UNESP - Universidade Estadual Paulista

“Júlio de Mesquita Filho”

Faculdade de Odontologia de Araraquara

Julianna de Oliveira Lima Parizotto

Propriedades mecânicas e biológicas dos alinhadores ortodônticos impressos de maneira direta em comparação com alinhadores termoformados

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Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara para obtenção do título de Doutora Ciências Odontológicas, na Área de Ortodontia.

Orientador: Prof. Dr. João Roberto Gonçalves
Coorientador: Prof. Dr. Jonas Bianchi

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Julianna de Oliveira Lima Parizotto

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2º Examinador: Prof. Dr. Luiz Gonzaga Gandini Junior.

3º Examinador: Prof. Dr. Luiz Geraldo Vaz.

4º Examinador: Profa. Dra. Patrícia Pigatto Schneider

5º Examinador: Prof. Dra. Daniela Gamba Garib Carreira

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DADOS CURRICULARES

Julianna de Oliveira Lima Parizotto

NASCIMENTO: 09/09/1992 – Campo Grande – Mato Grosso do Sul

FILIAÇÃO: Symonne Pimentel Castro de Oliveira Lima Parizottto e Valerio Antonio Parizotto.

2010/2014: Curso de Graduação em Odontologia: Faculdade de Odontologia da Universidade de São Paulo (FOUSP).

2016/2018: Curso de Especialização em Ortodontia: Hospital de Reabilitação de Anomalias Craniofaciais da Universidade de São Paulo (HRAC-USP).

2018/2020: Curso de Pós-Graduação: Mestrado pelo programa de Ciências Odontológicas. Área de concentração em Ortodontia: Faculdade de Odontologia de Araraquara – UNESP.

2020/atual: Curso de Pós-Graduação: Doutorado pelo programa de Ciências Odontológicas. Área de concentração em Ortodontia: Faculdade de Odontologia de Araraquara – UNESP.

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“Sua visão se tornará clara somente quando você olhar para dentro do seu coração. Quem olha para fora, sonha. Quem olha para dentro, desperta.”
Carl Jung*

* Jung CG. Practice of psychotherapy. Princeton: Princeton University Press, 2014. (Collected Works of C.G. Jung, 16).

Parizotto JOL. Propriedades mecânicas e biológicas dos alinhadores ortodônticos impressos de maneira direta em comparação com alinhadores termoformados [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2023.

RESUMO

A demanda cada vez maior por uma ortodontia estética e discreta favoreceu o crescimento da indústria dos alinhadores. No entanto, o fluxo de trabalho atual para confecção dos alinhadores, por termoformação, envolve a impressão tridimensional (3D) de modelos, cuja quantidade varia de acordo com a complexidade do caso. Todas as estruturas impressas 3D com resinas fotopolimerizáveis tradicionais (material de insumo para maioria das impressoras 3D) não podem ser reprocessadas. Esta característica combinada com o aumento da impressão 3D em todas as áreas, gera um grande desperdício destes materiais não reprocessáveis, com sérias implicações ambientais. Com isto, a produção por impressão direta dos alinhadores parece uma alternativa promissora por tornar o fluxo de trabalho para confecção dos alinhadores mais eficiente e diminuir o impacto ambiental. O objetivo deste trabalho foi testar as propriedades mecânicas e biológicas dos materiais para confecção de alinhadores por impressão direta. A amostra foi dividida em Grupo Controle (Zendura, termoformado) e 4 grupos experimentais de diferentes resinas para impressão direta dos alinhadores (S1, S2, S3 e TC-85). Para os testes mecânicos foi realizado teste de flexão em três pontos para avaliação da curva tensão/deformação, carga máxima e resistência a flexão. Para os testes biológicos, foi avaliada a adesão de biofilme (*Candida albicans* e *Streptococcus mutans*, simples e mistos) por meio dos testes contagem de unidade formadora de colônias (UFC) e AlamarBlue® e citotoxicidade por meio do teste de proliferação celular AlamarBlue®. O teste de Shapiro-Wilk foi utilizado para avaliar a normalidade e o teste F para avaliar a homocedasticidade dos grupos. Para a avaliação das propriedades biológicas e mecânicas, foram realizados ANOVA two-way e pós teste de Tukey. Para o ensaio de citotoxicidade foi realizado o teste de Kruskal-Wallis seguido do pós-teste de Dunn. Os alinhadores impressos de maneira direta exibem curvas de tensão-deformação mais planas, indicando uma melhor faixa elástica em comparação com os alinhadores termoformados. Todas as resinas usadas para impressão 3D direta de alinhadores aumentaram de espessura após a impressão. Além disso, os alinhadores impressos mostram menos formação de biofilme com *C. albicans* e *S. mutans*. O tempo entre a impressão 3D direta do alinhador e seu uso é importante e aumenta a adesão do biofilme misto. Todos os grupos apresentaram citotoxicidade semelhante, dentro de limites clínicos aceitáveis.

Palavras-chave: Aparelhos ortodônticos removíveis. Biofilmes. Estresse mecânico. Impressão tridimensional.

Parizotto JOL. Mechanical and biological properties of directly printed orthodontic aligners compared to thermoformed aligners [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2023.

ABSTRACT

The increasing demand for aesthetic and discreet orthodontics has favored the growth of the aligner industry. However, the current workflow for making aligners by thermoforming involves three-dimensional (3D) printing of models, the quantity of which varies according to the complexity of the case. All structures 3D printed with traditional light curing resins (input material for most 3D printers) cannot be reprocessed. This characteristic combined with the increase of 3D printing in all areas, generates a great waste of these non-reprocessable materials, with serious environmental implications. With this, direct printing production of aligners seems a promising alternative for making the workflow for making aligners more efficient and reducing the environmental impact. The objective of this work was to test the mechanical and biological properties of materials for making aligners by direct printing. The sample was divided into Control Group (Zendura, thermoformed) and 4 experimental groups of different resins for direct printing of aligners (S1, S2, S3 and TC-85). For the mechanical tests, a three-point bending test was performed to evaluate the stress/deformation curve, maximum load and rupture stress, and the indentation hardness test. For the biological tests, biofilm adhesion (Candida albicans and Streptococcus mutans, single and mixed) was evaluated using the colony forming unit count (CFU) and AlamarBlue® tests, and cytotoxicity using the AlamarBlue® cell proliferation test. The Shapiro-Wilk test was used to assess normality and the F test to assess the homoscedasticity of the groups. To evaluate the biological and mechanical properties, two-way ANOVA and Tukey's post test were performed. For the cytotoxicity assay, the Kruskal-Wallis test was performed followed by the Dunn post-test. Directly printed aligners exhibit flatter stress-strain curves, indicating a better elastic range compared to thermoformed aligners. All resins used for direct 3D printing of aligners increased in thickness after printing. Additionally, printed aligners show less biofilm formation with *C. albicans* and *S. mutans*. The time between direct 3D printing of the aligner and its use is important and increases the adhesion of the mixed biofilm. All groups showed similar cytotoxicity, within acceptable clinical limits.

