the good characteristics of PEEK the main disadvantage of this material is its hydrophobic behavior that makes this polymer inert resulting in low bioactivity. It is a matter of concern when the focus is the use of PEEK as biomedical implants because lower attachment of cells on its surface can hamper the osteointegration (Shimizu et al., 2016). Furthermore, PEEK has been used as abutments for implant-supported prosthesis, removable partial dentures and three-unit fixed dental prosthesis. However, the low surface energy of PEEK makes its surface modification necessary in order to obtain a good adhesion between the material and resins and cements used in dentistry (Rocha et al., 2016).

A great number of approaches have been applied in order to modify PEEK surface (Deng at al., 2015). Among them plasma treatment and coatings with nanohydroxyapatite particles, as well bulk PEEK modification with carbon fibers, are the most studied ways of PEEK surface modification when the target is the use as biomedical implants (Xu et al., 2015). Plasma treatment, sandblasting and etching with sulfuric acid 98 % and *piranha* solution, or the combinations of these techniques, are inside the options to modify PEEK surface when the target is to obtain a strong adhesion between PEEK and resins and cements (Stawarczyk et al., 2014). However, these techniques demand harsh conditions of reactions and use harmful agents (Ma et al., 2014). For this reason, an environmental friendly method could be an alternative to promote PEEK surface functionalization.

Catechols are small molecules found in a great variety of substances in nature and they are part of the structure of several molecules (figure 2). Their structure is compound by two neighboring hydroxyl groups linked to a benzene ring (Sedó et al., 2013). One reason for the great interest in these structures is due to strong adhesion that marine mussels show to hard substrates in moisture and saline habitats (Forooshani et al., 2017). One of the most important molecule responsible for the adhesion is DOPA (L-3,4-Dihydroxyphenylalanine) which is presented in great amounts in the mussel foot proteins mefp-3 and mefp-5 (Guo et al., 2017). In this context, several catechol-based biomimetic adhesives and coatings have been developed, to glue different types of substrates or to functionalize surfaces, respectively. Through an oxidation procedure the catechols can form reactive species which are responsible for the crosslinked bonds inside the adhesives and the attachment on different surfaces (Yang et al., 2014).

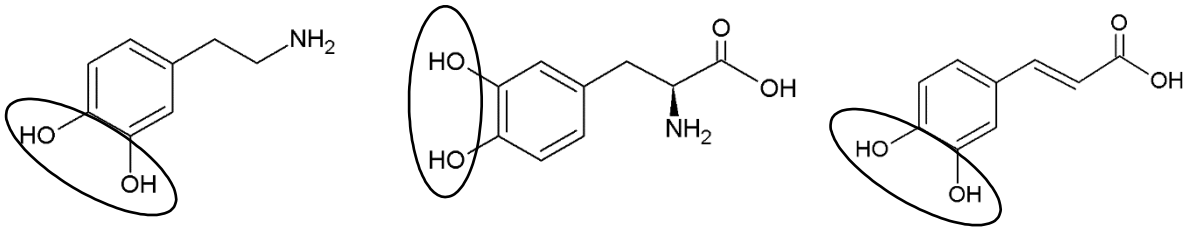

Figure 2: Structures of Dopamine (1), DOPA (2) and Caffeic acid (3) which present a catechol group (circle) in their compositions.

One way to oxidase catechols is through enzymes. Laccase is an enzyme produced mainly by fungus *Trametes versicolor* (Jones at al., 2012). This enzyme is also called polyphenol oxidase due to its capability to oxidize phenolic compounds (Baldrian et al., 2006). The phenol oxidation procedure occurs in the presence of molecular oxygen and the main subproduct is water (Mate et al., 2016). Laccase is consisted of four copper atoms which are surrounded by an amino acid (cysteine and histidine) network. The copper atoms are directly related to catechols oxidation withdrawing electrons from them and making possible the formation of the reactive catechols species mentioned above (Jeon at al., 2012).

In this context, the proposal of this study was to evaluate the influence of different surface treatments on the bond strength to a resin composite, surface characteristics and cell adhesion of PEEK. The surface treaments were carried out using different sources of catechol (Dopamine, DOPA and Caffeic Acid), laccase enzyme and aluminium oxide abrasive particles.

METHODOLOGY

In order to promote the functionalization of polyether ether ketone (PEEK), the enzyme laccase from the fungus *Trametes versicolor*, as well the catechols Caffeic acid, Dopamine hydrochloride and 3,4-Dihydroxyphenylalanine were purchased from Sigma-Aldrich. PEEK samples (medical grade) were obtained by cutting a PEEK bar in 1.2 x 1.0 x 0.1 cm discs with the aid of a diamond disc EXTEC DIA WAFER BLADE 4” x 0,12 x ½ (102 mm x 0,3 mm x 127 mm coupled to a metallographic cutter (Isomet 1000, Buehler Ltda; Lake Buff, IL, USA) set to 550 rpm. The obtained samples were submitted to a polishing step using #600, #1200 and

#2000 sandpapers coupled to a polishing machine (Arotec Ind. e Comércio; Cotia, SP, Brazil) for 1 minute each one under water cooling. In between the use of the different sandpapers the samples were submitted to ultrasonic bath in distilled water during 15 minutes in order to remove the remnants from their surfaces.

The samples were submitted to the following surface treatments: P- no functionalization; L- surface functionalization with laccase enzyme; CA-L-surface functionalization using Caffeic acid mediated by laccase; LDO-L-surface functionalization using DOPA mediated by laccase; DO-L-surface functionalization using Dopamine mediated by laccase; AO-sandblasting with aluminum oxide; AO-L-sandblasting with aluminum oxide followed by functionalization with laccase. Prior the modification procedures the PEEK samples were cleaned in 2% sodium dodecyl sulfate solution in ultrasonic bath for 10 minutes, followed by 15 minutes of autoclave and finally by 10 minutes in pure isopropanol ultrasonic bath.

A- Surface modification procedures

Surface functionalization with laccase

The laccase enzyme was dissolved in acetate buffer solution (104 mM acetic acid and 200 mM sodium acetate in deionized water, pH = 4.75) obtaining 150 µg/mL laccase solution. The PEEK samples were inserted in glass vessels containing the laccase solution while taking care for the total coverage of PEEK surface by the solution and left overnight on a platform shaker (110 rpm; Marconi Ltda.; Piracicaba, SP, Brazil). After removal from the solution the samples were washed with distilled water and gently air dried.

