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Influência da adição de politetrafluoretileno (PTFE) nas propriedades físicas, mecânicas e aderência microbiana em resinas acrílicas

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do Título de Doutor em Clínica Odontológica – Área de Concentração: Prótese Dental

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"…In the eyes of Spirit, little things count. What you give, you receive. Doing is understanding. And you can do anything when

you find the heart for it, and the courage.

Of course we make mistakes; it's how we learn. We're all in training.

Life can be difficult; what an opportunity! The Light will disturb us when

we're comfortable, and comfort us when we're disturbed.

We turn to Spirit for help when our foundations are shaking, only to find it is Spirit who is shaking them…

Trust yourself; trust the process that is your life."

Dann Millman, 1984. In: Peaceful of Warrior

RESUMO

Resinas acrílicas são materiais amplamente empregados na Odontologia, especialmente os polímeros a base de polimetilmetacrilato (PMMA) para confecção de próteses. Dentre as vantagens deste material estão à estabilidade de cor, propriedades ópticas, estética satisfatória, estabilidade dimensional, fácil manipulação e propriedades físicas adequadas para aplicações odontológicas. Entretanto, como desvantagem deste material, está à susceptibilidade à aderência e desenvolvimento de biofilme por microrganismos presentes na cavidade bucal, como fungos e bactérias. A aderência de microrganismo é o primeiro evento para o desenvolvimento do biofilme o qual está relacionado ao estabelecimento de quadros clínicos patológicos como estomatite protética. Recentemente, a adição de modificadores na composição da resina acrílica tem sido proposta com o objetivo de melhorar as características de superfície, e conseqüentemente controlar a aderência de microrganismos. Entretanto, a mudança na composição da resina acrílica não deverá comprometer as propriedades físicas. Politetrafluoretileno (PTFE) tem sido usado como modificador para confecção de stents e cateteres devido a biocompatibilidade, alta resistência a agentes químicos e baixa energia de superfície. Assim, o objetivo neste estudo foi avaliar as propriedades físicas, características de superfície, aderência de microrganismos e formação de biofilme de suas resinas acrílicas, uma polimerizada em água quente com ciclo longo e a outra polimerizada pelo ciclo curto, contendo 2% de PTFE em sua composição e comparar com as mesmas resinas sem adição de PTFE. Para isto, três estudos foram realizados: 1- Propriedades físicas da resina acrílica - espécimes das resinas foram confeccionados de acordo com a norma ISO 1567:1999 (n=10) para cada teste mecânico: dureza de superfície, resistência ao impacto, resistência a flexão e o módulo de elasticidade e carga máxima foram calculados; 2 – Características de superfície da resina acrílica e aderência de *Candida albicans*: espécimes retangulares (n=10) para cada resina foram confeccionados e avaliados quanto à rugosidade, ângulo de contato, energia livre de superfície e aderência de *Candida albicans.* As células aderidas foram expressas por unidade formadora

de colônia por área da superfície; 3- Biofilme formado na resina acrílica e sua composição: discos de resina acrílica (n=6) foram usados para formação de biofilme de *Streptococcus mutans* UA159, *Streptococcus sanguinis* ATCC 10556 e *Actinomyces naeslundii* ATCC 12104. A composição de polissacarídeos e unidades formadoras de colônia foram comparadas. Em todos os estudos, os resultados foram submetidos à análise variância a dois fatores e comparados pelo teste de Tukey ($p=0,05$). Os resultados não mostraram diferença estatisticamente significante para os valores de dureza de superfície ($p>0,05$). Entretanto, para os valores de resistência ao impacto e flexão para os grupos experimentais, contendo PTFE, apresentaram resultados significantemente menores ($p<0,05$) quando comparados aos grupos não adicionados de PTFE. Os valores de módulo de elasticidade da resina acrílica adicionada com 2% de PTFE polimerizada com o ciclo longo apresentaram os maiores valores comparados aos demais grupos ($p<0,05$). Não houve diferenças significativas para rugosidade, ângulo de contato e energia livre de superfície ($p>0,05$); houve uma tendência de redução de células de *C. albicans* no grupo com PTFE, mas não foi estatisticamente significante. Em relação, a formação e composição de polissacarídeo os resultados não apresentaram diferenças entre os grupos de resina acrílica ($p>0,05$). Os resultados sugerem que 2% de PTFE adicionada à resina acrílica não foi suficiente para promover mudanças nas propriedades mecânicas, aderência e composição do biofilme.

Palavras - chave: polimetilmetacrilato, *Candida albicans,* biofilme, bactéria.

ABSTRACT

Acrylic resins have been widely used in Dentistry, especially polymers of poly (methyl methacrylate) for fabricating dentures. This material has as advantages stable color and optical properties, satisfactory esthetic and dimensional stability, it is easy to be processed, and its physical properties have been proven adequate for dental applications. However, as disadvantage acrylic resins surface is susceptible to adherence and biofilm development of oral microorganism, such as fungi and bacteria. Microorganism's adherence is the first step to biofilm development, and it is related to pathogenic condition, leading to denture stomatitis. Recently, it had been proposed addition of modifiers to acrylic resin composition that could be able to improve its surface properties, and consequently controlling microorganisms' adherence. However, this change in composition of acrylic resin should not compromise its physical properties. Polytetrafluorethylene has been used as modifiers to fabricate medical devices such as stents and catheters because it is a biocompatible material, high resistance to chemical reagents and low surface energy. Thus, the aim of this study was to evaluate the physical properties, surface characteristics, adherence of microorganisms and biofilm formation of two acrylic resins, one polymerized by long and other polymerized by short cycle, with 2% (w/w) of PTFE added and to compare with the same resins with no PTFE added. For this, three studies were conducted: 1 – Physical properties of acrylic resins - specimens were prepared for each acrylic resin according to the ISO standard 1567:1999 (n=10) for each mechanical test: surface hardness, impact strength, flexural strength and flexural modulus and peak load were calculated; 2 – Characteristics of acrylic resin surface and adherence of *Candida albicans:* rectangular specimens (n=10) of each resin were prepared and evaluated for surface roughness, contact angle, surface free energy and *C.albicans* adherence. Adhered cells were expressed in colony forming units per surface area; 3- Biofilm formed on acrylic resins and its composition: acrylic resin discs (n=6) were used to *Streptococcus mutans* UA 159, *Streptococcus sanguinis* ATCC 10556 and *Actinomyces naeslundii* ATCC 12104 biofilm formation. Polysaccharide composition and colony forming units were

compared. For both three studies the results of were analyzed by Two-Way ANOVA and Tukey test (P<.05). The results showed no statistical significant differences for surface hardness (P>0.05). However, impact and flexural strengths values for experimental groups, with PTFE added were significantly lower (P<.05) when compared to the groups with no PTFE added. The flexural modulus values of acrylic resin containing 2% PTFE polymerized by long cycle was higher than other resins (P<0.05). No statistical differences were found for roughness, contact angle, surface free energy (P>.05); there was a reduction trend in *C. albicans* adherence in acrylic resin with PTFE added, but it was not statistically significant. Regarding, the formation and composition of polysaccharide matrix the results showed no differences among the acrylic resin groups ($P>0.05$). The results showed that the 2% of PTFEE incorporated to acrylic resins was not enough to affect the mechanical properties, adherence and composition of biofilm.

Keywords: polymethylmetacrilate, *Candida albicans, biofilms, bacteria.*

SUMÁRIO

INTRODUÇÃO GERAL

A resina acrílica, polimetilmetacrilato (PMMA) tem sido o material de escolha para confecção de próteses removíveis devido à estética, estabilidade dimensional, biocompatibilidade e de fácil manipulação técnica (Narva *et al.*, 2005). Apesar deste material possuir propriedades mecânicas aceitáveis, sua superfície é susceptível a colonização por microrganismos comumente encontrados na cavidade bucal, como bactérias e fungos, podendo representar um reservatório de microrganismos responsáveis por patologias locais e sistêmicas (Branting *et al.*, 1989; Douglas *et al.*, 1993; Jenkinson *et al.*, 1990; Kulak *et al.,* 1997; Morgan & Wilson, 2001; Gonçalves *et al.*, 2006; Marcos-Arias *et al.*, 2009).

Embora as propriedades mecânicas das resinas acrílicas sejam aceitáveis em algumas situações esta não é suficiente para evitar fraturas. Próteses removíveis parciais ou totais confeccionadas com este material são susceptíveis à fratura quando submetida à excessiva força mastigatória ou queda acidental decorrentes de flexões repetidas e impactos, respectivamente (Gutteridge, 1988; Jagger *et al.*, 1999; Jagger *et al.*, 2002; Stipho, 1998; Uzun & Hersek, 2002).

No que se refere à característica de superfície da resina acrílica, esta possui baixa hidrofobicidade e relativamente baixa dureza que facilita a alteração da rugosidade de superfície, especialmente pelo ato da escovação da prótese dental. Essas duas características facilitam a colonização por microrganismos (Epstein, 1990; Akpan & Morgan, 2002) que pode levar aos usuários de próteses confeccionadas com esse material a apresentarem quadros patológicos de reação inflamatória na mucosa bucal denominada de estomatite protética, caracterizada pela sensação de ardência, sangramento e halitose (Arendorf & Walker, 1987; Akpan & Morgan, 2002).

A estomatite protética possui etiologia multifatorial, destacando a presença de diferentes espécies de microrganismos associado a fatores locais e sistêmicos. Dentre os fatores locais destacam-se o uso de próteses removíveis, associado à negligência no controle da higiene oral, baixo fluxo salivar e alta

ingestão de carboidratos (Nikawa *et al*., 2001). Dentre os fatores sistêmicos estão o uso prolongado de antibióticos, terapia hormonal, *diabettes mellitus*, hipertensão arterial e quadros clínicos de imunossupressão (Nikawa *et al*., 2001; Akpan & Morgan, 2002; Ramage *et al*., 2004; Gonçalves *et al*., 2006).

A estomatite protética está fortemente associada à presença de *C. albicans* (Branting *et al*., 1989; Drake *et al*., 1992; Akpan & Morgan, 2002; Chandra *et al.,* 2005; Pereira-Cenci *et al*., 2008; Marcos-Arias *et al*., 2009), sendo que esta espécie de fungo é encontrado em até 65% de usuários de próteses removíveis (Arendorf & Walker, 1980). Além da presença de *C. albicans* na composição do biofilme encontrado nas superfícies de próteses, outros microrganismos como os estreptococos e actinomices (Theilade *et al*., 1983; Bagg & Silverwood, 1986; Jenkinson *et al.,* 1990; Radford & Radford, 1993; Grimaudo *et al*., 1996; Kulak *et al*., 1997) também são identificados nesse biofilme. A existência de agregação e co-agregação entre fungos e bactérias, fenômeno que possibilita mútuo benefício entre as espécies de microrganismos envolvidas favorece a permanência destes microrganismos no meio bucal (Holmes *et al*., 1995; Pereira-Cenci et al., 2007).