Keywords: Orthodontic appliances, removable. Biofilms. Stress, mechanical. Printing, three-dimensional.

SUMÁRIO

1 INTRODUÇÃO

A busca pela estética e conforto foi sempre presente em todos os âmbitos da odontologia. Devido ao avanço da tecnologia, as possibilidades de proporcionar tratamentos ortodônticos mais estéticos para o paciente aumentaram[1] com o surgimento dos bráquetes estéticos[2] e bráquetes linguais[3]. Apesar de existirem excelente bráquetes estéticos no mercado da ortodontia hoje em dia, ainda não é estético ou confortável o suficiente para alguns pacientes. Neste intuito, o interesse pelo tratamento com os alinhadores cresceu muito, tanto pelos ortodontistas como pelos próprios pacientes. Os alinhadores tornaram-se populares pois são aparelhos ortodônticos removíveis, com isso oferecendo mais liberdade ao paciente, e estéticos, visto que o material é quase transparente, por não haver fios ou bráquetes.

Apesar da grande evolução dos alinhadores ter ocorrido na última década, a ideia de alinhar os dentes com aparelhos consecutivos termoplásticos surgiu inicialmente em 1945 com Kesling[4], que utilizava os seus chamados “posicionadores” para pequenas irregularidades entre os dentes. Porém, como estes posicionadores eram fabricados por meio de setups sequenciais de cera, não foram incorporados na rotina clínica dos ortodontistas. Com os avanços tecnológicos, especialmente após a introdução do CAD/CAM[5] (*computer-aided design and computer-aided manufacturing* - design feito por computador e fabricação feita por computador) e sua aplicabilidade clínica, os antigos posicionadores Kesling tornaram-se uma proposta viável para tratamento ortodôntico. Em 1997, a Align Technology (Santa Clara, CA) introduziu os alinhadores Invisalign[6], produzidos pelo sistema CAD/CAM com software próprio que movem os dentes digitalmente em pequenos incrementos sequenciais. O tratamento com alinhadores, anteriormente restrito a casos de baixa complexidade, tornou-se uma opção para más oclusões mais complexas, devido às inovações tecnológicas incorporadas nos alinhadores como estagiamento, attachments e ao conhecimento adquirido[7,8].

O fluxo digital para confecção de alinhadores, com as tecnologias disponíveis atualmente segue a seguinte ordem: aquisição de imagens, setup virtual em software, impressão dos modelos exportados pelo software, termoplastificação destes modelos para originar os alinhadores e acabamento dos alinhadores. A aquisição de imagens é feita por meio do escaneamento intraoral ou do escaneamento do modelo de gesso, para criação de um arquivo dos modelos do paciente em formato STL (*Standard*

Tessellation Language - formato com dados do objeto tridimensional)[9]. Estes arquivos STL são inseridos em um software para realização do planejamento virtual e assim obtém-se a quantidade de modelos gerados, e consequentemente de alinhadores, que é determinada de acordo com parâmetros previamente estabelecidos de quantidade de movimento por alinhador[6]. O número de modelos a serem impressos, por meio de uma impressora 3D, depende da complexidade do caso. Casos simples podem gerar apenas 5 modelos, em um caso de tratamento em uma única arcada, por exemplo, porém, casos complexos envolvendo tanto a arcada superior quanto a inferior, podem chegar à 80 modelos.

Atualmente, após a impressão dos modelos, é feita a estampagem/plastificação destes para confecção do alinhador propriamente dito por meio de plastificadora. Os materiais termoplásticos comumente utilizados para confecção dos alinhadores são: polietileno tereftalato de etileno glicol (PET-G), polipropileno, policarbonato, poliuretanos termoplásticos (PU), entre outros[10]. Após este processo, é feito recorte e acabamento nos alinhadores e os modelos impressos são guardados e futuramente descartados.

A impressão 3D tem se mostrado cada vez mais útil, tanto na odontologia quanto em outras áreas. Hoje em dia já há casas construídas por meio de impressão 3D, veículos, ferramentas, além de prótese utilizadas em medicina. Na ortodontia, as impressoras mais utilizadas são: FDM (Fused deposition modeling), SLA (Estereolitografia) e DLP (Digital Light Processing)[11]. Estas impressoras, que trabalham por manufatura aditiva[12], variam de acordo com os seus métodos de construção de camadas, materiais e a tecnologia da máquina. O material de insumo da impressora FDM[13] é um filamento termoplástico, que apresenta um preço acessível, porém o tempo de impressão é maior e precisão é inferior quando comparada com as outras tecnologias citadas. A SLA[14] e DLP[15] diferem entre si pela forma de exposição à luz e consequentemente pelo tempo de impressão, sendo a DLP mais rápida, porém ambas utilizam de resinas fotopolimerizáveis líquidas como material de insumo.

No entanto, todas as estruturas impressas 3D formadas com resinas fotopolimerizáveis tradicionais não podem ser reprocessadas, pois as redes de polímeros são reticuladas covalentemente[16]. Essa natureza não processável, combinada com o aumento global da impressão 3D, está levando a um grande desperdício de materiais de impressão 3D, com sérias implicações ambientais[17]. Hoje já existem filamentos termoplásticos (para impressora FDM) recicláveis, que não são produzidos à base de petróleo, ou seja, são biodegradáveis[18,19]. Porém, eles possuem propriedades mecânicas limitadas e maior custo[19]. As resinas termofixas para impressoras SLA e DLP são infusíveis, insoluvéis e não recicláveis, uma vez que mesmo diante de uma temperatura e pressão elevadas, após o processo de polimerização ela não retornam ao estado de fluidez, pois sua estrutura tridimensional, reticulada com a formação de ligações cruzadas impede o deslizamento entre as cadeiais[20,21].

Neste contexto, a impressão 3D direta do alinhador seria ideal. Deste modo, seria diminuída uma etapa na produção, eliminando-se a necessidade de impressão de todos os modelos físicos dos alinhadores (economia de tempo e trabalho), visto que não seria necessário mais a plastificação. Com isso, elimina-se também a quantidade de lixo tóxico descartada durante o processo de confecção de alinhadores. Porém, há inúmeros desafios na criação e viabilização de uma resina para impressão de alinhadores, como: instabilidade dimensional, resistência baixa, porosidade, toxicidade, entre outros, além das limitações do processo de fabricação[22]. Esta resina precisaria apresentar principalmente três propriedades imprescindíveis: biocompatibilidade, flexibilidade ótima e transparência.

Até o momento, ainda não existem resinas para impressão direta de alinhadores com aprovação da ANVISA (Agência Nacional de Vigilância Sanitária) no Brasil, apenas a Tera Harz (Graphy, Seoul, Coreia do Sul) possui FDA (*Food and Drug Administration*) nos Estados Unidos e CE (Conformite Europeenne), além de possuir o FDA local da Coreia do Sul. Com isso, o objetivo desde trabalho é comparar as propriedades mecânicas e biológicas dos materiais considerados padrão ouro até o momento para confecção de alinhadores (termo-poliuretano – Zendura FLX) com as resinas para impressão direta de alinhadores e avaliar a viabilidade destes novos produtos, bem como suas limitações.