Surface functionalization with catechol mediated by laccase

A solution (1,5E-4 mol/L) of each catechol (Caffeic acid, DOPA or Dopamine) in phosphate saline buffer, pH = 7.4 (PBS, 0.01 M; Sigma Aldrich) was prepared. After the preparation of these solutions each one was inserted in glass vessels containing the PEEK samples, followed by the insertion of laccase solution (1,5 mg/mL in 0.01 M PBS) in those vessels containing the catechol and PEEK samples, respecting the ratio 10:1 of catechol/laccase in volume. The samples were left overnight inside the catechol/laccase solutions on a platform shaker (110 rpm) After removal from the solutions, the samples were washed with distilled water and gently air dried.

Sandblasting with aluminum oxide

Aluminum oxide particles (50 μm size) were used to sandblast PEEK samples in order to modify their surfaces by increasing the roughness. The sandblasting was performed on each sample belonging to this group for 30 seconds in a pressure of 2 bars. After the sandblasting the samples were air-blown for 60 seconds and immersed in isopropanol for 10 minutes in ultrasound bath.

Sandblasting with aluminum oxide followed by functionalization with laccase

The sandblasting of the samples belonging to this group was performed as described above. After the sandblasting the samples were inserted in a laccase solution (150 μg/mL of acetate buffer, pH 4.75) and left overnight in a platform shaker (110 rpm). After removal from the solution the samples were washed with distilled water and gently air dried.

B- Measurements

Contact angle

The contact angle measurements were made on modified and non-modified PEEK samples (n = 6). The contact angles were determined by the sessile drop method with distilled water in a goniometer (Digidrop; Tallaght, France) coupled with a camera (Pixelink; Ottawa, Canada). The contact angle was determined by the software AxioVision (Zeiss; Germany). Two measurements were performed for each sample and the contact angle value was regarded the average of these measurements.

Surface Roughness

The roughness of modified and non-modified PEEK samples (n = 7) was measured by a rugosimeter (SV-3100S4 Mitutoyo, Tokio, Japan) equipped with a diamond tip (0.5 μm radio). After the rugosimeter calibration the samples were parallel placed on the surface of the device. Three equidistant points passing through the center of the samples to the borders were measured. The precision of the equipment was 0.01 μm, with cutoff value of 0.25 mm and reading length of 1.25 mm. The roughness value (Ra) was regarded the mean of the three measurements of each sample.

X-ray photoelectron spectroscopy – XPS

XPS spectra of the samples were taken using a Kratos Ultra equipment (Kratos Analytical Ltd; Manchester, UK) using the following acquisition parameters: step (meV) 66.0 for C1s and 100.0 for N1s, hybrid lens mode, pass energy 20 eV or 40 eV (N1s spectra), excitation of photoelectrons by monochromatic Al radiation (300 W), dwell time 150 ms for all samples. The acquisition time varied according to the sample. The measurements were made on PEEK modified with the catechols or laccase using the same modification protocol described above, on the non-modified PEEK (pristine PEEK), grounded and cleaned PEEK (freshly PEEK) and PEEK immersed on phosphate buffer saline (PBS).

Microshear bond strength (MSBS)

The modified or non-modified PEEK samples were tested regarding the microshear bond strength to a resin composite (Z350XT Flowable, A1 shade; 3M-ESPE). Two fillings per PEEK sample (n = 7) were made on their respective surfaces using a matrix of perforated pasta (1mm height, 1.15 mm internal diameter; Furadinho 6, Pastifício Santa Amália, Machado, Minas Gerais, Brazil) (Theobaldo et al., 2016). The adhesive Single Bond 2 (3M-ESPE) was applied on PEEK surfaces before the filling and light-cured for 40 seconds using a 3rd generation light curing device (Valo-Ultradent). The PEEK samples were stored in distilled water for 2 hours at room temperature, in order to make the perforated pasta soft enough to be removed. After 24 hours, the microshear test was performed on a universal testing machine (EZ Test-L; Shimadzu Corporation, Tokyo, Japan) at a speed of 0.5 mm/min. The microshear bond strengths were given in Mega Pascals (MPa), according to the formula bellow:

$R = F / Area,$

where *R* is the bond strength in MPa, F is the force in Newtons (N), and A is the area of adhesion in mm^2.

The statistical analysis for contact angle, roughness and strength was performed using Tukey test and one-way ANOVA set in 5% significance level.

Cell viability assay

For the cell attachment tests PEEK was modified using laccase, Dopamine with laccase, DOPA with laccase, and Caffeic Acid with laccase. The modification procedures followed the parameters as described above. PEEK immersed in PBS was used as control. The applied growth medium was High Glucose Medium containing 10% fetal calf serum (FCS) and 1% (Penicillin-Streptomycin solution (P/S). The Peek sample disks (diameter = 2cm) were cleaned by rising several times with Ethanol followed by double distilled water (dd H_2O). After air drying of the samples, 1 x 10^4 cells (HT22, hippocampal neuronal cell line, P+8 (passage 8)) in one drop were pipetted on the samples in a 6-well plate. As a control 5 x 10^4 cells were used. After 6 hours, 2500µl medium was added to the samples to avoid desiccation of the cells. The cells were incubated for 24 h at 37°C and 5% Carbon dioxide (CO_2). After the incubation the medium was removed from the wells and 200µl of a 1% Brilliant cresyl blue-solution (Brilliant Blue C) was applied on the remaining cells and incubated for 1 min at room temperature. After the incubation the sample plates were washed with double distilled water. Pictures were taken with a Zeiss Axio IC/Scope.A1 microscope (Carl Zeiss Microscopy; LLC, USA) and analysed with software (ZEN, Carl Zeiss; LLC, USA). A 20x lens was used with a 5.0x zoom to analyse the cells adhered and their morphology.

RESULTS

The contact angle, roughness and microshear bond strenght values are described in table 1. The contact angle values showed different surface energy characteristics among the groups with statistical differences among all the groups ($p < 0.0001$), except between L and CA-L- which were similar. The group AO showed the highest contact angle values (86.9° ± 2.9) while the group AO-L showed the lowest values (20.6° ± 2.3). The contact angle values for unmodified PEEK (P) was lower than the sandblasted PEEK without laccase (AO) and higher than the laccase/catechols functionalized PEEK. The groups L and CA showed similar values for contact angle (64.3° ± 1.7 and 65.6° ± 0.8, respectively) and they presented higher values than the groups DO-L and LDO-L obtained (41.7° ± 1.2 and 35.0° ± 4.0, respectively).

Table 1: Contact angle, Roughness and Microshear Bond Strenght values (means and standard deviation) ($p < 0.0001$). Different letters showed statistical differences among the groups.