A aderência de microrganismos a um substrato é mediada por interações físico químicas como a hidrofobicidade (Colling *et al*., 2005), energia livre de superfície e diferenças na topografia (Pereni *et al*., 2006; Verran & Maryan, 1997). Quirynen *et al.* (1995), demonstram a relevância da rugosidade e da energia livre de superfície no desenvolvimento de biofilme, especialmente nos estágios iniciais de aderência de microrganismos. Porém, controvérsias ainda persistem quanto à relação direta entre a hidrofobicidade da superfície do material e aderência de microrganismos. Alguns autores encontraram resultados que sustentam a hipótese de que o aumento da hidrofobicidade do material polimérico causa diminuição da aderência bacteriana (Quirynen *et al*., 1989; Pereni *et al*., 2006). Entretanto é possível encontrar estudos que contradizem estes achados anteriores, onde se observou o aumentando a aderência de microrganismos com

o aumento da hidrofobicidade de superfície (Speranza *et al*., 2004; Chandra *et al*., 2005).

A colonização e desenvolvimento do biofilme podem ser descritos como uma seqüência de diferentes passos sendo a aderência na superfície do material a primeira etapa, seguida do crescimento e a estruturação do biofilme. Na estruturação do biofilme destaca-se a importância da presença de carboidratos que são usados pelos microrganismos para produção de ácidos ou então servindo como substrato para as enzimas extracelulares responsáveis pela síntese de polissacarídeos (Hamada & Slade, 1980; Schilling & Bowen, 1992).

Os polissacarídeos extracelulares podem ser solúveis (PECS) ou insolúveis (PECI), e estão relacionados ao acúmulo de microrganismos nas superfícies e estruturação do biofilme, desempenham importante papel nas interações de adesividade entre microrganismos e as superfícies existentes no meio bucal, seja estrutura dentária, material restaurador ou mucosa (Hamada & Slade, 1980; Schilling & Bowen, 1992; Colby & Russell, 1997). Os polissacarídeos intracelulares (PIC), por sua vez, servem como fonte endógena de carboidratos durante períodos de restrição de nutrientes (Paes Leme *et al*., 2006).

Dessa forma, estratégias para prevenir e/ou retardar a formação de biofilmes ou que facilitem a remoção deste devem ser estudadas. Dentre estas destaque deve ser dado a adição de modificadores na composição dos materiais odontológicos (Nomura *et al*., 1997; Steinberg & Eyal, 2002). O desenvolvimento de novos materiais a partir de combinações envolvendo dois ou mais polímeros, constitui uma estratégia capaz de reunir, em um único material, propriedades específicas de cada um dos componentes, uma vez que isto permite o desenvolvimento de materiais com propriedades diferentes aos materiais combinados (Oréfice & Moraes, 2004; Xie *et al*., 1999). Dentre esses materiais destaca-se o politetrafluoretileno (PTFE), um fluorpolímero que na forma de pó é comumente incorporado à matriz de polímeros para melhoria das propriedades de superfície do material. O PTFE é um material quimicamente inerte que apresenta alta estabilidade térmica e química, baixo coeficiente de fricção, boa

biocompatibilidade e alta hidrofobicidade com conseqüente baixa energia de superfície. Em acréscimo, esse material possui alto peso molecular e alta viscosidade, além de baixa solubilidade (Gumpenberger *et al*., 2003; Borkar *et al*., 2006; Gyo *et al*., 2008). Devido a essas propriedades sua aplicação na área de saúde tem aumentado como, por exemplo, na área médica na fabricação de "stents cardíacos", cateteres (Gumpenberger *et al*., 2003) e implante arterial (Chevalier *et al*., 2005), e na Odontologia é encontrada aplicação deste material na periodontia com aplicação na confecção de membranas usadas para reparação tecidual guiada.

Atenção especial deve haver para que a modificação de um material pela incorporação de outro, considerando as propriedades de superfície da resina acrílica no que tange à energia livre de superfície e rugosidade, a adição de PTFE na mesma poderia ser uma forma de melhorar essas características sem, contudo interferir com as propriedades mecânicas de resistência à flexão e impacto.

Assim, este trabalho objetivou avaliar e comparar duas resinas acrílicas termopolimerizáveis, uma polimerizada por ciclo longo e outra polimerizada por ciclo curto, adicionadas ou não de 2% de PTFE. Para isso, três estudos foram realizados:

Estudo 1- Propriedades físicas da resina acrílica – análise da dureza de superfície, resistência ao impacto, resistência a flexão, módulo de elasticidade e carga máxima.

Estudo 2- Características de superfície da resina acrílica e aderência de *Candida albicans*: avaliação da rugosidade, ângulo de contato, energia livre de superfície e aderência de *Candida albicans.*

Estudo 3- Avaliação da composição de biofilmes de *Streptococcus mutans* UA159, *Streptococcus sanguinis* ATCC 10556 e *Actinomyces naeslundii* ATCC 12104 formados sobre a superfície das resinas acrílicas.

CAPÍTULO 1

Polytetrafluorethylene added to acrylic resins: mechanical properties

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Abstract

Statement of problem. The addition of different polymers to denture base resins such as polytetrafluorethylene (PTFE) could be an option to modify acrylic resin surface.

Purpose. This study evaluated the surface hardness, impact and flexural strength, flexural modulus and peak load of two acrylic resins, one polymerized by a long cycle and another by a short polymerization cycle, with the addition of 2% polytetrafluorethylene, or without it.

Material and Methods. 40 specimens of each group were tested for hardness, discs measuring 30 mm in diameter and 5mm thick; impact and flexural strength tests, rectangular specimens measuring 50x6x4 mm and 64x10x3.3 mm respectively. Two acrylic resins were selected to test the effects of incorporating 2% polytetrafluorethylene: LC: acrylic resin polymerized by long cycle, LE: acrylic resin with 2% PTFE added, polymerized by long cycle; SC: acrylic resin polymerized by short cycle; SE: acrylic resin with 2% PTFE added, polymerized by short cycle. All tests were performed in accordance with the ISO1567:1999 Standard. The data were analyzed by ANOVA and Tukey test with the level of significance set at 5%.

Results. No statistically significant differences ($P>.05$) were found for surface hardness. Flexural strength, impact strength and peak of load values were higher for resins without added polytetrafluorethylene ($P<.05$). The flexural modulus value of acrylic resin with 2% PTFE added, polymerized by long cycle, was higher than that of the other resins ($P<.05$).

Conclusion. Within the limits of this study, the addition of polytetrafluorethylene did not improve the mechanical properties of the evaluated acrylic resins.

Clinical Implication: The mechanical properties of the evaluated acrylic resins were not enhanced with the addition of 2% of polytetrafluorethylene.

INTRODUCTION

Acrylic resin has been used for a long time as the best choice to fabricate full or partial dentures because of its esthetic qualities and ease of manipulation, nevertheless its mechanical properties should be improved.[1,2] Moreover, this material is a potential substratum for biofilm formation, leading to denture stomatitis.[3-6]

Alternatives to improve the physical and mechanical properties of acrylic resin have been described, and include the addition of co-polymers, cross-linking agents[7,10] and rubber substances in the form of butadiene styrene.[11,12] The use of the phosphate-containing monomer ethylene glycol dimethacrylate as substitute for the methyl methacrylate monomer to reduce microorganism colonization has also been described.[13] However, the addition of synthetic fluoropolymers, such as polytetrafluoroethylene (PTFE) particles, to the polymeric matrix of acrylic resin in order to control microorganism adhesion, unwanted creep and to improve wear and friction is still unexplored. There is little information available about PTFE for dentistry applications,[14,15] however, it is known that PTFE is chemically inert, biocompatible, highly resistance to chemical reagents, has high temperature tolerance, low surface energy and a low coefficient of friction which allows it to function as a sealing and lubricant pellicle.[16]

Considering PTFE characteristics, the addition of this synthetic fluoropolymers to methylmethacrylate might be capable of improving its surface properties, decreasing the surface wetting and the free surface energy, and consequently changing microorganism adherence, as well as slowing the degradation process of the acrylic resins in the oral environment. However, any change in the composition of acrylic resin should not compromise the physical/mechanical properties of this material. Impact and flexural strength are the two most important property requirements of denture base resins.[13] The former gives the required fracture resistance when a prosthesis accidentally dropped onto a hard surface, and flexural strength is responsible for the resistance to fracture under dynamic load.[8,12,13,17] In addition, hardness is a relevant surface

characteristic and it represents resistance to scratching, abrasion or cutting material. This characteristic is related to the material wear that can take place during daily denture brushing causing roughness, and facilitating microorganism adhesion.[18] Indeed, few studies have addressed the physical and mechanical proprieties of acrylic resin modified by PTFE.

Thus, the aim of this study was to evaluate surface hardness, impact and flexural strength, flexural modulus and peak of load of two acrylic resins modified by the addition of 2% PTFE.

MATERIAL AND METHODS

The materials used in this study are shown in Table I. Two acrylic resins with different polymerization cycles - long and short - were chosen because these two cycles are the ones most used by commercial laboratories.

Ten discs (50 mm in diameter) and ten rectangular specimens of each acrylic resin long (LC) or short (SC) polymerization cycle, with the addition of 2% PTFE powder (wt/wt) (Uniflon Fluormasters, Sao Paulo, Brazil) (LE and SE) or without it (LC and SC), measuring 50×6×4 mm and 64×10×3.3 mm were prepared for hardness, impact and flexural strength tests, respectively.[1,19]

Metal master patterns were individually invested with high-viscosity silicone (Zetalabor; Zermack S.p.A, Badia Polesine, Rovigo, Italy) and used to fabricate the specimens. Patterns were invested with Type III dental stone (Herodent Soli Rock; Rio de Janeiro, Brazil) in metal dental flasks (Uraby; DLC, São Paulo, Brazil). The acrylic resins were mixed in accordance with the manufacturers' instructions and packed into the silicone mold at the dough stage.

Table I: Acrylic resins and polytetrafluorethylene used in this study.