2 OBJETIVO

Comparar as propriedades mecânicas e biológicas dos materiais para confecção de alinhadores para impressão direta.

2.1 Objetivos Específicos

- Comparar a literatura disponível sobre as propriedades dos alinhadores impressos e dos alinhadores termoformados;
- Caracterizar mecanicamente as resinas para impressão direta de alinhadores;
- Caracterizar mecanicamente o material padrão ouro para confecção de alinhadores termoformados (Zendura FLX);
- Comparar propriedades mecânicas de resinas em desenvolvimento para impressão de alinhadores com a resina TC-85;
- Comparar propriedades mecânicas dos materiais para confecção dos alinhadores termoformados (Grupo Controle) com as propriedades das resinas para impressão direta de alinhadores (Grupos Experimentais);
- Avaliar adesão de biofilme somente com *Candida albicans*, somente com Streptococcus mutans e com os dois microrganismos juntos em todos os grupos;
- Comparar a adesão de biolfime (*C. albicans* e *S. mutans*) em todos os grupos em 2, 7 e 14 dias após a impressão;
- Avaliar a citotoxicidade dos alinhadores impressos, em comparação com o alinhador termoformado;
- Comparar a citotoxicidade da resina TC-85 com as outras resinas em desenvolvimento para impressão de alinhadores;
- Avaliar as limitações na confecção do alinhador impresso.

3 PUBLICAÇÕES

De forma a contemplar o objetivo geral deste trabalho, os objetivos específicos foram tratados na forma de três artigos distintos.

3.1 Artigo 1*

Direct 3D Printing of Clear Orthodontic Aligners: a narrative review

Abstract

Clear aligners have become popular in Orthodontics in recent years due to their advantages in aesthetics, comfort, and the convenience of removal for dental hygiene. Currently, different thermoplastic materials and vacuum-based methodology are used to manufacture clear aligners. This thermoforming process alters the materials mechanical properties such as thickness, and consequently affect the aligner performance. To enhance the aligner manufacturing process, make it more efficient and with less waste of time and material, the new technology of 3D printed aligners has emerged. This new technology aims to provide an aligner with memory shape, higher efficacy, and improved mechanical properties. Despite the advantages and possible benefits of 3D printed aligners, very limited technical and clinical data are available in the literature about them. The present article discusses the benefits of 3D printed aligners in comparison to thermoformed ones, presenting the current data on each of these techniques. In addition, the present review suggests that 3D printed aligners are superior than the thermoformed aligners especially due to its unique feature of memory shape, with less deformation. Despite of that, 3D printed aligners represent a complex method to control the thickness. The conclusion of this article is that 3D printed aligners are promising and further in vivo studies to test the interactions of 3D printed aligners with the oral microbiota and other biological features are needed, in order to assure this materials safety. Furthermore, 3D printed aligners they are much more eco-friendly than thermoformed ones.

Keywords: Removable Orthodontic Appliances; Biofilm; Cytotoxicity; Mechanical Stress; Three-Dimensional Printing.

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*Artigo escrito conforme as normas bibliográficas da revista *Orthodontics and Craniofacial Research* (OCR) para a qual será submetido.

Background

Although most people think that clear orthodontic aligners started in the early 90’s, the truth is they were invented way before, in 1945[1]. These ancient aligners, called positioners, were used to refine fixed orthodontics. At the time, patients were treated with bands on all teeth, so when the bands were removed, there was space left over from the bands in the interproximal teeth. With the objective of finishing these cases with space closure, setups and positioners were made. They performed the dental movement refining the previous treatment with fixed appliance.[1] To produce the positioners, it was necessary to mold the patient, and make a plaster cast followed by a manual setup[2] and positioner confection. If after this first positioner the teeth still needed correction, the process cited previously had to be performed again (mold, plaster model, manual setup and positioner confection).

Over time, treatment finishing was improved, as well as the orthodontic bonding materials. Therefore, bands in all teeth fell into disuse and bonded brackets took over to support wires made from stainless steel, which offered high strength, durability, reduced friction, and less food collection, and they were relatively easy to form. These positioners used for finishing tried to be used at the time by some orthodontists as a treatment, but there was a lot of frustration as the process was not easy and simple. And often when the orthodontist gets frustrated, he stops using that technique and goes back to what he is already used to. The next esthetic evolution in orthodontics, after bonded brackets, were the esthetic brackets which were made of unfilled polycarbonate that were later replaced by ceramic-reinforced, fiberglass-reinforced, and polycarbonate-reinforced brackets with a metallic insert to minimize creep deformation. After that, ceramic brackets (monocrystalline sapphire and polycrystalline ceramic) were used.

Following the evolution of dental materials for bonding, the technology has also improved over time. Although there are excellent aesthetic brackets on the orthodontic market nowadays, it's still not aesthetic or comfortable enough for some patients. In 1993, Sheridan et al.[3] suggested orthodontic setup with aligners, but it was necessary several molds, which still made the process very extensive. The proposed treatment involved a setup to treat the malocclusion, which usually needed interproximal reduction (IPR), and after that an acetate plate was thermoformed on the model with the new alignment and the same interproximal reduction performed on the model was reproduced in the mouth.

The introduction of CAD/CAM technology (computer-aided design and computer-aided manufacturing) in 1985[4,5] changed the course of dentistry for the better. The benefits associated with CAD/CAM in dentistry include: the access to new, industrially prefabricated, and controlled materials; an increase in quality and reproducibility and data storage commensurate with a standardized chain of production; an improvement in precision and planning, as well as an increase in efficiency. As a result of continual developments in computer hardware and software, new methods of production and new treatment concepts are to be expected[5].

With the clinical applicability of CAD/CAM, the old Kesling positioners have become a viable proposition for treatment orthodontic. In 1997, Align Technology (Santa Clara, CA) introduced the Invisalign aligners[6], produced by the CAD/CAM system with its own software that move the teeth digitally in small sequential increments. The treatment with aligners, previously restricted to cases of low complexity, has become an option for more complex malocclusions, due to the technological innovations incorporated in the aligners such as staging, attachments and acquired knowledge[7,8]. The orthodontic clear aligners became popular, by both orthodontists and by the patients themselves, because they are removable appliances that offer more freedom and aesthetics to the patient, as the material is almost transparent, and there are no wires or brackets.

Up until now, the use of 3D printing in orthodontics has mostly been limited to the fabrication of physical models of upper and lower dentition, which are clinically accurate for treating patients. However, with the limitations of conventional manufacturing methods for clear aligners such as customization, accuracy, speed, orthodontists have recently started exploring the use of Direct 3D Printing technology with biocompatible materials to produce clear aligners. Nasef et al.[9] were one of the firsts to attempt that, printing a 3D direct retainer in 2014. Since that, orthodontics came along testing new resins and methods to make this possible.