Groups	Contact Angle (°)	Surface Roughness (µm)	Microshear Bond Strenght
P	77.75 ± 0.80 b	0.07 ± 0.006 b	4.76 ± 0.52 b
CA-L	65.68 ± 0.84 c	0.08 ± 0.014 b	3.65 ± 0.54 b
DO-L	41.70 ± 1.26 d	0.06 ± 0.004 b	4.19 ± 0.36 b
LDO-L	35.03 ± 4.03 e	0.06 ± 0.005 b	4.54 ± 0.57 b
L	64.37 ± 1.70 c	0.06 ± 0.005 b	3.84 ± 0.38 b
AO	86.86 ± 2.39 a	0.42 ± 0.154 a	12.40 ± 1.86 a
AO-L	20.66 ± 2.86 f	0.50 ± 0.129 a	12.15 ± 2.34 a

The surface roughness results showed a significant increase of the roughness values when PEEK was sandblasted with aluminum oxide particles (AO and AO-L groups) ($p < 0.0001$). The roughness values did not differ among the modified PEEK (L, CA-L, DO-L and LDO-L) and unmodified one (P).

Regarding MSBS, the sandblasted PEEK (AO and AO-L) showed the best results, regardless if it was functionalized with laccase or not. These values were statistically higher ($p < 0.0001$) than the values obtained for P, L, CA-L, DO-L and LDO-L. The latest groups did not show significant differences among each other.

The binding energy (eV) and atomic concentrations (%) of the elements obtained by XPS are shown in tables 2 to 8, according to the groups. For XPS measurements, it could be observed an increase in the Nitrogen content (atomic concentration (%)) when PEEK was functionalized with dopamine (6.8 %) and DOPA (6.48 %) mediated by laccase in comparison with unmodified freshly grounded PEEK (0.18 %). The same has happened when PEEK was functionalized just with laccase (6.06 %). There was an increase in the Nitrogen contend for PEEK functionalized with caffeic acid (3.66 %). However, this increase was not as higher as for PEEK functionalized with Dopamine, DOPA, and laccase. The unmodified PEEK presented insignificant amounts of Nitrogen in its surface as already mentioned above. PEEK immersed in buffer solution present a small amount of Nitrogen (0.79 %), also insignificant. The same trend could be observed for Oxygen content (%) on PEEK surface. The unmodified PEEK presented 13.92 % of Oxygen while Dopamine, DOPA and laccase presented 17.24 %, 17.10 % and 15.80 %, respectively. On the

other way, the Carbon content (%) decreased when PEEK was functionalized with Dopamine (75.75 %), DOPA (75.57 %) and laccase (77.95 %). The unmodified freshly grounded PEEK presented the highest C content (87.76 %) followed by unmodified pristine PEEK (83.83 %). Caffeic acid presented 81.66 % of Carbon content.

Table 2: Binding energy and atomic concentration of elements obtained by XPS for pristine PEEK.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	529.544	13.92
N 1s	396.788	1.46
C 1s	281.792	83.83
P 2p	130.388	0.03
Si 2p	99.752	0.40

Table 3: Binding energy and atomic concentration of elements obtained by XPS for freshly grounded PEEK.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	530.432	11.57
N 1s	397.676	0.18
C 1s	282.263	87.76
P 2p	130.388	0.00
Si 2p	100.196	0.17

Table 4: Binding energy and atomic concentration of elements obtained by XPS for PEEK immersed in PBS.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	529.544	14.89
N 1s	396.344	0.79
C 1s	281.792	83.80
P 2p	130.338	0.11
Si 2p	99.752	0.18

Table 5: Binding energy and atomic concentration of elements obtained by XPS for PEEK functionalized with laccase.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	529.100	15.80
N 1s	396.788	6.06
C 1s	281.792	77.95
P 2p	130.338	0.03
Si 2p	99.752	0.04

Table 6: Binding energy and atomic concentration of elements obtained by XPS for PEEK functionalized with Dopamine.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	529.100	17.24
N 1s	396.788	6.80
C 1s	282.236	75.75
P 2p	132.164	0.03
Si 2p	99.864	0.08

Table 7: Binding energy and atomic concentration of elements obtained by XPS for PEEK functionalized with DOPA.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	528.656	17.10
N 1s	396.788	6.48
C 1s	281.792	75.57
P 2p	130.832	0.04
Si 2p	99.308	0.07

Table 8: Binding energy and atomic concentration of elements obtained by XPS for PEEK functionalized with Caffeic acid.

Peak	Position BE (eV)	Atomic Concentration (%)
O 1s	529.100	14.45
N 1s	396.788	3.66
C 1s	281.792	81.66
P 2p	130.388	0.02
Si 2p	99.752	0.11

The images of cell viability assay are shown in figure 3 (A,B, C, D and E). It could be observed that the cells proliferated in substrates, except on PEEK modified with Caffeic Acid (3-C) which presented only few cells adhered. The aspect of the cells spread on the surfaces were not favorable on unmodified PEEK (3-A) and Dopamine-modified PEEK (3-D) as it can be seen by the cytoplasm:nucleo ratio of the cells. PEEK modified with laccase (3-B) and DOPA (3-E) with laccase presented the best pattern of cell proliferation which can be seen by the flattened way of proliferation and by the cytoplasmic processes presented in these cells.