Material	Processing method	Chemical composition	Manufacturer
Polymethyl methacrylate,	Hot water bath 9 h at 74ºC	Powder: Polymethylmethacrylate, ethylacrylate, benzoyl peroxide, organic pigments Liquid: Methylmethacrylate, topanol stabilizer	Classico Ind. e Com, São Paulo, Brazil.
Polymethyl methacrylate,	Hot water bath 20 min at 100ºC	Powder: Copolymer (methyl-n- butyl) methacrylate, benzoyl peroxide, mineral dyes Liquid: Methylmethacrylate, ethyleneglycol, dimethacrylate, N,N dimethyl-p-toluidine, hydroquinone methyl ether	Dentsply International, York, PA, USA.
Polytetrafluoroethylene	-------	Powder: polytetrafluoroethylene	Uniflon Fluormasters, Sao Paulo, Brazil.

All flasks, containing PMMA with 2% PTFE added (LE) or without it (LC) with long polymerization cycle, were placed in a polymerizing unit (Termotron P-100; Termotron Equipments Ltd, Piracicaba, Brazil) contained with water at 74ºC for 9 hours. Flasks containing PMMA with 2% PTFE added (SE) or without it (SC) with short polymerization cycle were immersed in boiling water for 20 minutes. Afterwards, all flasks were allowed to bench cool for 2 hours, then opened, and the specimens were finished using progressively smoother aluminum oxide papers (grit 320, 400 and 600) in a horizontal polisher (Arotec APL-4; São Paulo, Brazil). Subsequently, polishing of the specimen surfaces to be evaluated for surface hardness was completed on a bench lathe (P134R; Nevone, Sao Paulo, Brazil)

with a roller brush, pumice stone paste (Herjos lot 05794; Probem, Sao Paulo, Brazil) and water for 15 sec, followed by a wet polishing wheel and a chalk (Probem, Sao Paulo, Brazil) and water slurry for 15 sec. followed by a polishing machine with diamond paste (Extec Corp, Enfild, Connecticut, USA) and a cotton disk (Extec Corp, Enfild, Connecticut, USA). After finishing procedures, the specimens were ultrasound cleaned (Thornton T 740, Thornton-Inpec Eletronica LTDA, Vinhedo, Brazil) for 20 minutes and then immersed in distilled water at 37°C for 48 ± 02 hours.

The specimens were divided into 4 groups (n=10 per group): LC- acrylic resin polymerized by long cycle; LE- acrylic resin with 2% PTFE added polymerized by long cycle; SC- acrylic resin polymerized by short cycle; and SE- acrylic resin with 2% PTFE added polymerized by short cycle.

Surface hardness test

Surface hardness (SH) of the acrylic resin discs (Ø 30x5 mm) was performed with a microhardness tester (Shimadzu HMV-2000, Kyoto, Japan), using a Knoop indenter with a 25 g load for 5 s. Fifteen indentations were made on each specimen at a distance of 300 µm between them and the average was considered the microhardness value for the specimen (kg/mm^2).[20-23]

Impact strength test

The impact strength test was performed according to ISO standard 1567:1999/Amd.1:2003(E),[9,24] using an impact test machine (AIC - EMIC, São Jose dos Pinhais, Brazil) by the Charpy method with a pendulum of 0.5 J, in which the specimens were horizontally positioned with a distance of 40 mm between the 2 fixed supports.

Flexural strength test

Flexural characteristics such as flexural strength (Mpa), flexural modulus (Mpa) and peak load (N) were determined by the 3-point bending test using a

universal testing machine (Instron Model 4467, Instron Industrial Products, PA, USA) calibrated with a 500 kgf load cell and a crosshead speed of 5 mm/min. The flexural testing device consisted of a central loading plunger and 2 polished cylindrical supports, 3.2 mm in diameter and 10.5 mm long.[24] The distance between the centers of the supports was 50 mm. The compressive force was applied perpendicular to the center of the specimens until fracture occurred.[1,7,12,24-27]

Statistical Analysis

Statistical analysis was performed with SAS software (SAS Institute Inc., version 9.0, Cary, NC, USA) using a level of significance fixed at 5%. Analysis of Variance (ANOVA) was used to test the null hypothesis that assumed there were no differences among the groups with added PTFE or without it. Data that violated the assumptions of equality of variances and normal distribution of errors were transformed. The flexural strength was transformed by square root after that all data were analyzed by two way-ANOVA and pos hoc Tukey test.

RESULTS

Two-way ANOVA indicated significant differences for impact and flexural strength ($P<.05$) (Table II) but not for surface hardness. Surface hardness showed no statistical differences ($P>.05$) among all groups (LC=20.4 ± 0.35 Kg/mm^2, SC=20.5 ± 0.33 Kg/mm^2, LE=20.2 ± 0.24 Kg/mm^2; SE= 20.5 ± 0.43 Kg/mm^2).

Table II: Two-way ANOVA for surface hardness, impact strength, flexural strength, flexural modulus and peak load.

Dependent variable	Source	DF	Sum of Squares	Mean square	F value	*P**
Surface hardness	Acrylic resin	1	0.402	0.402	3.31	0.077
	PTFE	1	0.136	0.136	1.12	0.298
	Acrylic resin * PTFE	1	0.172	0.017	0.14	0.709
	Error	36	4.374	0.121		
Impact strength	Acrylic resin	1	2.256	2.256	3.68	0.063
	PTFE	1	53.592	53.592	87.30	<0.0001
	Acrylic resin * PTFE	1	2.550	2.550	4.15	0.049
	Error	36	22.099	0.614		
Flexural strength	Acrylic resin	1	3793709.8	3793709.8	5.13	0.030
	PTFE	1	120593250.1	120593250.1	163.10	<0.0001
	Acrylic resin * PTFE	1	750985.3	750985.3	1.02	0.320
	Error	36	26617857.5	739384.9		
Flexural modulus	Acrylic resin	1	382906.624	382906.624	50.49	<0.0001
	PTFE	1	92217.609	92217.609	12.16	0.0013
	Acrylic resin * PTFE	1	152349.649	152349.649	20.09	<0.0001
	Error	36	273017.642	7583.823		
Peak load	Acrylic resin	1	562.725	562.725	4.89	0.033
	PTFE	1	20505.954	20505.954	178.14	<0.0001
	Acrylic resin * PTFE	1	2009.164	2009.164	17.45	0.0002
	Error	36	4143.918	115.109		

The mean and standard deviation values for impact and flexural strength, flexural modulus and peak load are presented in Table III.

All materials evaluated with regard to impact strength fulfilled the requirements of ISO 1567 (> 2 KJ/m^2). However, the impact strength values for short cycle resin with 2% PTFE added were significantly lower P<.049) when compared with those of the other groups.

Flexural strength of LE and SE was significantly lower than that of LC and SC (P<.05). Significantly higher flexural modulus was obtained for LE when compared with the other groups (P<.0001).

There was a general trend towards decreasing peak load values in the groups with the addition of PTFE, and the LC group exhibited higher peak load (N) in comparison with the other groups (Table III).

Table III: Impact strength, flexural strength, flexural modulus and peak load values (Mean ± SD) of acrylic resins (n=10).

Acrylic Resins	Impact strength (KJ/m^2)	Flexural strength (MPa)	Flexural modulus (MPa)	Peak load (N)
LC	6.1 ± 0.5 a	86.8 ± 3.5 a	1895.0 ± 83.1 a	137.7 ± 11.9 a
LE	4.3 ± 1.0 b	65.6 ± 7.5 b	2121.5 ± 67.9 b	78.3 ± 8.5 b
SC	6.1 ± 0.6 a	91.8 ± 4.1 a	1822.8 ± 48.7 a	116.0 ± 10.1 c
SE	3.3 ± 0.9 c	68.2 ± 7.2 b	1795.4 ±128.2 a	84.9 ± 12.0 b

Distinct letters represent statistically differences among acrylic resins (Tukey test, *P*<.05).

DISCUSSION

The addition of PTFE powder to acrylic resin in order to change its surface characteristic to avoid microorganism adhesion could cause deleterious effects on its mechanical properties. However, in this study, addition of PTFE did result in a significant change in impact and flexural strength. The values of experimental groups were still in accordance with ISO 1567:1999 standard.

The surface hardness results showed that the addition of PTFE to both resins polymerized by a long or by a short cycle, did not cause any change in this physical property. This result could favor the addition of PTFE, but the same was not observed for impact and flexural strength although hardness is a good measurement of material strength.

According to the results, the addition of PTFE to acrylic resins did not improve the impact and flexural strengths of resins. These results could be explained because the mixture of resin with PTFE probably did not provide a homogeneous formulation.[16] A primary obstacle to achieving substantially complete and thorough distribution of a fine PTFE powder throughout the resin has been recognized because of blending difficulties. This can be explained by the fact that PTFE presents extreme chemical and thermal stability, very high molecular weight and consequently has an extremely high melt viscosity and negligible solubility.[15] Thus, the PTFE powder was probably not totally incorporated into the polymeric matrix in a homogeneous manner. Thus, the PTFE could behave as a barrier that did not allow the product obtained to react homogeneously during the impact and flexural strength tests. Therefore, under tensile stress, or even an impact force, the acrylic resin with PTFE added elongates and breaks under a lower load in comparison with acrylic resin without any PTFE added.

Although the results obtained by the experimental groups with regard to strengths, the values are in accordance with the requirements of ISO 1567, which stipulate values of $\geq$2 kJ/m^2 for impact strength and values of $\geq$ 65 Mpa for flexural strength.

The improvement in flexural modulus is important because it reflects the rigidity of the denture base acrylic resin and assures the integrity of the supporting ridge and tissue.[1,8,24] In this study the long cycle with added PTFE obtained the highest value (2121.5±67.9 Mpa) showing that this property was the only one positively affected by the PTFE. This result could be due to the highly cross-linked polymer structure. It can be assumed that the presence of PTFE powder in the acrylic resin polymerized by long cycle improved its rigidity. This result could be

due to the long polymerization cycle being better able to a reach higher temperature affecting the melting point of the PTFE and its initial linking to the polymer network microstructure. Whereas, this did not occur for the peak load, which was the highest recorded (138±12 N) in the long cycle group without the addition of PTFE ($P<.05$). Although the groups with the addition of PTFE powder showed the lowest values, they could not be considered unacceptable since the mean peak load of acrylic resin should not be less than 55 N.

Furthermore, one limitation of the present study was that only the addition of 2% of PTFE was tested. Thus, it is imperative to conduct additional studies involving the incorporation of different percentages and methods of incorporating the PTFE powder into acrylic resin, as well as, to develop studies focused on longer polymerization cycles and higher polymerization temperatures. In addition to this, further studies are necessary with regard to the surface properties related to the adherence of microorganisms and biofilm development.