Main text:

Thermoformed Aligners

The manufacture of thermoformed aligners begins with the acquisition of images through an intraoral scanner. Before, digital models generated by desktop-scanning of conventional impression/plaster models were accepted. However, nowadays impression/plaster models are not indicated for the manufacture of aligners since there are many variables that can contribute to the failure of the final product, such as an error in the impression, inadequate preparation of the impression material, syneresis and imbibition of the material and inadequate plaster casting[10]. Therefore, intraoral scanning done with care and attention is the best way to acquire images for aligners, because in addition to having fewer variables, it is proven to be more effective[11].

The second step, after acquiring the digital models, is the digital setup. Some of the most common softwares for orthodontic setup are Bluesky Plan, Archform, 3Shape Clear Aligner Studio, Maestro 3D, Nemocast and Archform. These softwares all work in similar ways, by sequentially moving the teeth in small amounts with consecutive aligners to reach to the final orthodontic outcome. Each movement performed within the software (in/out, left/right, up/down, root tip, torque and rotation) has parameters that enables the software algorithm to define the quantity of aligners for each case. One of the advantages of the digital setup is the treatment individualization to improve the effectiveness, such as: addition of attachments[12], staging of complex orthodontic movements and reduction of the movements velocity[13]. After the digital setup, the number of aligners refers to the number of models generated by the software that will be 3D printed and then thermoformed.

Within the thermoforming process, there are variables that affect the final product, such as: quality of the thermoforming machine (vacuum or positive pressure), ability and experience of the thermoforming machine operator, material composition and thickness. The positive pressure thermoforming process enables higher fidelity of the thermoformed object[14], and should be preferred. The majority of current thermoformed aligners are made from thermoplastic polyurethanes, modified polyethylene terephthalate glycol (PETG), copolyester polypropylene, polycarbonate, and many other materials.[15,16] Before thermoforming, all these materials have a determined thickness (usually approximately 0.75mm for aligner therapy), but after the thermoforming process material properties change due to the heat generation. Min et al.[17] evaluated the level of forces delivered by thermoformed orthodontic aligners and reported that the thickness of the thermoplastic material affected the amount of deflection after repeated loading and stress. Besides that, for different materials and thicknesses, significant changes occur in the material transparency, water absorption and solubility, surface hardness, flexure and elastic moduli, and tensile and flexural forces, after thermoforming.[18] Transparency, one of the key elements for the aligner, decreases with a decrease in thickness after thermoforming, water absorption properties and water solubility increases, besides the modification in surface hardness.[18,19]

The shrinkage and expansion of the material that occurs during the thermoforming process affects the orthodontic force delivered from the clear aligners to the teeth. In a study evaluating the fit of the clear aligners fabricated with thermoplastic materials, all materials showed various amounts of shrinkage and expansion[18], which can alter their thickness and physical properties[20]. The average thickness of PETG specimens thermoformed is only 54.7% of the thickness before thermoforming (0.75 mm before thermoforming, 0.41mm after)[20]. Min et al.[17] also reported a 57.5% decrease in thickness of the materials that were thermoformed.

Johal et al.[21] compared the fit of four different commercially available thermoform retainer materials. After scanning and analyzing the materials with a co-ordinate measuring machine to calculate their respective fit, statistically significant differences were observed in the fit behavior of all four thermoform materials being tested.[21] In addition, because the aligner material is viscoelastic, loss of mechanical properties over time (stress relaxation) can decrease the prediction of the orthodontic forces[16,20]. This stress release can cause significant changes in the behavior of the polymers at 24 hours from the application of orthodontic loads, which may influence the predictability of the treatment[16].

Although thermoformed aligners have been used for over a decade, it is imperative to assess the cytotoxicity of these materials to provide a safe orthodontic treatment for the patients. Martina et al.[22] evaluated the in vitro cytotoxicity of different thermoplastic materials for clear aligners on human primary gingival fibroblasts (HGFs). The materials investigated in the study were Duran (Scheu-Dental GmbH, Iserlohn, Germany), Biolon (Dreve Dentamid GmbH, Unna, Germany), Zendura (Bay Materials LLC, Fremont, CA, USA), and SmartTrack (Align Technology, San Jose, CA, USA) and all of them presented a slight cytotoxicity. Biolon was the most cytotoxic when compared to the other materials, and the thermoforming process increased the cytotoxicity. Although intraoral environment cannot be perfectly reproduced with in vitro studies, as the cytotoxicity level of the materials were low , their clinical use may be considered safe[22].

3D Printed Aligners

The manufacturing process of 3D printed aligners is fastest then the thermoformed aligners because there are more steps involved such as 3D printing of a large number of models, thermoforming, cutting and polishing. Instead printing the dental models, this new approach prints the final product (the orthodontic aligner). After being 3D printed, the aligner, as all 3D printed objects, goes through post-processing (specific washing and post-curing). In both methods, errors in the 3D printing process leads to an inadequate final product, but the more steps involved in the manufacturing, more variables to control and the failure possibility is increased. With the aligners manufactured by direct 3D printing, the thermoforming variable is eliminated and so the errors that comes with it.

The first two steps of the direct 3D printed aligner are similar to the thermoformed mentioned before: image acquisition and digital setup. While there are many CAD softwares for treatment planning and digital setup of 3D models for aligners, there are few softwares that allow the exportation of printable aligners instead of the printable models to thermoform, and this is the one difference in the digital setup step of the manufacturing of direct 3D printed aligners: the software must have the printable aligner exportation tool where you design the edges and thickness of the aligner.[23] Maestro 3D (Ortho Studio v.5.2, AGE Solutions S.r.l., Pontedera, Italy), Blue Sky Plan (v.4.9.4, Blue Sky Bio, Illinois, United States), Deltaface © (Limoge, France), LuxCreo (Belmod, California, United States) are a few of the softwares that have a module that can also design aligners and export them in a format convenient for direct 3D printing, most of the softwares only export the 3D dental models (Fig. 1). Export the aligners directly form the digital setup software is the most fast and efficient option, but it is still possible to export all the models and design the aligner in another software like 3Shape Ortho System™ - Appliance Designer™ (Copenhagen, Denmark) or even a license free software like Meshmixer (Autodesk Inc., California, United States).