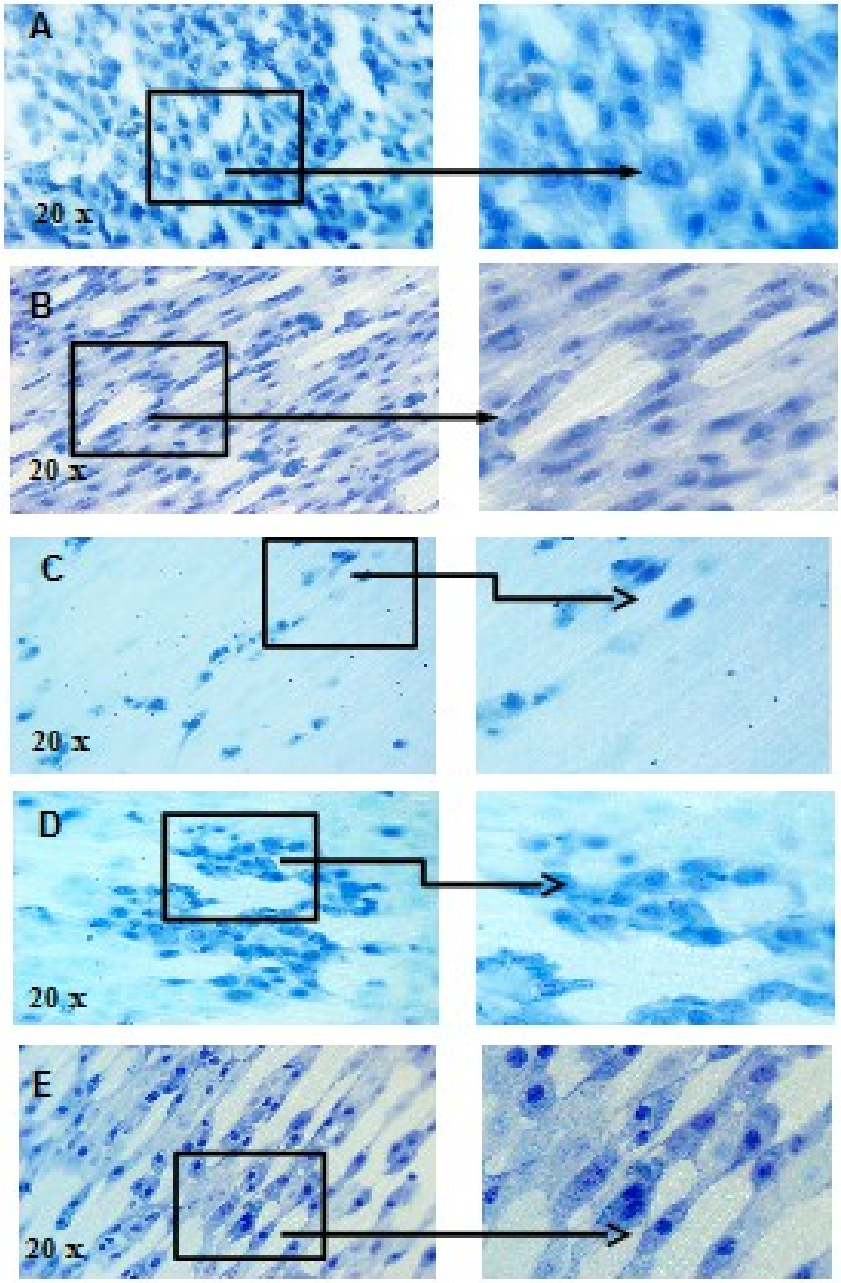

Figure 3: Pattern of cell adhesion presented by modied and unmodified PEEK (pictures on left side): A) unmodified PEEK; and PEEK modified by B) laccase; C) Caffeic Acid with laccase; D) Dopamine with laccase; and E) DOPA with laccase. The pictures on right side represent the 5 x zoom from the rectangle defined area.

DISCUSSION

Several attempts have been made to modify PEEK surface in order to improve the attachment of cells on its surface or to improve its adhesion to resin-based materials. Nonetheless, these techniques use toxic solvents and harsh conditions of reaction (Ma et al., 2014). Unlike these techniques, the present study proposed a way to modify PEEK surface characteristics, such as wettability or roughness, to make it suitable to be used as implants or in dental prosthesis.

For XPS analysis, PEEK samples were pretreated and coated with different catechols in the presence of PBS and the enzyme laccase. In a first measurement, pristine PEEK was evaluated. The formula of PEEK is $C_{19}H_{12}O_3$ of each repetition unit. This means the polymer ratio of Carbon (C) to Oxygen (O) is nearly 6 to 1. The value is exactly reflected in the atomic concentration of the XPS obtained. There was a similar ratio of Carbon and Oxygen in the XPS obtained for the freshly grounded PEEK. But, additionally, the values for Nitrogen (N) and Silicon (Si) were reduced. This is a clear indication for a successful cleaning process by grounding, because Silicon is a very common impurity in technical processes and elevated Nitrogen values can be caused by nitrogen deposition.

Additionally, the absorbance of laccase on the PEEK surface was specified. Laccase was exposed over 16 h in acetate buffer to grounded PEEK surfaces. It is known that laccase has the tendency to adsorb irreversibly in water-based solvents on hydrophobic surfaces. Laccase is an enzyme composed mainly of Carbon, Oxygen and Nitrogen. The impact of the adsorption is clearly detectable. The carbon signal decreased and the Nitrogen signals increased. This means that the laccase partially covers the PEEK surface. The XPS value for laccase on a carbon free surface (silicon wafer) is for O 1s 27.3%, N 1s 5.4% and C 1s 66.5% (Ureña et al., 2016).

In a last step, the grounded substrates were coated with three different catechols (Dopamine, DOPA (3,4-Dihydroxyphenylalanine) and Caffeic acid) in PBS in the presence of laccase. In the case of Dopamine and DOPA no significant changes could be observed in the XPS spectra in comparison to the obtained laccase spectrum. A slight increase of the Oxygen content was observed with a decrease of the Carbon content in both cases. But no real proof is given, if the surface is coated with a mixture of laccase and catechol or just the laccase is on the surface. Both catechols and laccase contain Nitrogen, Oxygen and Carbon. In the

case of a catechol coating a closed mono- or multilayer is not present. Otherwise the ratio of the Carbon to Oxygen must be close to 2:1 or 4:1 in the case of DOPA and Dopamine, respectively. Caffeic acid shows a different picture of adsorption on the surface. In this case a significant decrease of the Oxygen and Nitrogen values and an increase of the Carbon content were detected. This could be caused by a hindered adsorption of the laccase on the surface caused by the Caffeic acid.

In summary grounding before adsorption improves the quality of the PEEK surface. The laccase was identified without any doubts on the PEEK surface. But the combinations of laccase and catechols have shown no significant changes in the spectra in comparison to the pure adsorbed laccase beside the combination with Caffeic acid.

For further investigations Time-of-Flight Ion Mass Spectrometry (TOF-SIMS) technique could be an alternative method for the surface evaluation (Bellu et al., 2003) as it could provide useful information about the molecular fragments adhered on the surface (Kingshott et al., 2011).

The contact angle values obtained in this study are consistent with the XPS spectra obtained. This last one showed an increase in the Nitrogen atomic concentration for the groups containing Dopamine and DOPA together with laccase. The contact angle values showed a great increase in the wettability when PEEK was functionalized with Dopamine and DOPA mediated by laccase. This hydrophilic behavior obtained can be due the high amounts of hydrophilic groups such as -NH_2, -OH and -COOH on the surface (Jiang at al., 2010), which cannot be found in unmodified PEEK. PEEK has in its repetition unit two aromatic rings which make it very hydrophobic. It is important to observe the differences in contact angle when PEEK was sandblasted with aluminum oxide followed, or not, by functionalization with laccase. When PEEK was sandblasted without laccase there was an increase in the contact angle values, probably because more hydrophilic contaminations of the surface were removed. Some authors reported an increase of wettability after sandblasting PEEK (Rocha et al., 2016). In this study PEEK sandblasted samples were washed with isopropanol and distilled water in ultrasonic bath after the sandblasting procedure. This cleaning procedure could be able to remove all the remained aluminum oxide layer formed after the sandblasting which is responsible for the increase of wettability. The functionalization of sandblasted PEEK with laccase made it highly hydrophilic. The roughness created by sandblasting allowed

for the entrapping of laccase molecules inside the porous formed. In this case the hydrophobic moiety of laccase was interacting with the hydrophobic aromatic rings of PEEK while the hydrophilic moiety was turned out, decreasing the contact angle value (Draghici et al., 2014).