CONCLUSION

The results suggest the addition of PTFE powder did not improve the physical and mechanical properties of the acrylic resins studied; however the requirements of ISO 1567 were fulfilled.

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CAPÍTULO 2

Polytetrafluoroethylene added to acrylic resin: surface properties and *Candida albicans* adherence

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Abstract

Purpose: This study evaluated the surface properties and *Candida albicans* adherence on two acrylic resins, one polymerized by long cycle and another by short polymerization cycle, added (experimental groups) or not added (control groups) with 2% PTFE (wt/wt).

Methods: The specimens were divided into 4 groups (n=10 per group): 1- acrylic resin polymerized by long cycle; 2- acrylic resin polymerized by long cycle added with 2% PTFE; 3- acrylic resin polymerized by short cycle; and 4- acrylic resin polymerized by short cycle added with 2% PTFE. After finishing and polishing, all specimens were assessed for their surface roughness (μm), contact angle (^{o}) and surface free energy (erg/cm^{-2}). Saliva-coated specimens were submitted to the adherence assay with *C. albicans* (ATCC 90028). Adhered cells were detached from the acrylic resin surface by ultrasonic waves at 7 watts for 30 seconds in phosphate buffered saline solution (PBS). This cell solution was serially diluted in PBS and plated on Sabouraud agar. The results were expressed in colony forming units per surface area (CFU/mm^2). The data was analyzed by two-way ANOVA. The significance limit was set at 5%.

Results: No statistical differences were found for roughness, contact angle and surface free energy. There was a reduction trend in *C. albicans* adherence in PTFE added to resins, but it was not statistically significant.

Clinical Significance

Polytetrafluoroethylene added in small amount to acrylic resin denture base was not efficient to reduce *Candida albicans* adherence.

Introduction

Denture stomatitis is the most common oral candidiasis in removable denture wearers and it is associated with the presence of *Candida* species on denture base surface. The prosthetic device in the oral environment allows fungi to adhere and form biofilm, serving as an effective reservoir of microorganisms that has important clinical implications [1]. *Candida* presence on tissue-fitting surface over denture base may cause only local damage on mucosa as a localized erythema, or, in immunocompromised patients, yeasts could cause systemic infection, being one of the most common bloodstream infections with high mortality rate [2, 3].

Although *Candida* species are the main pathogens responsible for the development of oral candidiasis, *Candida albicans* is the predominant isolate in these infections [4]. This fungus expresses several virulence factors that contribute to pathogenesis and easily colonizes denture base surface. It is well known that the microorganisms adhere directly to the acquired pellicle formed over the denture surface. Therefore the composition and surface properties of acrylic resin could facilitate fungi adhesion [5, 6].

Correlations between *C. albicans* adhesion and surface properties have been investigated in an attempt to prevent, or reduce, microorganism adhesion on prosthetic device [7]. One of these properties is the surface free energy (SFE), a non specific adherence mechanism, which is utmost important during the initial microorganism adhesion. A linear relationship between SFE on various types of substratum and *C. albicans* adherence has been demonstrated [5]. The substrata with high SFE values will be more hydrophilic and the adherence of more cells will be expected. Surface roughness, is also related to microorganism adhesion, irregularities and porosities that provide microorganism mechanical retention and protection from shear forces, thus rougher surfaces usually exhibit higher yeast counts [8, 9].

One approach to prevent or reduce microorganism adhesion on biomaterials is to modify their surface properties by altering the material

composition [6]. Polytetrafluoroethylene (PTFE) is a biocompatible fluoropolymer which could be added to acrylic resin in an attempt to improve surface properties. PTFE is extensively used in the medical area as implant material or a component of a range of clinical dentistry devices. It has several unique characteristics such as high resistance to chemical reagents, tolerance to high temperatures, and low surface energy [10]. The addition of PTFE particles in acrylic resin could decrease the surface wetting and the free energy improving better surface properties and consequently lower yeasts adherence.

Indeed, a wide number of studies with different material compositions have been carried out to reduce microorganism adherence. However, no study evaluating acrylic resin with PTFE addition was found. Thus, the aim of this study was to evaluate the surface roughness, contact angle and surface free energy and *C. albicans* adherence on acrylic resin modified by PTFE addition.

Material and methods

Experimental design

This *in vitro* study was a completely randomized and blinded design using surface roughness, contact angle, surface free energy and adhered cells of *C. albicans* as dependent variables. Two heat-polymerized acrylic resins, one with long, and another with short polymerization cycle, added with 2% PTFE or not, were used to fabricate the specimens. The specimens were divided into 4 groups (n=10 per group): 1- acrylic resin polymerized by long cycle; 2- acrylic resin polymerized by long cycle added with 2% PTFE; 3- acrylic resin polymerized by short cycle; and 4- acrylic resin polymerized by short cycle added with 2% PTFE. After finishing and polishing, surface roughness, contact angle and surface free energy were measured. All specimens were immersed in clarified saliva for 30 minutes and submitted to the adherence assay with the *C. albicans* suspension for 2 hours. Next, the specimens were immersed in a phosphate buffered saline solution (PBS) and adhered cells were detached from the acrylic resin surface by

ultrasonic waves at 7 watts for 30 seconds. This solution was serially diluted in PBS and plated on Sabouraud agar. After incubation, the colony forming units (CFU) were counted and the results expressed in CFU/mm^2.

Preparation of acrylic resin specimens

The acrylic resins and polytetrafluoroethylene used in this study are listed in Table 1.

Table 1: Acrylic resins and polytetrafluorethylene used in this study:

Material	**Processing method**	**Chemical composition**	**Manufacturer**
Polymethyl methacrylate,	Hot water bath 9 h at 74ºC	Powder: Polymethylmethacrylate, ethylacrylate, benzyl peroxide, organic pigments Liquid: Methylmethacrylate, topanol stabilizer	Classico Ind. e Com, Sao Paulo, Brazil.
Polymethyl methacrylate,	Hot water bath 20 min at 100ºC	Powder: Copolymer (methyl-n- butyl) methacrylate, benzoyl peroxide, mineral dyes Liquid: Methylmethacrylate, ethyleneglycol, dimethacrylate, N,N dimethyl-p-toluidine, hydroquinone methyl ether	Dentsply International, York, PA, USA.
Polytetrafluoroethylene	----------	Powder: polytetrafluoroethylene particles	Uniflon Fluormasters, Sao Paulo, Brazil.

Forty rectangular specimens measuring (20 x 12 x 2 mm) were prepared for each acrylic resin according to the manufacturers' recommendations adding 2% PTFE powder (wt/wt) or not. Metal master patterns were included in laboratory silicone (Zetalabor, Zhermarck SpA, Badia Polesine, Rovigo, Italy). Silicone moulds were invested in metal dental flasks (Uraby; DLC, Sao Paulo, SP, Brazil) with Type III dental stone (Herodent Soli-Rock; Vigodent, Rio de Janeiro, RJ, Brazil). After the gypsum had set completely, acrylic resin was packed in the moulds. Next, the metal flasks were placed in a polymerizing unit (Termotron P-100; Termotron Equipments Ltd, Piracicaba, SP, Brazil) filled with water at 74ºC for 9 hours or at 100ºC for 20 minutes, for acrylic resin of long and short polimerization cycle, respectively.

All flasks were allowed to bench cool for 2 hours, and the specimens were removed. Each specimen was trimmed and finished using aluminum oxide papers (320, 400 and 600 grit; Carbimet, Buehler, Lake Bluff) in a horizontal polisher (model APL-4; Arotec, Sao Paulo, SP, Brazil). For mechanical polishing, a brush wheel with pumice slurry and a felt wheel with chalk powder were used. The specimens were stored in deionized distilled water at 37ºC for 48 hours in order to release the residual monomer [8, 11].

Surface roughness

Surface roughness (µm) of the acrylic resin specimens was measured using a profilometer (Surfcorder SE 1700; Kosaka Laboratory Ltd, Kosaka, Japan) with a 0.01 µm resolution, calibrated at a specimen length of 0.8 mm at a speed of 0.5 mm/s. Three readings were made on different areas of each specimen and a mean value was calculated [11, 12].

Contact angle and surface free energy measurements

Contact angle (º) was measured by dispensing a droplet (15 µL) of deionized distilled water on the specimen surface. Photographs (Nikon 5700, Nikon, Japan) of the droplets were taken immediately and contact angles were

measured (AutoCAD R14; Autodesk, Inc, San Rafael, CA, USA) from the left boundaries of the magnified picture to the point of air-water-specimen intersection. This procedure was carried out 3 times for each specimen. The surface free energy (erg/cm^{-2}) was calculated using cosine θ of contact angle values obtained previously [11]. After surface roughness, contact angle and surface free energy measurements, the specimens were immersed in sterile distilled water for 20 minutes before the adherence assay to remove the surface contaminants by sonication [11] (Thornton T 740; Thornton-Inpec Eletronica Ltda, Vinhedo, SP, Brazil).

Human saliva collection and preparation for the adherence assay

All specimens were saliva-coated previously to the adherence assay. Human whole saliva was collected from only one healthy volunteer during all the experiment. The saliva donor had not used antibiotics, mouth rinses, or any other medication known to affect salivary composition and flow in the past 3 months. He also provided written informed consent previously approved by the Local Ethics Committee of Piracicaba Dental School, UNICAMP. Saliva was collected during masticatory stimulation with Parafilm M (American Can Co., Greenwich, CT, USA) in an ice-chilled polypropylene tube, and clarified by centrifugation at 10,000 g for 5 minutes at 4ºC [15]. The supernatant was collected and placed into sterile petri dishes where all specimens rested on the bottom with polished surface facing up and left for 30 min to form acquired pellicle. After this period, specimens were removed and immediately used in the adherence assay [11, 13].

Inoculum and growth conditions

Candida albicans (ATCC 90028) was reactivated from its original culture at −70°C, first in Sabouraud broth (Difco Laboratories, Detroit, MI, USA) and next, plated on Sabouraud agar (Difco Laboratories, Detroit, MI, USA). Single colonies were inoculated into 10 ml of Sabouraud broth and incubated. Cells were harvested in the late exponential growth phase, washed with PBS (pH 7.2) twice

and re-suspended spectrophotometrically in Sabouraud broth to a concentration of 1 to 5 x 10^6 cells/ml (0.38 at 520 nm) [8, 13].

Adherence assay and yeast counts

The saliva-coated specimens were placed on the bottom of sterile petri dishes with polished surfaces facing up and covered with a suspension of Sabouraud broth containing *C. albicans*. Specimens were incubated for 2 hours at 37°C to promote yeast adherence [11, 14, 15]. All adherence assays were performed in triplicate in three independent experiments on different days. During the adhesion process the specimens were kept undisturbed.