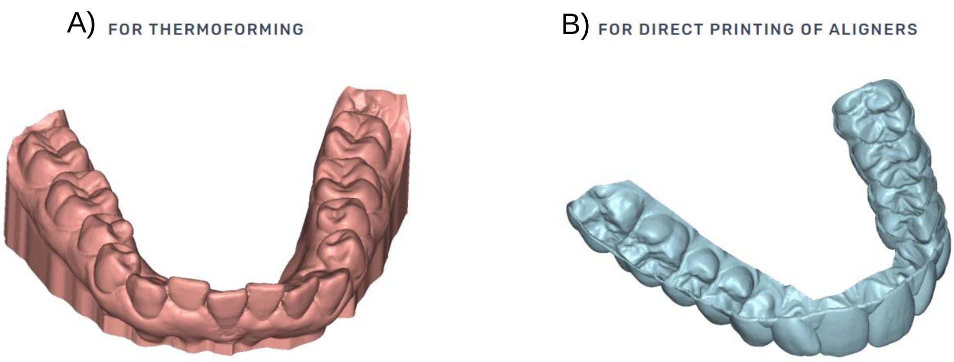

Figure 1 – Example of export options in the suitable softwares for 3D printed aligners. A) Exporting the models for thermoforming. B) Exporting the aligner that you design in the software. Deltaface©

After the digital setup and aligner design in the software and exportation of the files, the next step is 3D printing. However, models and aligners have inherent geometric differences and one of them is the shell-like morphology of aligners compared with solid models, which may affect especially the accuracy of the intaglio (inside) surface of the aligners, responsible for the teeth movement[24]. Boyer et al. evaluated the effect of print orientation on the dimensional accuracy of direct 3D printed aligners and observed that the ones printed parallel or perpendicular (90 and 0 degrees) to the printing platform show less dimensional variation. Aligners printed with the intaglio oriented away and angled (135 degrees) from the printing platform present larger dimensional variation: 3.5 times larger standard deviation than the 90 orientation[24] (Fig. 2). Although this study's results recommend printing the aligners at a 90 angle and guide us towards an accurate 3D printed aligner, it was performed with Grey resin to improve the optical scanning of the material. This Grey resin is not indicated to print aligners and is not biocompatible, which may interfere with the dimensional accuracy of the material (clear and flexible resins, such as the aligner resin, behave differently as materials then opaque and hard resins)[24].

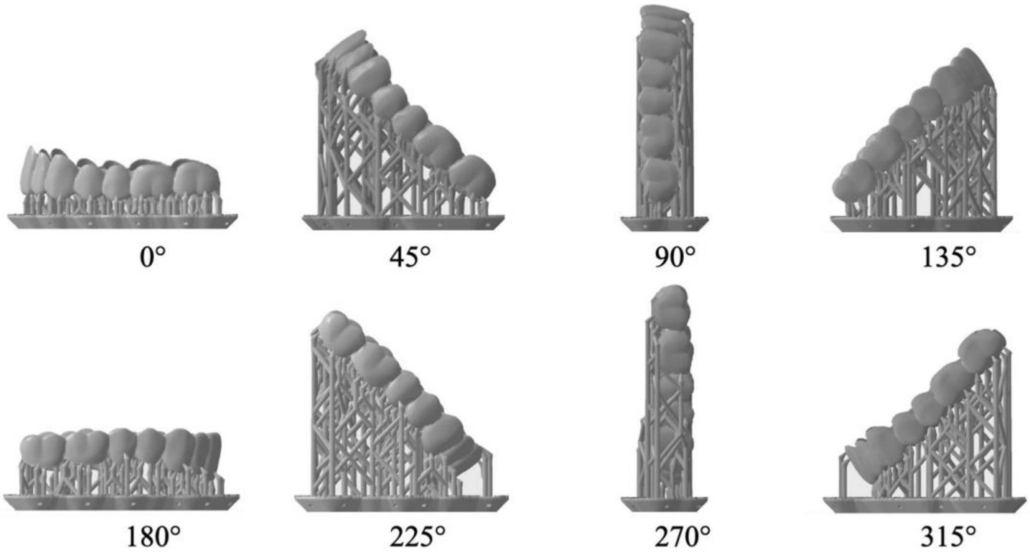

Figure 2: Eight orientations evaluated for 3D printing aligners, by Boyer et al.[24]

The quality of the 3D printed aligner is dependent on the choice of the 3D printer[25] and the resin. Among the various 3D printing methods available, Digital Lighting Process (DLP)(Fig. 3) and Material Jetting (PolyJet) produce more surface accuracy for orthodontic purposes than the Digital Lighting Synthesis (DLS) and Stereolithography Apparatus (SLA)[26]. Liquid Crystal Display (LCD) 3D printers are not as accurate as SLA printers, but its variations are within the clinically acceptable range for orthodontic models[27].

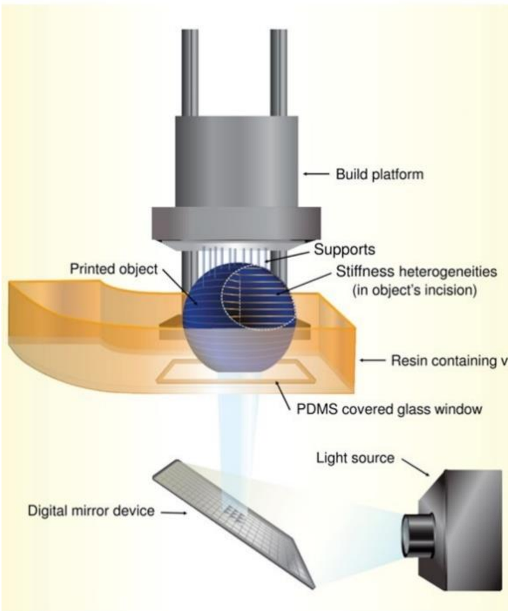

Figure 3: Schematic representation of layer-by-layer photopolymer printing (digital lighting process – DLP) in the bottom-up configuration[28].

The directly printed aligner must be transparent, biocompatible, flexible, and dimensionally stable. These features are complex to develop all together, and that is why there are not many available options of resins to directly print aligners. So far, the only one with Food and Drugs Administration (FDA), Korean's Food and Drugs Administration (KFDA) and Conformite Europeenne (CE) approval is the Tera Harz TC-85 (TH) resin, by Graphy, and therefore is the only one with a few studies comparing its properties with thermoformed aligners. The TH resin is composed of aliphatic vinyl ester-urethane monomers, with acrylate and/or methacrylate functionalization.[29] Considering that 3D printing resins are toxic before the printing process, the evaluation of the toxicity and potential hazards to the patient are necessary.[23] The toxicity of the 3D printing resins decreases with the post-curing process, and that's why attention should be brought to this important step of the manufacturing.

The post-curing process in 3D printed aligners plays a crucial role in enhancing the mechanical and biocompatibility properties. Kim et al found that materials properties such as the Vickers hardness, the degree of conversion, and biocompatibility of the 3D printed resins increased significantly around the beginning of the post-curing time, and then increased more gradually as the post-curing time increased further[30]. The post-processing of the 3D printed aligners involves the following steps: removal from the 3D printer, cleaning (remove the uncured resin of the surface), removal of supporting structures and post-polymerization (UV chamber)[31] (Fig. 4). Unlike conventional post-curing process for 3D printed dental models, the 3D printed aligner cannot be washed with alcohol, it must be centrifuged to remove the remaining liquid resin, as recommended by the manufacturer. Isopropyl alcohol (IPA) is the most commonly used solvent for eliminating excess of uncured resin[32]. However, it is suggested that IPA has low compatibility with acrylate based polymer and might induce polymer swelling instead of dissolution[33,34]. Besides, as IPA evaporates from the swollen polymer surface, the polymer chains can undergo reorganization, and therefore change the surface's solubility and lead to surface fracture of the 3D printed objects[34]. IPA cleaning method, consequently, has a harmful effect on the surface properties of the 3D-printed aligners[33]. For these reasons, centrifugation is a better cleaning method for 3D printed aligners as it separates the liquid part (unpolymerized residual resin) from the solid. Park et al[33] showed that 3D printed aligners that underwent centrifugation after 3D printing achieved greater translucency, however, median thickness was significantly higher after centrifugation, when compared to 3D printed aligners that underwent the IPA cleaning method.