For cell viability assays, different fluorescent dyes can be used to make possible the visualization of the adherence and morfology of the cells on a surface. Nonetheless, in the present study the use of fluorenscent dyes in visible region light was not feasible due to the high autofluorescence of PEEK (Althaus et al., 2012). In this case, the dye Brilliant Crezyl Blue was used. Brilliant Crezyl Blue is a fluorescent probe that displays strong fluorensence emission in the wavelenght between 626 and 670 nm which is very close to red region (Zheng et al., 2000). PEEK funcionalized with DOPA-laccase and only laccase showed the best pattern of cell adhesion. As shown by contact angle measurements, PEEK had a more hydrophilic behavior after immersed in catechol-containing solutions (DOPA, Dopamine and Caffeic Acid) with laccase or just with laccase. Hydrophilicity as well surface energy are of great importance for cells attachment and spreading on surfaces. It is known that cells adhere and spread better on more hydrophilic surfaces than on hydrophobic ones (Anselme, 2000).

Despite the functionalization of PEEK using catechols-containing substances and laccase, the roughness of the samples was mandatory to obtain higher MSBS values. The highest MSBS values were obtained for sandblasted PEEK regardless the functionalization with laccase or not. Several studies reported an increase in bond strength values when the roughness of the samples increased by sandblasting, plasma treatment, chemical attack, or the combination of both. In the present study, it means that the adhesive entrapped inside the porous created by the sandblasting procedure and its curing inside these porous was the parameter responsible to create a reasonable adhesion between PEEK and a resin composite (Rosentritt et al., 2015; Schmidlin et al., 2010). The functional groups attached on PEEK surface after the functionalization procedures had no effect on the bond strength as they presented similar results from unmodified PEEK. It is important to mention that the adhesive Adper Single Bond 2 was used and its composition is basically methacrylate monomers which could be incompatible with the functional groups on PEEK surface. One way to overcome this problem is maybe the use of epoxide-based adhesives. In this case the opening of the cyclic ring of the epoxy

group by the -NH_2 and -OH groups inside the laccase that could provide interaction between the adhesive and the functionalized PEEK surface. However further studies should be carried out to prove this concept.

CONCLUSION

It was concluded that PEEK can be functionalized by laccase-mediated oxidation of catechols or just with laccase. The PEEK modification procedure which presented the best bond strength results was sandblasting with aluminum oxide, regardless the functionalization with laccase. The modification with DOPA with laccase, and only with laccase presented the best cell adhesion.

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2.2 Chapter 2:

"INFLUENCE OF ACID SOLUTIONS AND SANDBLASTING ON SURFACE ROUGHNESS AND BOND STRENGTH OF POLYETHER ETHER KETONE"

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ABSTRACT

The aim of the present study was to evaluate the influence of acid solutions and sandblasting on surface roughness and bond strength to resin composite of polyether ether ketone. For this PEEK samples (1.0 x 0.85 x 0.1 cm) were used after polishing and cleaning procedures. The samples were submitted to the following surface treatments: SA- sulfuric acid; PS- piranha solution; and SAO- aluminium oxide sandblasting. Untreated PEEK samples (P) were used as control. After the treatments procedures, the surface roughness (μm) and the microshear bond strength (MPa) were measured. For the bond strength, two fillings with a composite resin (Z350 flowable) were made on PEEK samples, using two different adhesives (Adper Single Bond 2 or Visio.link). The results for surface roughness show statistical differences among the groups ($p < 0.0001$), where SAO showed the higher values and P and PS the lower values. There was no significant difference between P and PS. For microshear bond strength, there was no significant difference among the groups tested, except for PS when Single Bond was used ($p < 0.001$). In this case, PS presented the lower bond strength values. All untreated samples (group P) presented pre-test failures. Results suggested that the acid solutions and sandblasting were proper to increase the bond strength of PEEK to resin composite for both adhesive systems, except when piranha solution was used with Single Bond. PEEK without surface treatment is not proper to be bond to resin composites.

Key words: polyether ether ketone. sulfuric acid. sandblasting. piranha solution.

INTRODUCTION

Polyether ether ketone (PEEK) is a semicrystalline and thermoplastic polymer developed in the beginning of 80's,[1] whose use is increasing in the medical and dental field in order to replace metallic devices.[2] Altough metals have proper mechanical properties and friction-resistance, and usually do not present toxicity, they have the disadvantage to have high elastic modulus, which do not match with the bone and dentin and may cause stress shielding effect and lead to long-term prosthesis and implants failures.[3]

In contrast to the high elastic modulus of metals, the low elastic modulus of PEEK, around 3.6 GPa, is similar to cortical bone and dentin.[4] Besides this advantage, PEEK also has high thermal stability, hardness and proper strength, which make it very attractive to be used in dentistry as implants,[5] abutments,fixed and removable prosthesis,[6] and root canal posts .

Despite the good characteristics PEEK can offer, it has the disadvantage to have its surface chemically inert, with low reactivity.[7] This behavior impairs the adhesion of PEEK to several resin-based materials used in restorative dentistry and prosthesis.[8] Regarding the long-term durability as the main factor that measures the success of adhesive bonding, PEEK surface treatments have been proposed to overcome its lack of adhesion. A strong adhesion can be obtained through reactive groups present on the surface, and as well, by the high surface roughness, that increases the surface contact, and thus, the mechanical anchorage of the adhesive.[9] Among of the available surface treatments, sulfuric acid, “piranha” solution, and sandblasting with abrasive particles, show to be proper alternatives to modify the surface characteristics of PEEK, increasing its roughness, and achoring reactive groups on its surface.[10]

In this context, the objective of the present study was to evaluate the influence of the PEEK surface treatment with sulfuric acid, “piranha” solution, and aluminion oxide sanblasting on the bond strength of PEEK to a flowable composite resin. The hypothesis was that these three surface treatments provides an improvement of the bond strength of PEEK to a composite resin used in dentistry.

METHODOLOGY

Sample preparation

PEEK samples (medical grade) were obtained by cutting a PEEK bar in 1.2 x 1.0 x 0.1 cm discs with the aid of a diamond disc EXTEC DIA WAFER BLADE 4” x 0,12 x ½ (102 mm x 0,3 mm x 127 mm coupled to a metallographic cutter (Isomet 1000, Buehler Ltda; Lake Buff, IL, USA) set to 550 rpm. The obtained samples were submitted to a polishing step using #600, #1200 and #2000 sandpapers coupled to a polishing machine (Arotec Ind. e Comércio; Cotia, SP, Brazil) for 1 minute each one under water cooling. In between the use of the different sandpapers the samples were submitted to ultrasound bath in distilled water during 15 minutes in order to remove the remnants from their surfaces. Before the surface treatments procedures, PEEK samples were cleaned with pure isopropanol, followed by distilled water, both for 15 minutes in ultrasound bath.