After the yeast adhesion, each specimen was aseptically removed and washed with PBS twice in a standard fashion, by gentle immersion in a new petri plate with sterilized PBS for 2 seconds [15], to remove loose and non-adhered cells. Each specimen was placed inside a cryogenic tube containing a pre determined sterile PBS volume of 5.5 ml and adhered microorganisms were detached from the resin surface by ultrasonic waves at 7 watts for 30 seconds (Sonifier 150™, Branson Ultrasonics Corporation, Danbury, CT, USA). The ultrasonic energy has previously shown to have no detrimental effect on microbial viability [16]. The sonicated suspension of cells was serially diluted in PBS and drops of 20 µl of each dilution were plated Sabouraud agar in triplicate. Plates were incubated at 37°C in aerobic atmosphere for 48 hours. Colony forming units (CFU) were counted using a stereomicroscope, and the results were expressed in CFU/mm^2 [15].

Statistical analysis

Statistical analysis were done using SAS software (SAS Institute Inc., version 9.0, Cary, NC, USA) employing a significance level fixed at 5%. The null hypothesis assumed no differences between the acrylic resins added with PTFE and the ones not added with it. Data that violated the assumptions of equality of variances and normal distribution of errors were transformed. The roughness

surface data was transformed to the inverse (1/Ra) and data of yeast count was transformed to square root. After that, all data was analyzed by two-way ANOVA.

Results

The two-way ANOVA results are on table 2.

Table 2: Two-way ANOVA in accordance with acrylic resin with and without 2% PTFE:

Dependent variable	*df*	Sum of Squares	Mean square	F	*P*
Surface roughness	3	0.0056	0.0019	0.10	0.9615
	36	0.7007	0.0195		
	39	0.7063			
Contact angle	3	41.720	13.907	1.54	0.2197
	36	324.133	9.004		
	39	365.853			
Surface free energy	3	9.266	3.089	0.84	0.4796
	36	131.963	3.666		
	39	141.229			
C. albicans CFU/mm^2	3	207767554	69255851	1.56	0.2149
	36	1593983160	44277310		
	39	1801750714			

There was no statistical difference between the resins polymerized by long and short polimerization cycle added with PTFE ($p>0.05$) or not. The mean values and standard deviation are presented in Table 3.

Table 3: Surface roughness, contact angle and surface free energy values of the acrylic resins (Mean ± SD; n=10)

Acrylic Resin	Surface Roughness (μm)	Contact angle (°)	Surface Free Energy (erg/cm^2)
Long cycle (Control)	0.43 ± 0.13	64.8 ± 2.4	43.7 ± 1.5
Long cycle 2% PTFE	0.39 ± 0.10	63.5 ± 2.7	44.5 ± 1.7
Short cycle (Control)	0.41 ± 0.18	63.9 ± 4.2	44.3 ± 2.7
Short cycle 2% PTFE	0.41 ± 0.14	66.2 ± 2.3	42.8 ± 1.4

C. albicans counts ranged from 5 to 25x10^3 CFU per square millimeter. Although the counts of *C. albicans* adhered on acrylic resins surface were similar among the materials with no statistical difference, Fig. 1 shows an adherence reduction tendency in both acrylic resin added with 2% PTFE.

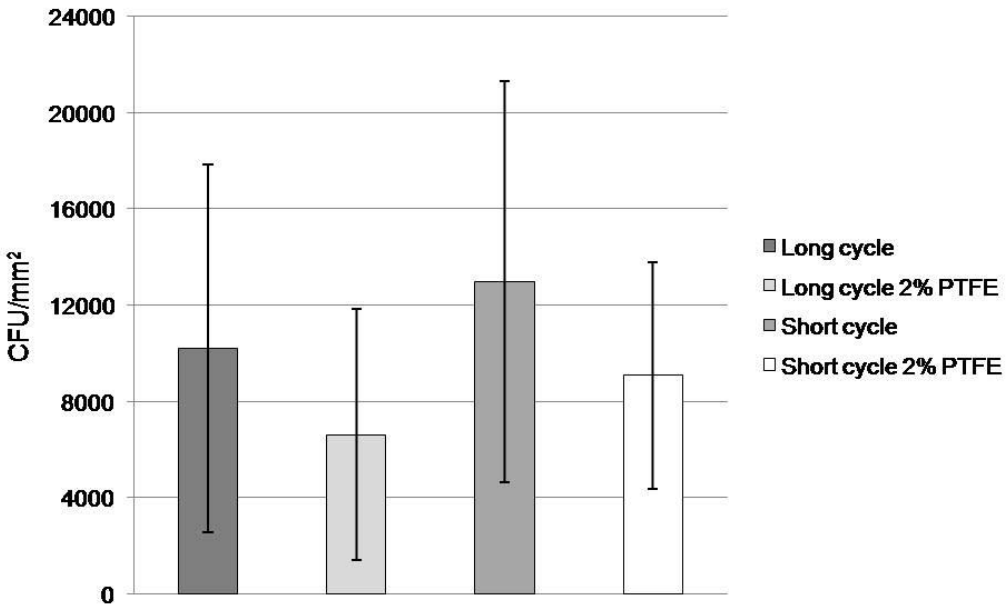

Fig. 1: *C. albicans* CFU counts per acrylic

Discussion

The purpose of this study was to evaluate surface properties and *Candida albicans* adherence on acrylic resins modified by PTFE addition.

The present study was performed mimicking a clinical condition. The adherence assay was conducted on heat acrylic resins, routinely used to make dentures, added with PTFE or not. It was hypothesized that PTFE addition to acrylic resin could change resin surface properties and difficult *Candida albicans* adherence.

The results showed that the acrylic resins added with 2% PTFE (wt/wt) or not did not differ regarding all the variables evaluated. Contact angle and surface free energy data found in this study are in accordance with those found by others researchers that evaluated distinct acrylic resins [8, 9, 11]. Gyo *et al.* (2008) evaluated PTFE addition, not in acrylic resin, but in composite resin and found significant higher contact angle values when 21.3% of PTFE (wt/wt) was added to this material. This different behavior in relation to contact angle values observed could be explained by the amount of PTFE added. In the present study only 2% of PTFE was used because another study was conducted, and it was verified that more than 2% of PTFE addition decreased the mechanical properties of acrylic resins.

Surface roughness also favors microorganism adhesion, surface porous and irregularities facilitate the entrapment of microorganisms and protect them from shear forces [12, 17, 18]. The PTFE acrylic resins presented similar surface roughness to that without PTFE. Although roughness values (0.39 - 0.43 µm) were higher when compared with other studies [8, 11], no statistical difference was found among the groups. Consequently the similar roughness values did not interfere with the adhesion pattern of microorganisms among the groups.

Despite of the fact that no statistical difference was found for *C. albicans* adherence, the acrylic resins with PTFE tend to decrease microorganism adhesion. In this study, the mean *C. albicans* counts ranged from 6,618 to 12,963 CFU/mm^2, showing high values when compared to other studies. Moura *et al.* (2006), Pereira-

Cenci *et al.* (2007) and Serrano-Granger *et al.* (2005) found *C. albicans* counts of 28, 10.8 and 50.3 cells/mm^2, respectively. In these studies the cells were stained and counted per fields on acrylic resin surface by an optical microscope. The differences found between the studies could probably be due to the counting methods. Serrano-Granger *et al.* (2005) reported difficulties with the method used by them, since there was a wide variation in the adhesion pattern among the fields on the same specimen and, besides that, clusters of yeast difficult an accurate count, underestimating the number of cells.

On the other hand, a study of Henriques *et al.* (2004) using an epifluorescence microscopy to enumerate the cells showed *C. albicans* counts of 16,209 to 17,916 cells/mm^2. This higher count is, in accordance with our findings, showing a great number of cells adhered to the surface. These differences in the cells counts could also be attributed to the different strains of *C. albicans*, the adhesion assay period, the medium used and, mainly, by the method used to enumerate de microorganisms. Although quantification of CFU of detached cells by ultrasonic waves is time-consuming and laborious, its method enumerates cells directly.

Considering the limitations of this study, in which a low amount of PTFE was added to acrylic resins; only an ATCC 90028 laboratory was used; the experimental length was only evaluated with adhered cells; and also taking into consideration the *in vitro* nature of this investigation, we come to the conclusion that further studies are necessary to find a better way for PTFE to be added to denture base materials. Acrylic resins with modified surface properties could be promising to decrease microorganism levels, especially the *Candida* species, which is strongly related to patients with denture stomatitis. Additionally, only one laboratorial strain was used to evaluate *C. albicans* adherence in this study. Although the *C. albicans* ATCC 90028 is widely used in laboratorial assays, further studies are suggested to evaluate the adherence ability of *Candida* clinical strains.

In this study, it could be concluded that the amount of 2% of PTFE added into acrylic resins was not enough to change the surface properties or to interfere with *Candida albicans* adherence.

Acknowledgement

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CAPÏTULO 3

Biofilms formed on acrylic resin with or without polytetrafluorethylene

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Running Title: Biofilm on modified acrylic resin

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Abstract

The aim of study was to evaluate the bacterial biofilm formation and polysaccharide composition on acrylic resin without or with 2% polytetrafluorethylene (PTFE). Specimens (10 x 2 mm) of two different PMMA were fabricated (n=6): LC: acrylic resin polymerized by long cycle; LE: acrylic resin polymerized by long cycle with 2% PTFE; SC: acrylic resin polymerized by short cycle; SE: acrylic resin polymerized by short cycle with 2% PTFE. Biofilms of *Streptococcus mutans*, *Streptococcus sanguinis* or *Actinomyces naeslundii* were formed on each specimen for 5 days. At the end of the experimental period, the biofilms were analyzed for: biomass (dry weight), number of viable cells, and polysaccharides content soluble (WSP) and insoluble (INS) extracellular and iodophilic (IPS) polysaccharides. The data were analyzed by ANOVA followed by Tukey test with significant level set at 5%. For surface roughness no statistical difference was observed between groups (p >0.05). There was no statistical difference on the biofilms biomass. The concentration of WSP and INS were not significantly different between groups (p >0.05). The amount of IPS was lower in short cycle 2% PTFE, however the difference among the groups was not significant (p >0.05). There was no difference on total microorganisms (CFU/mm^2) (p >0.05). The addition of 2% PTFE powder was insufficient to cause effect on formation and composition of biofilms polysaccharide matrix.