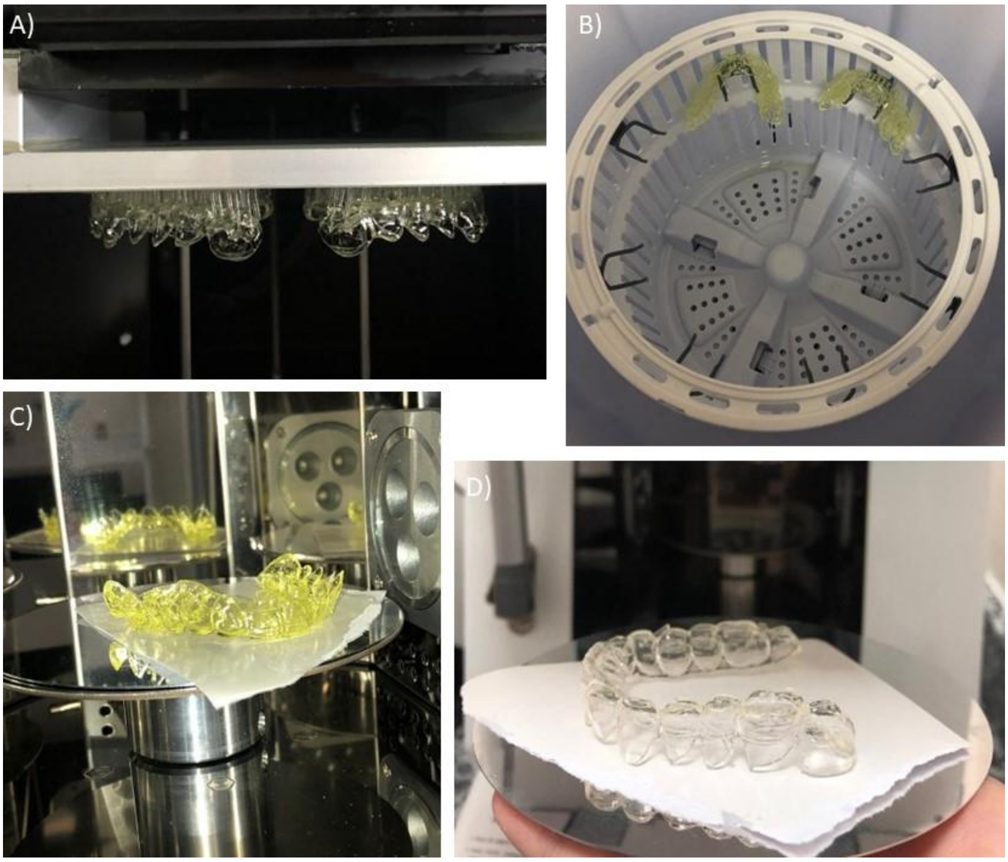

Figure 4: Post-processing of 3D printed aligners. A) Removal from the 3D printed, followed by supports removal. B) Centrifugation method for removal of residual resin. C) 3D printed aligner inside the UV curing chamber before post-curing. D) 3D printed aligner after post-curing in the UV chamber.

Differences in polymerization rates can alter the properties of the 3D printed material, as well as their color tone[30]. Mechanical properties can be improved when a sufficient post-curing time is applied to achieve homogeneous polymerization in both deep and superficial layers of the 3D printed object. For 3D printed crowns, for example, an adequate post-curing time of at least 60 min (post-curing equipment CureM D102H (SONA Global, Seoul, Korea) with a UV intensity of 220 $\mu W \cdot cm^{-2}$ and an internal temperature of 60 °C) is required to improve the overall clinical performance of the printed material[30]. The post-cure process of the 3D printed aligners is performed with an UV chamber with nitrogen. Under nitrogen conditions, the UV chamber prevent the formation of an oxygen inhibition layer, allowing the surface of the object to polymerize[28,33]. Injecting inert nitrogen gas to exclude oxygen can also improve mechanical properties and surface smoothness of the 3D printed aligners[33].

As residual monomers are toxic, the degree of conversion (the proportion of polymerized monomers after 3D printing) of the 3D printed material is important for its biocompatibility. Willi et al[29] evaluated the degree of conversion and elution of target substances from 3D printed aligners. The results showed a degree of conversion of around 83%, indicating efficient polymerization. However, concerns arise from the quantifiable amounts of urethane dimethacrylate (UDMA) monomer leached from the aligners. UDMA has been associated with cytotoxic effects, and its leaching from the aligners into the oral environment could have adverse effects on oral tissues and overall health[35]. While the 3D printed aligners are BPA-free[36], the variability in UDMA leaching may pose potential health hazards upon repeated intraoral exposure. Therefore, thorough post-curing is essential to ensure biocompatibility and safety.

One significant advantage of directly printed aligners lies in their design flexibility, allowing for adjustments in material thickness and precise control of the offset between the aligner and dentition. Despite the benefit of being customizable, thickness is still thickness is still a complex factor to be controlled for 3D printed aligners because after printed they are thicker than their intended print design[37]. Edelman et al[38] observed 0.274mm maximum thickness increase in 3D printed aligners, however, the resins used in the study are not suitable for 3D printed aligners since they are neither flexible nor transparent. Although it is theoretically possible to set the thickness of 3D printed aligners for comparison with thermoformed aligners, the recommendations for effective TC-85 3D-printed aligners have a lower limit of 0.5 mm[33]. Therefore, considering this increase in the thickness of the 3D printed aligner, studies have used the planned thickness of 0.5mm[20,33], assuming that after 3D printing it will be slightly larger, thus becoming similar to what is clinically desired.

Shape memory is a unique and favorable feature of 3D printed aligners. Owing to this shape memory property, especially for TC-85 resin the aligners can constantly apply orthodontic forces to the teeth under normal body temperature conditions without force decay caused by the deformation of the aligners[20]. Therefore, the aligners can apply a constant orthodontic force to the teeth. The clinical advantage is that before inserting the aligners, patients can immerse them in warm water to render them flexible, which can reduce the discomfort and provide a better fit[20]. If the aligner is deformed along the dentition, a process called loss of tracking, its original printed shape and stiffness will be recovered at 37 °C[20]. This shape memory feature may even indicate that fewer aligners will be needed throughout the orthodontic treatment, as their activation can be recovered.