Surface treatments procedures

The PEEK samples were submitted to the following surface treatments procedures: SA – treatment with sulfuric acid P.A. (Neon; Suzano, SP, Brazil) for 60 s, rinsed with distilled water for 30 s, and air dried for 10 s; PS – treament with “piranha” solution. This solution was obtained by the mix of sulfuric acid and hydrogen peroxide 35% P.A. (Neon; Suzano, SP, Brazil) in a ratio of 10:3 of sulfuric acid to hydrogen peroxide. The treatment was carried out for 30 s, followed by distilled water rinse for 30 s, and air drying for 10 s; SAO – sandblasting with aluminium oxide (50 μm size particles). The sandblasting was performed for 30 seconds in a pressure of 2 bars. After the sandblasting the samples were air-blown for 60 seconds and immersed in isopropanol for 10 minutes in ultrasound bath. The untreated PEEK samples (P) were used as control.

Surface roughness measurement

The roughness of treated and untreated PEEK samples (n = 10) was measured by a rugosimeter (SV-3100S4 Mitutoyo, Tokio, Japan) equipped with a diamond tip (0.5 μm radio). After the rugosimeter calibration the samples were parallel placed on the surface of the device. Three equidistant points passing through the center of the samples to the borders were measured. The precision of the equipment was 0.01 μm, with cutoff value of 0.25 mm and reading length of 1.25 mm.

The roughness value (Ra) was regarded the mean of the three measurements of each sample.

Microshear bond strength test

The treated and untreated PEEK samples were tested regarding the microshear bond strength (n = 10) to a resin composite (Z350XT Flowable, A2 shade; 3M-ESPE, Saint Paul, MN, USA). Two fillings per PEEK sample (n = 10) were made on their respective surfaces using a matrix of perforated pasta (1mm height, 1.15 mm internal diameter; Furadinho 6, Pastifício Santa Amália, Machado, Minas Gerais, Brazil).[11] The adhesive Adper Single Bond 2 (3M-ESPE; Saint Paul, MN, USA) or the adhesive Visio.link (Bredent GmbH & Co.KG; Senden, Germany) were applied on PEEK surfaces before the filling and light-cured for 90 seconds using a 3rd generation light curing device (Valo-Ultradent). The PEEK samples were stored in distilled water for 2 hours at room temperature, in order to make the perforated pasta soft enough to be removed. After 24 hours, the microshear test was performed on a universal testing machine (Instrom; Grove City, PA, USA) at a speed of 1.0 mm/min. The microshear bond strengths were given in Mega Pascals (MPa), according to the formula bellow:

$$R = F / Area,$$

where *R* is the bond strength in MPa, F is the force in Newtons (N), and A is the area of adhesion in mm^2.

Statistical analysis

The obtained data were statistically analysed by ANOVA and Tukey test set at 5 % of significance.

RESULTS

The results obtained for surface roughness (table 1) show statistical differences among all groups, except for P and PS, which were similar between each other ($p < 0.0001$). The group SAO presented the higher values, while PS and P presented the lower.

Table 1: Average and standard deviation of surface roughness for the groups: P- untreated PEEK; SA- sulfuric acid treatment; PS- piranha solution treatment; SAO- aluminium oxide sanblasting treatment.

Groups	Surface Roughness (μm)
P	0.0972 ± 0.04 c
SA	0.7365 ± 0.15 b
PS	0.1856 ± 0.04 c
SAO	1.4342 ± 0.25 a

Different letters show statistical difference among the groups

The microshear bond strength results (table 2) showed no difference among the tested groups, regardless the adhesive system used, except when the adhesive Single Bond was used on samples treated with piranha solution ($p < 0.001$). In this case, Single Bond used with PS obtained the lower results. All samples belonging to the group P presented pre-test failures, when both adhesive systems were used.

Table 2: Average and standard deviation of microshear bond strength (MPa) for the groups. P- untreated PEEK; SA- sulfuric acid treatment; PS- piranha solution treatment; SAO- aluminium oxide sanblasting treatment.

	P	SA	SAO	PS
Single Bond	-------	10.4 ± 3.7 aA	9.8 ± 4.8 aA	3.1 ± bB
Visio.link	-------	11.5 ± 6.2 aA	8.4 ± 2.5 aA	10.2 ± 7.2 aA

Different letters show statistical difference among the groups (uppercase letters) and adhesive systems (lowcase letters)

DISCUSSION

The synthetic polymer PEEK is becoming increasingly focus of research in medicine and dentistry. The reason of the great interest in this material is the good mechanical and physical properties it can offer that place it as an opportunity to replace metallic devices.[12] However, PEEK surface is known to be inert, and it characteristic can jeopardize the reactivity of PEEK with great range of materials, such as composite resins, and living organisms.[13,14] In this respect, the present study proposed to evaluate three different methods based in acid solutions (sulfuric acid and piranha solution) or sanblasting (aluminium oxide sandblasting) to treat PEEK surface in order to increase its reactivity to composite resins. The hypothesis was that these treatments could improve the bond strength of PEEK to composite resins. Whereas all untreated PEEK samples presented pre-test failures, oppositely to treated samples, the given hypothesis was accept.

The modification of PEEK surface can be demonstrated by changes on surface roughness after the treatments proposed. Especially for aluminium oxide sanblasting and sulfuric acid, PEEK roughness changes were significant. Aluminium oxide sandblasting has already been used to increase the bond strength of prosthetic components to resin-based cements. The reason is due to the surface microporosities created after the sandblasting, what increase the contact area of the respective component with the cement.[15,16] Sulfuric acid, as others acidic substances, can chemically modify surfaces, which creates a rougher surface.[17] It is known that rougher surfaces provide better adhesion due to higher surface area and mechanical interloking of the adhesive inside the porous created.[18]

In the present study, the rougher surfaces provided by the surface treatments with aluminium oxide sandblasting and sulfuric acid were responsible for the improvement of the bonding behavior of PEEK. These treatments increased the bond strength of PEEK, regardless the adhesive systems used, Single Bond or Visio.link. Besides the porous created by etching, sulfuric acid might attack PEEK ether and carbonyl groups, anchoring functional groups, which are responsible for the increase of polarity and thus the adhesive diffusion into PEEK porous.[19] This fact was also responsible for the proper bond strenght provided by the treatment with sulfuric acid.