Keywords: acrylic resin, PTFE, polysaccharide, biofilm, bacteria

Introduction

Denture biofilm contains several strains of bacteria, such as *Streptococcus mutans, Streptococcus sanguinis*, *Streptococcus salivares, Actinomyces viscosus* and *Actinomyces naeslundii*[1-6] and it is frequently associated with fungi, i.e *Candida albicans.*[7-10] Once *C.albicans*' cells attach to the bacteria and the surface, their proliferation could be enhanced by metabolic products of oral bacteria such as polysaccharides.[7,11] In addition, denture is indicated as the main source of those microorganisms[7,12] and commonly related to inflammatory process affecting the oral mucosa, known as denture stomatitis.[13]

Acrylic resin polymethyl methacrylate (PMMA) belongs to the most common group of material used for removable denture. Recently, the addition of modifiers that interfere with the specific interactions between microorganisms and polymer surface has been proposed.[14-16] One of the main modifiers suggested is polytetrafluorethylene (PTFE). PTFE is an fluorpolymer containing fluorine atoms. This fluorpolymer presents biocompatibility and remain stable in almost all chemical environments.[17-19] PTFE is extensively used in medical area as implant material or component of a range of devices in clinical dentistry.[20]

The oral cavity is a challenging environment for the long term persistence of bacteria and fungi. Fluctuations in nutrient supply, temperature, pH, and the shear force of salivary flow have selected a biofilm community adapted to high cell density, species diversity, and dynamic growth conditions.[21,22] Bacterial production of extracellular polymeric substance (EPS) matrix is also very important for their interaction with the environment.[23,24]

Bacteria from biofilms synthesize extracellular polysaccharides from carbohydrates using enzymes, i.e. sucrose and glucosyltransferases (GTFs), which is one of the most critical virulence factor involved in the formation of the pathogenic biofilm.[25,26] Polysaccharides were also shown to activate macrophage and induce inflammatory immune responses of squamous tissue.[27,28] In the oral cavity, water-soluble (WSP) and water-insoluble (INS) extracellular polysaccharides promote bacterial accumulation to the tooth surface, and influence

the physical and biochemical properties of biofilm; the iodophilic polysaccharides (intracellular polysaccharides - IPS) serve as an endogenous source of carbohydrates that can be metabolized to produce acids during periods of nutrient limitation.[29,30] Therefore, interfering with the polysaccharide production and composition in the biofilm matrix is a valuable way to control the formation of a pathogenic biofilm.

Thus, it is important to determine how oral bacteria interact with acrylic resin used in oral rehabilitation.[10,31,32] Considering the central role of the polysaccharide matrix in the pathogenicity of dental biofilm, there is a lack of information regarding the polysaccharide formation and composition of biofilms formed on those dental materials. Several studies have been performed to analyze adherence and biofilm development on resin materials, however they are limited to cellular viability and biofilm morphology.[10,33]

The hypothesis is that there is an alteration in the resin surfaces' characteristics when modifiers, such as polytetrafluorethylene, are added to the PMMA, which interferes with the bacterial biofilm on this surface. In this study, we evaluated the bacterial biofilm formation and polysaccharide composition of three common oral bacteria (*Streptococcus mutans*, *Streptococcus sanguinis* or *Actinomyces naeslundii)* on acrylic resin with or without polytetrafluorethylene (PTFE).

Material and Methods

Preparation of acrylic resin specimens

Two heat polymerized acrylic resins were used in this study: long cycle resin (Classico Ind. Com, Sao Paulo, Brazil), prepared in hot water bath for 9 h at 74ºC; and short cycle resin (Dentsply International, York, PA, USA), prepared in hot water bath for 20 min at 100ºC. Discs (2.5 x 1.2 x 0.2 mm) were prepared for each acrylic resin according to the manufacturers' recommendations. For the two heat polymerized acrylic resins, PTFE 2% powder (wt/wt) was added or not. The specimens were divided into 4 groups (n=6 per group), classified according to the

dental material: LC: acrylic resin polymerized by long cycle; LE: acrylic resin polymerized by long cycle added 2% PTFE; SC: acrylic resin polymerized by short cycle and SE: acrylic resin polymerized by short cycle added 2% PTFE.

Initially, wax cylindrical discs patterns (10 mm in diameter and 2 mm in thickness) were prepared with the aid of an aluminum matrix. After, they were placed in metallic flasks. They were then completed with Type III dental stone (Herodent Soli-Rock; Vigodent, Rio de Janeiro, RJ, Brazil). After the gypsum had set completely, it boiled out to soften and eliminate the wax. Acrylic resin was packed in the moulds. Next, the metal flasks were placed in a polymerizing unit (Termotron P-100; Termotron Equipments Ltd, Piracicaba, SP, Brazil) filled with water at 74ºC for 9 hours or at 100ºC for 20 minutes, to acrylic resin of long and short polimerization cycle, respectively.

All flasks were allowed to bench cool for 2 hours, and the specimens were removed. Each specimen was trimmed and finished using aluminum oxide papers (320, 400 and 600 grit; Carbimet, Buehler, Lake Bluff) in a horizontal polisher (model APL-4; Arotec, Sao Paulo, SP, Brazil). For mechanical polishing, a brush wheel with pumice slurry and a felt wheel with chalk powder were used. The specimens were stored in distilled water at 37ºC for 48 hours to release the residual monomer.[9,34]

Surface roughness

To avoid the surface roughness (µm) of the acrylic resin to interfere with the biofilm formation, it was standardized by finished using aluminum oxide papers (320, 400 and 600) grit. The specimens' roughness were measured using a profilometer (Surfcorder SE 1700; Kosaka Laboratory Ltd, Kosaka, Japan) with a 0.01 µm resolution, calibrated at a specimen length of 0.8 mm at a speedy of 0.5 mm/s. Three readings of both sides were made on different areas of each specimen and a mean value was calculated.[9,34,35] We aimed to have similar roughness to all the specimens, to be able to evaluate how the other surfaces' characteristics were interfering with the polysaccharide matrix.

Biofilm preparation and analysis

This *in vitro* study had a completely randomized design conducted in 3 different and independent assays for each bacterium. Monospecies biofilms of *S. mutans* UA159 ATCC 700610, *S. sanguinis* ATCC 10556 or *A. naeslundii* ATCC 12104, were formed for 5 days on acrylic resin discs, previously sterilized by ethylene oxide[36], and placed in a vertical position using a disc holder.[37]

Before biofilm formation, salivary pellicle was formed on the discs. Saliva was collected from one donor according to protocols approved by Research and Ethics Committee of New York University and Dental School of Piracicaba, State University of Campinas. Stimulated whole saliva was collected on ice after chewing parafilm, and clarified by centrifugation at 3.800 g, 4°C, 10 min[37] and filter-sterilized.[29] Immediately, it was used for pellicle formation during 1 hour (37°C, at orbital shaker).

After 1 hour of salivary pellicle formation, the discs were dip-washed three times in absportion buffer. The biofilms were then grown in tryptone yeast extract containing sucrose 1%, and incubated at 37°C, 5% CO_2. The culture medium was replaced daily until 5 days of biofilm formation.[29] The discs with 5 days-old biofilms were gently washed in physiological saline (0.89% NaCl, w/v) for removal of loosely adherent material. Then, the discs were placed in sterile glass tube with 5 ml sterile saline solution and they were subjected to sonication during 10 minutes to harvest adherent cells. The discs were removed from the tubes and the biofilm suspension subjected to sonication at 8 watts for 5 seconds (Sonifier 150™, Branson Ultrasonics Corporation, Danbury, CT, USA).[16] The homogenized suspension was used for: biomass (dry weight), number of viable cells, and polysaccharides composition (water soluble and insoluble polysaccharides and iodophilic polysaccharides) by means of colorimetric methods estimated by the phenol sulfuric method[38] as detailed elsewhere.[29] The bacterial viability was measured inoculating an aliquot of the bacterial suspension in blood agar. Plates were incubated at 37°C in 5% CO_2 for 48 hours. Colony forming units (CFU) were

counted using a stereomicroscope, and the results were expressed in CFU/mm^2.[10,39]

Statistical analyses

Data are presented as means ± standard deviation (SD). An exploratory data analysis was performed to determine the most appropriate statistical test: the assumptions of equality of variances and normal distribution of errors were also checked. Statistical analyses were done using SAS software (SAS Institute Inc., version 9.0, Cary, NC, USA) and conclusions are based on a significance level of 5%. Data that violated the assumptions of equality of variances and normal distribution of errors were transformed. The biomass (dry weight), water soluble were transformed by square root and the colonies count data were transformed by log data, after that all data were analyzed by two way-ANOVA.

Results

Regarding surface roughness (Ra value - µm) measurements no statistical difference was observed between control and experimental groups (p >0.05). The Ra values were 0,24±0,05 for LC and 0,25±0,04 for other groups, which suggested that the addition of 2% PTFE did not change de surface topography characteristics.

Table 1 shows data of biomass (dry-weight) according to the groups. The addition of 2% PTFE to acrylic resin did not produced difference on the amount of biomass in biofilm. As expected, among the bacteria, *S. mutans* produced more biofilm mass (p <0.05).

Table 1: Means (± SD) of biomass (dry-weight) in mg/total biofilm dry weight:

Acrylic Resin	*S. mutans*	*S. sanguinis*	*A. naeslundii*
Long cycle (Control)	2.88 ± 1.52	1.58 ± 0.89	1.46 ± 0.59
Long cycle 2% PTFE	2.54 ± 0.47	1.43 ± 0.65	1.86 ± 0.32
Short cycle (Control)	2.23 ± 0.54	1.96 ± 0.71	1.36 ± 0.53
Short cycle 2% PTFE	3.64 ± 2.09	2.04 ± 0.67	1.96 ± 0.47

$p<0.05$, ANOVA, comparison for all pairs using Tukey test.

The concentration of soluble EPS (Table 2) was higher to *S. sanguinis* and not significantly different between the control group and resin added PTFE ($p>0.05$). However, higher concentration of insoluble EPS was observed to *S. mutans*, but no significant difference was found. Regarding IPS, the results were numerically lower in short cycle 2% PTFE added, also the difference between the groups was not significant ($p>0.05$).