Overall, 3D printed aligners are promising, as their mechanical properties are comparable or even possibly superior to thermoformed aligners. Studies on its biocompatibility show positive results, however further research, especially in vivo, is necessary to assess the interactions of 3D printed aligners with the oral microbiota. In addition, 3D printed aligners offer a greater ecological benefit than thermoformed ones, as they generate much less waste while offering a more efficient workflow for the orthodontist and the patient.

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3.2 Artigo 2*

Mechanical properties of different resins for directly printed of aligners

ABSTRACT

Introduction: The current thermoplastic materials used for clear aligners manufacturing are associated with disadvantages, such as environmental impacts, a complex manufacturing process and potential printing inaccuracies. Tridimensional printed aligners have been introduced but require high-quality printers and resins. Properties like dimensional and color stability, flexibility and biocompatibility are crucial for achieving proper orthodontic movements. This study aims to compare the mechanical properties of thermoformed aligners with different types of resins used for directly printed aligners. Methods: The sample was divided into five groups: Control Group (Zendura FLX), S1, S2, S3, and TC-85. Fifteen specimens were prepared for each group. For the experimental groups, samples with the same dimensions as the control group were virtually designed and 3D printed using different resins. All specimens underwent a three-point flexural strength test to evaluate stress-strain curve, maximum load, and flexural strength. Statistical analysis was performed using ANOVA and Tukey's test. Results: 3D printed aligners showed different mechanical properties compared to the thermoformed aligner material. The Zendura FLX specimens had a greater elastic deformation and a greater tension than the other groups. TC-85, S1 and S3 showed lower maximum load. Flexural strength for the control group was statistically different for experimental groups. All experimental groups showed thicker specimens than the intended 0.75mm, while the control group had a reduction in thickness. Conclusions: 3D printed aligners exhibit flatter stress-strain curves, indicating a better elastic range compared to thermoformed aligners. All resins used for direct 3D printing of aligners increased in thickness after printing.

Keywords: Removable Orthodontic Appliances; Mechanical Stress; Three-Dimensional Printing.

* Artigo escrito conforme as normas bibliográficas da revista *American Journal of Orthodontics and Orthodontics and Dentofacial Orthopedics* para a qual será submetido.

INTRODUCTION

Most patients that seek for orthodontic treatment are looking for improve their aesthetic at the end of the treatment. However, many patients, mainly adolescents and adults, have a higher demand for alternatives that are more aesthetic than conventional brackets. The technologies improvements and the aid of CAD/CAM have made possible for orthodontists to have more treatment options, such as clear aligners. These advancements, however, don't exempt the clinician to do an appropriate diagnostic to provide the best treatment planning.

Currently, different thermoplastic materials and vacuum-based methodology are used to manufacture clear aligners. However, these materials can have an important environmental impact since in many countries they aren't considered as recyclable items and must be burned, which causes damages for the environment[1]. Furthermore, these materials may also have a few more disadvantages and inconveniences such as a long and complex manufacturing process, some of the material properties that may interfere in the orthodontic movement, 3D printing models may produce some inaccuracies, mainly in crowding areas[2–5].

Accordingly, 3D printed clear aligners have been introduced to avoid the problems cited before and to improve the accuracy of the treatments[6,7]. In both methods, problems during the 3D printing may harm the results[7]. However, 3D printed aligners involve less procedures in the manufacturing process and dismiss the thermoforming process, that many times are performed by a human, makes it easier to have a more satisfactory product at the end.

The aligners must be printed in good quality printers and resins to provide dimensionally stable, flexible, transparent, and biocompatible aligners[8–11]. It's important that the material that are used to print the aligners have the right mechanical properties that will give the proper characteristics to provide the best and most predictable orthodontic movements[12]. Lee et al.[13] assessed the thermo-mechanical properties of 3D printed aligners and observed that the yield strength and elastic modulus were higher in PET-G (thermoformed aligner) than in 3D printed aligners (resin Tera Harz) TC-85 , and the elastic range was significantly larger in TC-85 (4.65%) than in PETG (3.92%). In addition, the stress relaxation and creep behavior of TC-85 shows greater flexibility and improved fitting after used.[13]

Nowadays, only Tera Harz TC-85 resin (Graphy) have FDA approval to be used, because it fits the proper features and is not toxic for patients. Since there are still a lack of quantitative and qualitative materials for 3D printing, more options are needed to make it more accessible to produce 3D printed aligners[12]. Accordingly, this study aimed to compare the mechanical properties of thermoformed aligners to different types of resins used for directly printed aligners.

MATERIALS AND METHODS

Preparation of the specimens

The sample was divided in Control Group (Zendura FLX) and Experimental Groups (S1, S2, S3 and TC-85 Groups). (Table 1). Fifteen specimens were prepared for each group.

For the preparation of the test specimens of the Control Group (Zendura FLX), rectangular samples (10 x 40 mm)[14] of each material were cut from the 125-mm disks provided by the respective manufacturer, after thermoforming process. To prepare the test specimens for the Experimental Groups of 3D printing resins (S1, S2, S3 and TC-85), samples with the same dimensions as the Control Group and a thickness of 0.75mm were designed virtually using the MeshMixer® software. After that, they were printed by the MiiCraft 125 Ultra® 3D printer (digital lighting process high resolution 3D printer) and they all went through the same postprocessing method, which included 4 minutes through the Centrifuge and then 5 minutes on each side through the N_2 with ultraviolet (UV) light chamber (CureM, Graphy Inc., Korea).

A digital caliper was used to verify the dimensions and uniformity of each sample at three different points, especially specimens' thickness. The specimens' thickness was measured at: E1 at one end of the specimen, E2 in the middle of the specimen and E3 at the other end of the specimen. This evaluation was carried out to certify the thickness uniformity after the 3D printing of the specimens.

Mechanical Tests

The fifteen specimens from each group were submitted to a three-point flexural strength test, using a universal testing machine (Model DL 2000, microprocessed, brand EMIC, São José dos Pinhais, Brazil) with a load cell of 200kgf and test speed 5mm/minute (Fig. 1). The length of the gap between the supports for the specimens were 16 mm, according to the ANSI/ADA Specification (American National Standards Institute/American Dental. The variables to be evaluated by the three-point flexion tests will be: stress-strain curve, maximum load and flexural strength.

Statistical analysis

Statistical analysis included initially descriptive statistics with mean and standard deviation after appropriate outlier checks. The Shapiro-Wilk test was used to assess normality and the Brown-Forsythe test to assess groups homoscedasticity.

To evaluate the thickness (T1, T2 and T3), maximum load and flexural strength from each group, the data were subjected to one-way ANOVA complemented by Tukey's posttest. All statistical analyses were performed with a significance level of 5% using SPSS software.

RESULTS

Stress-strain curve:

The stress-strain curve is a graphical description of the deformation behavior of a material under uniaxial tensile loading. After submitted to a three-point flexural strength test, stress-strain curve was obtained for each group (Fig. 2).