In contrast to etched and sandblasted treated PEEK, untreated PEEK had pre-test failures for all samples belonging to this group. Besides the lower surface roughness shown by untreated PEEK, the lack of functional groups on PEEK surface which could react with methacrylate-based adhesives might be the reason for those premature failures.[20]

Piranha solution, a combination of sulfuric acid with hydrogen peroxide, used for descontamination and cleaning of surfaces. In the structure of PEEK, piranha solution can remove organic remnants, increase the surface polarity and break aromatic bonds, which can increase the bonding properties of PEEK.[10] However, the ability of piranha solution in the improvement of bond characteristics of PEEK is controversal in the literature.[21]

In the present study, the results obtained showed that for piranha solution only the adhesive Visio.link could improve the bond strength, and they were similar to the results obtained for sandblasted and sulfuric acid etched PEEK. In contrast, when Single Bond was used after piranha solution, the bond strength values dropped considerably. Visio.link is an adhesive compound by pentaerythritol triacrylate, dimethacrylate and methyl methacrylate (MMA) monomers. The dymethacrylates present in the composition of Visio.link might play a significant role on the bonding properties of PEEK as it can act as a linker between the functional groups provided by piranha solution and the composite resin used. In the case of Single Bond, the polyacrylic and polyitaconic acids present in its composition could react with the dimethacrylate also present, which could prevent the reaction of the dimetacrylate with functional groups in the surface, or with the composite resin.[21]

Several studies have shown different methods to modify PEEK surface in order to change its lack of reactivity. Most of these studies test acid solutions and sandblasting with abrasive particles to improve the adhesion of PEEK to other types of polymers.[22] As it was already mentioned, the surface roughness have a important hole on the bonding properties of many types of substrates.[23] The results obtained in the present study corroborate this statement. It is important to observe that the porous created on the surface by the methods proposed here and the interlocking and polymerization of the adhesive into these porous were the main responsible for the increase of bonding properties of PEEK. When the surface roughness is not high

enough to promote mechanical bonding, enough amount of functional groups should be present to allow the chemical bonding of PEEK and the adhesive. In this case, the ideal adhesive system should be carefully selected. In this direction, further studies are necessary.

CONCLUSION

It was concluded that the acid solutions and sandblasting were proper to increase the bond strength of PEEK to resin composite for both adhesive systems, except when piranha solution was used with Single Bond. PEEK without surface treatment is not proper to be bond to resin composites.

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3 DISCUSSION

This thesis tested different approaches to modify PEEK surface in order to chage its characteristcs. PEEK is known to be a bioinert polymer, with hydrophobic behavior. It means that altough the good mechanical properties PEEK presents, it is difficult to adhere cells or resins on its surface, what can limit its use in medical and dental field (Peng et al., 2017).

In the Chapter 1 was proposed an enzymatic way to modify PEEK surface in order to increase the bond strength to resin composite, improve the surface characteristics and cell adhesion of this polymer. In a first measurement, for XPS analysis, PEEK samples were pretreated and coated with different catechols in the presence of PBS and the enzyme laccase. In a first measurement, pristine PEEK was evaluated. The formula of PEEK is $C_{19}H_{12}O_3$ of each repetition unit. This means the polymer ratio of Carbon (C) to Oxygen (O) is nearly 6 to 1. The value is exactly reflected in the atomic concentration of the XPS obtained. There was a similar ratio of Carbon and Oxygen in the XPS obtained for the freshly grounded PEEK. But, additionally, the values for Nitrogen (N) and Silicon (Si) were reduced. This is a clear indication for a successful cleaning process by grounding, because Silicon is a very common impurity in technical processes and elevated Nitrogen values can be caused by nitrogen deposition.

Additionally, the absorbance of laccase on the PEEK surface was specified. Laccase was exposed over 16 h in acetate buffer to grounded PEEK surfaces. It is known that laccase has the tendency to adsorb irreversibly in water-based solvents on hydrophobic surfaces. Laccase is an enzyme composed mainly of Carbon, Oxygen and Nitrogen. The impact of the adsorption is clearly detectable. The carbon signal decreased and the Nitrogen signals increased. This means that the laccase partially covers the PEEK surface. The XPS value for laccase on a carbon free surface (silicon wafer) is for O 1s 27.3%, N 1s 5.4% and C 1s 66.5% (Ureña et al., 2016).

In a last step, the grounded substrates were coated with three different catechols (Dopamine, DOPA (3,4-Dihydroxyphenylalanine) and Caffeic acid) in PBS in the presence of laccase. In the case of Dopamine and DOPA no significant changes could be observed in the XPS spectra in comparison to the obtained laccase spectrum. A slight increase of the Oxygen content was observed with a

decrease of the Carbon content in both cases. But no real proof is given, if the surface is coated with a mixture of laccase and catechol or just the laccase is on the surface. Both catechols and laccase contain Nitrogen, Oxygen and Carbon. In the case of a catechol coating a closed mono- or multilayer is not present. Otherwise the ratio of the Carbon to Oxygen must be close to 2:1 or 4:1 in the case of DOPA and Dopamine, respectively. Caffeic acid shows a different picture of adsorption on the surface. In this case a significant decrease of the Oxygen and Nitrogen values and an increase of the Carbon content were detected. This could be caused by a hindered adsorption of the laccase on the surface caused by the Caffeic acid.

In summary grounding before adsorption improves the quality of the PEEK surface. The laccase was identified without any doubts on the PEEK surface. But the combinations of laccase and catechols have shown no significant changes in the spectra in comparison to the pure adsorbed laccase beside the combination with Caffeic acid.

For further investigations Time-of-Flight Ion Mass Spectrometry (TOF-SIMS) technique could be an alternative method for the surface evaluation (Bellu et al., 2003) as it could provide useful information about the molecular fragments adhered on the surface (Kingshott et al., 2011).

The contact angle values obtained in this study are consistent with the XPS spectra obtained. This last one showed an increase in the Nitrogen atomic concentration for the groups containing Dopamine and DOPA together with laccase. The contact angle values showed a great increase in the wettability when PEEK was functionalized with Dopamine and DOPA mediated by laccase. This hydrophilic behavior obtained can be due the high amounts of hydrophilic groups such as -NH_2, -OH and -COOH on the surface (Jiang at al., 2010), which cannot be found in unmodified PEEK. PEEK has in its repetition unit two aromatic rings which make it very hydrophobic. It is important to observe the differences in contact angle when PEEK was sandblasted with aluminum oxide followed, or not, by functionalization with laccase. When PEEK was sandblasted without laccase there was an increase in the contact angle values, probably because more hydrophilic contaminations of the surface were removed. Some authors reported an increase of wettability after sandblasting PEEK (Rocha et al., 2016). In this study PEEK sandblasted samples were washed with isopropanol and distilled water in ultrasonic bath after the sandblasting procedure. This cleaning procedure could be able to remove all the

remained aluminum oxide layer formed after the sandblasting which is responsible for the increase of wettability. The functionalization of sandblasted PEEK with laccase made it highly hydrophilic. The roughness created by sandblasting allowed for the entrapping of laccase molecules inside the porous formed. In this case the hydrophobic moiety of laccase was interacting with the hydrophobic aromatic rings of PEEK while the hydrophilic moiety was turned out, decreasing the contact angle value (Draghici et al., 2014).