Table 2: Means (± SD) of extracellular polysaccharide concentration (µg/mg dry weight) and relative percentage (%) of soluble and insoluble in 5 old day's biofilm according to acrylic resin groups. Means (± SD) of extracellular polysaccharide concentration (µg/mg dry weight) (n=6):

	Acrylic Resin	Extracellular polysaccharide (EPS)		*Intracellular polysaccharide (IPS)*
		Soluble	*Insoluble*	
S. mutans	**Long cycle (Control)**	210.65 ± 91.14 (38%)	328.42 ± 60.67 (62%)	450.11 ± 191.72
	Long cycle 2% PTFE	241.19 ± 84.68 (40.5%)	374.50 ± 169.86 (59.5%)	493.32 ± 244.53
	Short cycle (Control)	204.08 ± 62.98 (37.5%)	379.53 ± 186.19 (62.5%)	485.65 ± 238.19
	Short cycle 2% PTFE	172.66 ± 30.78 (30%)	419.21 ± 121.39 (70%)	336.56 ± 140.01
	Acrylic Resin	Extracellular polysaccharide (EPS)		*Intracellular polysaccharide (IPS)*
		Soluble	*Insoluble*	
S. sanguinis	**Long cycle (Control)**	376.89 ± 77.87 (57.5%)	304.90 ± 235.51 (42.5%)	79.29 ± 60.72
	Long cycle 2% PTFE	445.34 ± 155.76 (57%)	400.91 ± 110.72 (42%)	74.63 ± 21.71
	Short cycle (Control)	411.17 ± 120.53 (59%)	338.93 ± 171.82 (41%)	96.93 ± 18.34
	Short cycle 2% PTFE	455.56 ± 108.34 (64%)	256.20± 121.68 (36%)	94.0 60.69
	Acrylic Resin	Extracellular polysaccharide (EPS)		*Intracellular polysaccharide (IPS)*
		Soluble	*Insoluble*	
A. naeslundii	**Long cycle (Control)**	57.77 ± 32.51 (43%)	72.51 ± 23.43 (57%)	198.17 ± 36.88
	Long cycle 2% PTFE	59.38 ± 26.61 (55%)	69.68 ± 43.80 (45%)	217.80 ± 113.25
	Short cycle (Control)	67.05 ± 20.61 (39%)	105.86 ± 37.36 (61%)	217.71 ± 36.81
	Short cycle 2% PTFE	53.81 ± 48.45 (58%)	81.61 ± 72.86 (42%)	132.64 ± 114.89

$p > 0.05$, ANOVA, comparison for all pairs using Tukey test.

The counts of total microorganisms in the 5 days-old biofilms sucrose dependent were formed on all samples surface in a similar design for all tested resin group. There was no difference in the amount of CFU/mm^2 between the control and experimental group ($p > 0.05$).

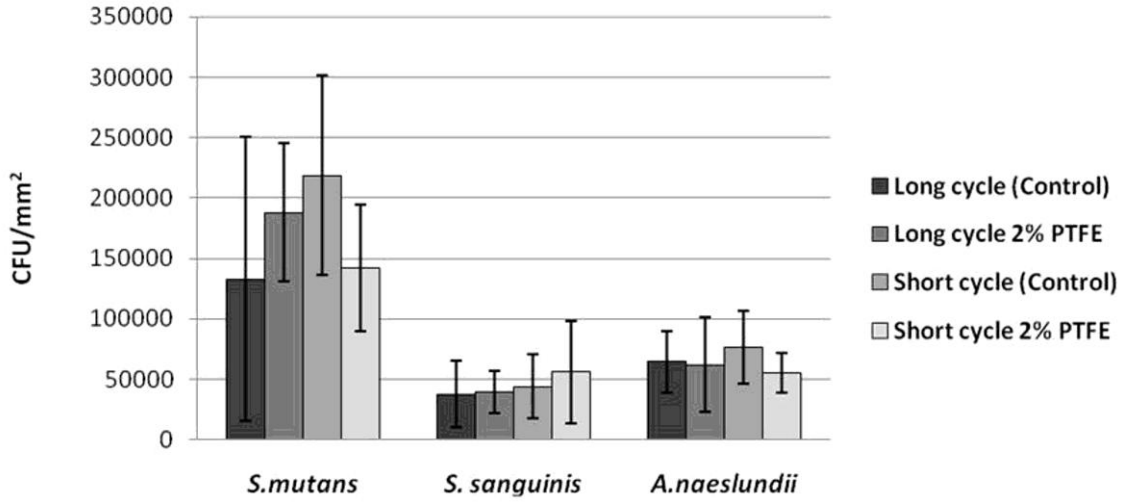

Figure 1: Microbiological analysis of dental biofilm according *S. mutans*, *S. sanguinis* and *A. naeslundii* CFU counts per acrylic resin surface area (mm^2) of the acrylic resins (Mean ± SD; n=6). The CFU counts per acrylic resin surface area were not significant among acrylic resin groups (n=6; p >0.05, two-way ANOVA, comparison for all pairs using Tukey test).

Discussion

Considering the fact that few studies in the literature have addressed biofilm analyses on restaurative material [18] in the present study, the surface proprieties of 2% PTFE incorporated into acrylic resin were investigated in relation to biofilm formation and polysaccharide composition.

To avoid mechanical influence, the roughness was standardized. It is known from literature that surface roughness encourages the adhesion and proliferation of microorganisms.[31,40,41] Roughness values were homogenous among the groups (±0,25 µm), consequently did not interfere with the biofilm patter and to able to show how the chemical characteristics of resins would affect the biofilms. It is kwon that roughness plays a fundamental property on biofilm formation and composition. The roughness values were higher whem compared with other studies.[9,34]

The presence of this biofilm is commonly related with denture stomatitis disease affecting denture wearers, characterized by an erythema on mucosa confined to the area covered by the complete denture.[4,5,12,13,42,43] It has been suggest that microorganisms that adhere to the surface and structured into biofilm may be better protected from any washing action and have been shown to resist antimicrobials.[44] Inhibition or at least decrease the bacteria polysaccharide production, particularly extracellular polysaccharide synthesized by enzymes impart structural integrity and bulk to biofilms[25,45,46] could play a significant role in preventing the presence of this pathogenic biofilm on surface of denture material. A slight change was found for short cycle 2% PTFE resin for WSP, however it was not statistical different.

Despite of the fact that no statistical difference was found, our data tends to decrease values of iodophilic polysaccharides for short cycle 2% PTFE added in all microorganisms analyzed. The effort to decrease this type of polysaccharide is to attempt the fact iodophilic polysaccharides promote lower fasting pH during periods of nutrient deprivation and *Candida* cells that are commonly find on denture biofilm were able to persist and grow at a low pH increasing colonization by these microorganisms.[30,47]

Dentures wears frequently report difficulties chewing foods that are hard or fibrous in texture[48,49] and instead increasing their consumption of softer foods such as processed foods, refined carbohydrates (sugar), and soft drinks, cholesterol, and fat.[49] Sucrose dependent biofilm were formed on the surfaces of all materials during 5 days to simulate the same that happens *in vivo*. Furthermore, sucrose had a considerable influence on number of bacterial cell and when associated with fungi species are also lead a significant increase in the number of viable cells.[11] Therefore, the results of this study may not show differences among the acrylic resin with or without PTFE added, when different types the polysaccharide were analyzed. Those data could be explained by the fact that 2% of PTFE added to acrylic resin was not enough to change the surface resin characteristic and produce effect at biofilm development. In this study, the

presence of PTFE into acrylic resin may not cause effect at biofilm development process, resulting in a accounting of viable bacteria cells similar among the groups, in agreement with Gyo *et al.* 2008, that incorporated PTFE into resin composite and submitted the material into *S. mutans* biofilm formation, who showed that the resistance against biofilm formation was not improved.

The investigation of the interface between polymeric surface and bacterial wall is gaining a great relevance. A strategy to prevent biofilm formation would be to physically and chemically modify the surface of a restorative material in order to inhibit bacterial adherence to the surface.[14,16] Correlations between bacterial adhesion and various surface characteristics (chemical composition, surface free energy, surface roughness, and presence of function groups of surface) may be an option an attempt to reduce bacterial adhesion through surface modification. The results had shown that this amount of incorporated powder of PTFE was not sufficient to cause effect on formation and composition of polysaccharide matrix of biofilm.

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DISCUSSÃO GERAL

O desenvolvimento de novos materiais a partir de associações entre dois ou mais polímeros pode ser considerado uma estratégia capaz de modificar as propriedades mecânicas ou proporcionar modificações nas características de superfície destes materiais, e conseqüentemente poderão refletir nas interações entre a superfície e microrganismo. Estudos buscando o conhecimento do que ocorre na interface entre superfície polimérica e microrganismos, sejam bactérias ou fungos, são também relevantes na Odontologia.

Dessa forma, foi necessária a avaliação não só os fatores relacionados à aderência de microrganismos, mas também a investigar se a incorporação de PTFE a resina acrílica a base de PMMA poderia causar algum efeito nas propriedades físicas e mecânicas, essenciais para bom desempenho clínico. De maneira geral, os resultados encontrados em relação às propriedades físicas e mecânicas avaliadas estão de acordo com o estabelecido pela norma ISO 1567:1999.

Considerando os resultados obtidos de dureza de superfície, a adição de PTFE, em ambos os tipos de resina de ciclo longo e curto, não causou nenhuma mudança nesta propriedade. A característica de dureza de superfície está relacionada com a resistência de desgaste do material, e conseqüentemente a manutenção da sua rugosidade de superfície. Este resultado mostrou que a adição de PTFE à resina acrílica não prejudicou a dureza de superfície, entretanto o mesmo não foi observado para resistência ao impacto e flexão.

De acordo com os resultados, a adição de PTFE a resina acrílica reduziu a resistência ao impacto e a flexão das resinas avaliadas. Resultados estes que podem ser justificados devido à mistura resultante entre resina e PTFE não ser uma mistura homogênea (Khan *et al.*, 2009). A dificuldade de formação de blendas poliméricas homogêneas pode ser explicada devido ao fato do PTFE apresentar estabilidade química e térmica, alto peso molecular e baixa solubilidade (Borkar *et al.*, 2006), assim o PTFE não foi completamente incorporado de maneira homogênea na matriz polimérica. Desta forma, este

polímero adicionado pode atuar como uma barreira para que o material reaja de forma homogênea durante a realização dos ensaios de resistência a flexão e ao impacto.

Entretanto, apesar da resina do grupo experimental sofrer maior alteração quanto à adição do PTFE, todos os grupos apresentaram valores de resistência ao impacto $\geq$ 2 kJ/m^2, e para os valores de resistência flexural > 65 MPa estabelecidos como adequados pela norma ISO 1567:1999.