For cell viability assays, different fluorescent dyes can be used to make possible the visualization of the adherence and morfology of the cells on a surface. Nonetheless, in the present study the use of fluorenscent dyes in visible region light was not feasible due to the high autofluorescence of PEEK (Althaus et al., 2012). In this case, the dye Brilliant Crezyl Blue was used. Brilliant Crezyl Blue is a fluorescent probe that displays strong fluorensence emission in the wavelenght between 626 and 670 nm which is very close to red region (Zheng et al., 2000). PEEK funcionalized with DOPA-laccase and only laccase showed the best pattern of cell adhesion. As shown by contact angle measurements, PEEK had a more hydrophilic behavior after immersed in catechol-containing solutions (DOPA, Dopamine and Caffeic Acid) with laccase or just with laccase. Hydrophilicity as well surface energy are of great importance for cells attachment and spreading on surfaces. It is known that cells adhere and spread better on more hydrophilic surfaces than on hydrophobic ones (Anselme, 2000).

Despite the functionalization of PEEK using catechols-containing substances and laccase, the roughness of the samples was mandatory to obtain higher MSBS values. The highest MSBS values were obtained for sandblasted PEEK regardless the functionalization with laccase or not. Several studies reported an increase in bond strength values when the roughness of the samples increased by sandblasting, plasma treatment, chemical attack, or the combination of both. In the present study, it means that the adhesive entrapped inside the porous created by the sandblasting procedure and its curing inside these porous was the parameter responsible to create a reasonable adhesion between PEEK and a resin composite (Rosentritt et al., 2015; Schmidlin et al., 2010). The functional groups attached on PEEK surface after the functionalization procedures had no effect on the bond strength as they presented similar results from unmodified PEEK. It is important to mention that the adhesive Adper Single Bond 2 was used and its composition is

basically methacrylate monomers which could be incompatible with the functional groups on PEEK surface. One way to overcome this problem is maybe the use of epoxide-based adhesives. In this case the opening of the cyclic ring of the epoxy group by the -NH_2 and -OH groups inside the laccase that could provide interaction between the adhesive and the functionalized PEEK surface. However further studies should be carried out to prove this concept.

In the chapter 2 was proposed three different methods based in acid solutions (sulfuric acid and piranha solution) or sanblasting (aluminium oxide sandblasting) to treat PEEK surface in order to increase its reactivity to composite resins. The hypothesis was that these treatments could improve the bond strength of PEEK to composite resins. Whereas all untreated PEEK samples presented pre-test failures, oppositely to treated samples, the given hypothesis was accept.

The modification of PEEK surface can be demonstrated by changes on surface roughness after the treatments proposed. Especially for aluminium oxide sanblasting and sulfuric acid, PEEK roughness changes were significant. Aluminium oxide sandblasting has already been used to increase the bond strength of prosthetic components to resin-based cements. The reason is due to the surface microporosities created after the sandblasting, what increase the contact area of the respective component with the cement (Okuyama et al., 2016; Rocha et al., 2016). Sulfuric acid, as others acidic substances, can chemically modify surfaces, which creates a rougher surface (Pourkhalili et al., 2016). It is known that rougher surfaces provide better adhesion due to higher surface area and mechanical interloking of the adhesive inside the porous created (Stawarczyk et al., 2013).

In the present study, the rougher surfaces provided by the surface treatments with aluminium oxide sandblasting and sulfuric acid were responsible for the improvement of the bonding behavior of PEEK. These treatments increased the bond strength of PEEK, regardless the adhesive systems used, Single Bond or Visio.link. Besides the porous created by etching, sulfuric acid might attack PEEK ether and carbonyl groups, anchoring functional groups, which are responsible for the increase of polarity and thus the adhesive diffusion into PEEK porous (Stawarczyk et al., 2014a). This fact was also responsible for the proper bond strenght provided by the treatment with sulfuric acid.

In contrast to etched and sandblasted treated PEEK, untreated PEEK had pre-test failures for all samples belonging to this group. Besides the lower surface roughness shown by untreated PEEK, the lack of functional groups on PEEK surface which could react with methacrylate-based adhesives might be the reason for those premature failures (Stawarczyk et al., 2014b).

Piranha solution, a combination of sulfuric acid with hydrogen peroxide, used for descontamination and cleaning of surfaces. In the structure of PEEK, piranha solution can remove organic remnants, increase the surface polarity and break aromatic bonds, which can increase the bonding properties of PEEK (Silthampitag et al., 2016). However, the ability of piranha solution in the improvement of bond characteristics of PEEK is controversal in the literature (Uhrenbacher et al., 2014).

In the present study, the results obtained showed that for piranha solution only the adhesive Visio.link could improve the bond strength, and they were similar to the results obtained for sandblasted and sulfuric acid etched PEEK. In contrast, when Single Bond was used after piranha solution, the bond strength values dropped considerably. Visio.link is an adhesive compound by pentaerythritol triacrylate, dimethacrylate and methyl methacrylate (MMA) monomers. The dymethacrylates present in the composition of Visio.link might play a significant role on the bonding properties of PEEK as it can act as a linker between the functional groups provided by piranha solution and the composite resin used. In the case of Single Bond, the polyacrylic and polyitaconic acids present in its composition could react with the dimethacrylate also present, which could prevent the reaction of the dimetacrylate with functional groups in the surface, or with the composite resin (Uhrenbacher et al., 2014).

Several studies have shown different methods to modify PEEK surface in order to change its lack of reactivity. Most of these studies test acid solutions and sandblasting with abrasive particles to improve the adhesion of PEEK to other types of polymers (Taufall et al., 2016). As it was already mentioned, the surface roughness have a important hole on the bonding properties of many types of substrates (Zhou et al., 2014). The results obtained in the present study corroborate this statement. It is important to observe that the porous created on the surface by

the methods proposed here and the interlocking and polymerization of the adhesive into these porous were the main responsible for the increase of bonding properties of PEEK. When the surface roughness is not high enough to promote mechanical bonding, enough amount of functional groups should be present to allow the chemical bonding of PEEK and the adhesive. In this case, the ideal adhesive system should be carefully selected. In this direction, further studies are necessary.

4 CONCLUSION

It was concluded that PEEK can be treated by the methods proposed in both Chapters. The PEEK surface treatment which presented the best bond strength results was sandblasting with aluminium oxide, regardless the functionalization with laccase (Chapter 1) and sandblasting with aluminium oxide and sulfuric acid (Chapter 2). The surface treatment with DOPA with laccase, and just with laccase presented the best cell adhesion (Chapter 1).

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