O aumento do valor do módulo flexural de um material é característica importante, pois reflete na rigidez da base da prótese, além de auxiliar na manutenção da integridade do rebordo alveolar e da mucosa (John *et al.*, 2001; Zappini *et al,.* 2003; Pfeifer *et al.,* 2005). Neste estudo a resina de ciclo longo adicionada de PTFE obteve os maiores valores (2.121,5 ± 67,9 MPa) mostrando que esta propriedade foi positivamente afetada pela adição de PTFE. Este fato pode ser justificado devido à presença de maior número de ligações cruzadas na estrutura do polímero, responsável por aumentar a rigidez, o que pode ter sido resultado da polimerização de ciclo longo na formação de ligações cruzadas, as quais refletiram na microestrutura do polímero. O oposto ocorreu com a carga máxima suportada pelo material antes da fratura, já que os maiores valores foram obtidos (138 ± 12N) no grupo de ciclo longo sem incorporação de PTFE. Contudo os grupos com PTFE mostraram os menores valores, porém são considerados aceitáveis desde que sejam maiores que 55N.

As características da superfície do substrato incluindo rugosidade de superfície, energia livre, ângulo de contato (Carlen *et al.,* 2001, Chandra *et al*., 2005, Morgan & Wilson, 2001; Moura *et al.,* 2006), são apontadas na literatura como fatores importantes no processo de aderência e desenvolvimento do biofilme. Assim, os resultados encontrados de rugosidade, ângulo de contato e energia livre de superfície para as resinas com e sem adição de PTFE, foram similares e estão em consonância com dados da literatura (Moura *et al*., 2006; Pereira-Cenci *et al*., 2008). Entretanto, quando a quantidade de PTFE incorporada à resina for substancialmente maior (21,3%) Gyo *et al.,*2008 encontraram valores

de ângulo de contato maiores, entretanto é importante destacar que a resina utilizada foi resina composta.

Quanto à aderência de *C. albicans,* não foi encontrada diferença estatisticamente significante entre as resinas testadas, entretanto houve uma tendência de diminuição dos valores nos grupos com adição de PTFE. Em relação ao número de células de *C. albicans* encontradas decorrentes do processo de aderência, variaram de 6,618 a 12,963 CFU/mm^2, sendo valores superiores a outros estudos (Serrano-Graner *et al*., 2005; Pereira-Cenci *et al.*, 2007), nos quais os método de contagem das células aderidas foi por meio de do uso de microscópio óptico. Entretanto o método de quantificação de CFU por meio de remoção das células por ultrasom consome maior tempo além de ser exigir mais etapas quando se comparado ao método de contagem celular direta.

Neste estudo, as características de superfície da resina acrílica após a adição de PTFE foi investigada, avaliando a formação e composição de polissacarídeo em biofilme neste material, considerando o fato que poucos estudos na literatura abordaram este tipo de análise (Gyo *et al.,* 2008). A avaliação da composição de polissacarídeo foi realizada em biofilme desenvolvido durante 5 dias na presença de sacarose, com o intuito de simular o que acontece *in vivo.* Microrganismos aderidos à superfície e organizados em biofilme estão mais protegidos da ação de remoção e ainda apresentam maior resistência a ação de antimicrobianos (Redding *et al*., 2009). Inibir ou ao menos diminuir a produção de polissacarídeo, particularmente os extracelulares que são os principais responsáveis pela estruturação do biofilme (Bowen, 2002; Colby & Russell, 1997; Hamada & Slade, 1980), pode ser uma estratégia significativa na prevenção do desenvolvimento do biofilme patogênico na superfície de materiais restauradores como a resina acrílica estudada. Neste estudo, houve a tendência de redução dos valores de polissacarídeos solúveis na resina de ciclo curto com PTFE, entretanto não foi estatisticamente significante a diferença.

Apesar de não ter sido encontrada diferença estatisticamente significante, os dados apresentaram diminuição dos valores para os

polissacarídeos intracelulares na resina de ciclo curto com 2% de PTFE em todos os microrganismos analisados. A importância em reduzir esse tipo de polissacarídeo está baseada no fato de que este é capaz de promover queda no pH durante períodos de restrição de nutrientes e é conhecido o fato que espécies de *Candida* que são comumente encontradas em biofilme de próteses são capazes de resistir e crescer em ambientes de baixo pH, favorecendo desta forma a colonização destes microrganismos (Paes Lemes *et al*., 2006; Wargo & Hogan, 2006).

Neste estudo, a presença de PTFE na resina acrílica não foi capaz de causar efeito no processo de desenvolvimento do biofilme, resultando em contagem de unidades formadoras de colônia similares entre os grupos, em concordância com os dados obtidos por Gyo *et al.,* 2008, estudo o qual foi desenvolvido para analisar o efeito da adição de PTFE em resina composta, e submetida a formação de biofilme de *S. mutans. O*s resultados mostraram que não houve diferença na formação de biofilme. Assim, estes dados encontrados podem ser explicados que apenas 2% de PTFE adicionado a resina acrílica não foi o suficiente para causar diferenças na superfície a resina e produzir efeito no desenvolvimento do biofilme.

Com base nos resultados encontrados, há a necessidade de novos estudos avaliando a incorporação diferentes porcentagens de PTFE, bem como estudos que investiguem ciclos de polimerização capazes de resultar melhor incorporação da partícula na matriz polimérica. Desta forma, com base nos dados obtidos de aderência inicial de *C.albicans* sugere resultados promissores com maiores concentração de pó adicionado a resina acrílica quanto à aderência microrganismo e desenvolvimento de biofilme.

CONCLUSÃO GERAL

Considerando as limitações desse estudo, a adição de PTFE não melhorou as propriedades físicas e mecânicas das resinas acrílicas estudadas; entretanto permaneceram dentro do padrão estabelecido pela norma ISO 1567. O presente estudo sugere que a adição de 2% de PTFE em resinas acrílicas de ciclo longo e curto ainda não foi suficiente para promover mudanças nas propriedades de superfície e interferir na aderência de *C. albicans*. Adicionalmente, a adição de PTFE foi insuficiente para modificar composição de biofilmes de *Streptococcus mutans* UA159, *Streptococcus sanguinis* ATCC 10556 e *Actinomyces naeslundii* ATCC 12104.

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ANEXO 1 – Certificado de aprovação do Comitê de Ética em Pesquisa:

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Avaliação in vitro do comportamento mecânico e adesão de microorganismos em resina acrílica modificada com politetrafluoretileno**", protocolo nº **112/2006**, dos pesquisadores **ALTAIR ANTONINHA DEL BEL CURY e FABIANA GOUVEIA STRAIOTO**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 13/09/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**In vitro analysis of the mechanical behavior and adherence of microorganisms on polytetrafluoroethylene modified acrylic resin**", register number **112/2006**, of **ALTAIR ANTONINHA DEL BEL CURY and FABIANA GOUVEIA STRAIOTO**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 13/09/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2 – Comprovante da submissão do artigo ao periódico:

ENC: Manuscript Received

De: Altair Cury <altcury@fop.unicamp.... Exibir contato
Para: Fabiana Gouvela <fabianagouvela@yahoo.com.br>

Quinta-feira, 11 de Fevereiro de 2010 13:13:17

Profa Dra. Altair A. Del Bel Cury
Department of Prosthodontics and Periodontology
Piracicaba Dental School, University of Campinas
P.O. Box 52
13414-903, Piracicaba, SP, Brazil
Phone: #55-19-21065294

De: JPD JPD [mailto:JPD@mail.mcg.edu]
Enviada em: quinta-feira, 11 de fevereiro de 2010 12:01
Para: altcury@fop.unicamp.br
Assunto: Manuscript Received

Dear Dr. Del Bel Cury:

The manuscript entitled "Polytetrafluorethylene added to acrylic resins: mechanical properties," which you recently submitted to the Journal, has been received and assigned number 18685. Please refer to this number in all correspondence relating to your article. All articles are considered in the order in which they were received.

Thank you for your interest in The Journal of Prosthetic Dentistry.

Drew Landrum
Editorial Assistant

Journal of Prosthetic Dentistry
MCG School of Dentistry
1120 15th. St. AD2943
Augusta, GA 30912-1255
Phone: (706) 721-4558
Fax: (706) 721-4571
Email: jpd@mcg.edu

ANEXO 3 – Comprovante do aceite da publicação do artigo no periódico:

[Fwd: RE: AJD Paper - Status]

De: "altcury@fop.unicamp.br" <altcury@fop.unicamp.... Exibir contato
Para: pedroricomini@gmail.com; fabianagouveia@yahoo.com.br
Cc: altcury@fop.unicamp.br
untitled-2 (5KB)

Congratulations! The MS was accepted for publishing.
Regards,
Altair

---------- Mensagem Original ----------
Assunto: RE: AJD Paper - Status
De: "Franklin Garcia-Godoy" <godoy@amjdent.com>
Data: Dom, Julho 26, 2009 11:53 pm
Para: altcury@fop.unicamp.br

Dr. Cury:

I am pleased to inform you that the revised version of your paper has been accepted for publication in the American Journal of Dentistry. Before publication you will receive galleys for your approval.

Again, thank you for considering the American Journal of Dentistry for publication of your work.

Sincerely,

Prof. Dr. Franklin Garcia-Godoy

Editor

ANEXO 4 – Comprovante do aceite da publicação do artigo no periódico:

Manuscript submitted to Journal of Biomedical Materials Research: Part B - Applied Biomaterials - JBMR-B-10-0083, Author...

De: "JBMRB@wiley.com" <JBMRB@wiley.co... Adicionar a contatos
Para: fabianagouveia@yahoo.com.br
Quinta-feira, 11 de Fevereiro de 2010 18:50:10

11-Feb-2010

Manuscript number: JBMR-B-10-0083

Dear Ms. Straioto:

We are pleased to receive your manuscript entitled "Influence of polytetrafluorethylene incorporated into acrylic resin on biofilm" by Straioto, Fabiana; Murata, Ramiro; Del Bel Cury, Altair; Duarte, Simone. We will be sending it out for review shortly.

To track the progress of your manuscript through the editorial process using our new web-based system, simply point your browser to:

http://mc.manuscriptcentral.com/jbmr-b

and log in using the following user ID and password:

(User ID): fabianagouveia@yahoo.com.br
(Password): Your Password: fa001716

Please remember in any future correspondence regarding this article to always include its manuscript ID number JBMR-B-10-0083.

If you experience problems associated with the submission web site, please click on the "Get Help Now" link at
http://mc.manuscriptcentral.com/jbmr-b

Thank you for submitting your manuscript to JBMR Part B, Applied Biomaterials.

Dr. Jeremy Gilbert
Journal of Biomedical Materials Research: Part B - Applied Biomaterials Editor-in-Chief