This graph evaluates stress related to deformation: to what extent the material deformation is elastic (reversible) and when plastic deformation (irreversible) begins. When the stress begins to decrease, after its maximum load peak, this is where plastic deformation begins, and the material has already lost its mechanical properties. Therefore, the higher the curve, the greater the tension, the more resistant the material. However, the stiffer the material, the smaller the elastic deformation and the greater the plastic deformation.

The Zendura FLX specimens all had greater elastic deformation, compared to the other groups, with approximately 6mm of elastic deformation before plastic deformation. In groups TC-85, S1, S2 and S3 the plastic deformation started approximately at 3mm or less (Fig. 2). However, Zendura(FLX) presented a greater tension than the other groups, of 127.70MPa.

Maximum load

The average maximum load for the Zendura FLX group was 12.67N, for the TC-85 group was 6.2N, S2 was 13.33N, S1 was 5.2N and S3 was 9.26N. (Fig. 3). When maximum load was compared between each group, TC-85 was statistically different from S3 and S2, but not from S1. S3 was statistically different from TC-85 and S1; S1 was statistically different from S2 and S3 and S2 was statistically different from S1, S3 and TC-85.

TC-85, S1 and S3 had lower maximum load, which means they are not as resistant and as rigid as Zendura FLX and S2. But when the stress-strain curve of these three groups (Fig. 2) is compared, we can observe that TC-85 and S1 group had greater elastic deformation (the curve is flatter) then S3.

Flexural strength (MPa)

Flexural strength, also known as rupture stress or modulus of rupture (MOR), is the strength/tension of a material as determined by flexural test before rupture. It is calculated by the formula:

$$MOR = (3 * P * L) / (2 * b * d^2)$$

Where, M = Modulus of Rupture, Rupture Stress or Flexural Strength; P = Breaking Load; L = Distance Between Knife Edges on which the Sample is Supported; b = Average Specimen Breadth d = Average Specimen Depth.

The mean flexural strength for the control group (Zendura FLX) was 121.70MPa, for TC-85 group was 16.58MPa, for group S1 was 18.12MPa, for group S2 was 32.75MPa and for group S3 was 26,03MPa. Control group was statistically different for all groups. TC-85, S1, S2 and S3 were not statistically different from each other, but TC-85 and S1 had the lower values (Fig. 4).

Thickness

At T1, all experimental groups were statistically different from each other, except for groups S1 with S3. At T2 and T3, groups S2 with S3 and groups S1 with TC-85 presented no statistically significant difference (Fig. 5). All the means values of experimental groups' thickness tested were at least 14% above the ideal 0.75mm planned thickness. TC-85 was the one with the least difference (14% thicker), while S3 presented approximately 33% thicker, S2 was 37% and S1 was 21.3% thicker. Control group (Zendura FLX), differently from experimental groups, presented medium thickness reduction of 33% from the intended value of 0.75mm, and therefore was statistically different from TC-85, S1, S2 and S3 in T1, T2 and T3.

DISCUSSION

This study aimed to compare the mechanical properties of thermoformed aligners with different types of resins used for 3D printed aligners. However, the comparison between mechanical properties of these materials with previous articles becomes complex due to the lack of standardized protocols for the three-point bending tests[15,16]. Elkholy et al[16] proposed recommendations for test parameter standardization for thermoplastic aligner materials in order to facilitate comparison between studies.

To enhance the aligner design to its best performance, numerical simulations through parametric modeling and finite element analysis have shown that the aligners best performance is dependent on the aligner geometry and auxiliary element configuration[17]. In comparison with the thermoformed aligners, 3D printed aligners with Tera Harz (TC-85) resin have demonstrated better geometric stability at high temperatures without heat shrinkage due to its' viscous nature, in which the strain is gradually recovered, and the stress relaxation is increased[13]. When the newly developed resins were compared in this study (S1, S2 and S3), S1 showed better results among them and its' mechanical properties more similar to TC-85. Resins S1 and S3 were composed of the same monomer, a more viscous one than that used for the composition of S2. The main difference between S1 and the other tested resins was the amount of this viscous monomer, a higher percentage of this monomer was used in its final composition. In agreement with Lee et al[13] regarding the viscous behavior of Tera, S1 also presented a more viscous behavior than the other resins and as this characteristic is positive and favors the shape recovery, S1 shows promising results.

The tests were performed with samples of flat 40mmx10mm length-to-width in all Groups, in accordance with International Organization for Standardization (ISO 527-

3) that recommends specimens with approximately 4 x 1 length-to-width ratio[14]. This sample with flat shape is preferred when it comes to mechanical tests, but also samples from fabricated aligners (curve surface) have been used because it is closer to the clinical situation[18,19]. Shirey et al[18], have found that, despite being similar to the clinical use, the aligner shaped sample, especially in the maxillary central incisor region, were unable to be appropriately secured on the testing machines, resulting in slippage of the specimen and therefore high levels of variance on the results.

The stress-strength curve is a well-known graphical way to show the reaction of a material when a load is applied and its elastic properties. The aligner material must follow the same orthodontic premise as the fixed appliance: to exert light and constant forces. The higher the stress-strength curve, the stiffer and stronger the material. Therefore, the ideal curve for an aligner material is flat, representing better elastic range. Lombardo et al[15] compared the stress-strength curve of four different materials for thermoformed aligner and none of them presented the desired flat curve. In this study, the Zendura FLX (thermoformed aligner material) curve also did not present a flat curve, similar to the S2 and S3 results as well. The only materials that presented the ideal flat curve were TC-85, S1and S2 resins for the 3D printed aligners (Figure 2). Lee at al[13] also compared a thermoformed aligner material (PETG) with 3D printed aligners with TC-85 resin and their stress-strength curve was similar to ours, showing that the stiffness of PETG was significantly higher than the TC-85, but, despite the stress-strain curve design being similar, our curve presented greater discrepancy between thermoformed and 3D printed aligners. This discrepancy occurred mainly because to calculate the tension/flexural strength value (y axis of the graph) the thickness data are considered and in this study the initially idealized thickness for the printed aligners was 0.75mm, different from Lee et al[13] that recommended 0.5mm of thickness for 3D printing of these materials. Thus, compared to the thermoformed aligners, the 3D printed aligners present better ability to exert light and continuous forces over time and due to its larger elastic range more tooth movement can be performed without permanent deformation[13].

Part of the success of orthodontic treatment with aligners is the materials' capacity to maintain their shape and mechanical properties without deforming or fracturing when force is applied. The maximum load, or ultimate strength, is the amount of load the material can sustain without deformation or rupture. Zendura FLX and S2 had the highest maximum loads, and therefore were the most resistant materials, while TC-85, S1 and S3 had lower maximum loads (Fig. 3). The flexural strength is proportional to the maximum load, but in addition to the load, it considers the dimensions of the specimen, such as thickness. TC-85 and S1 results for this variable were also the lowest, with mean values of 15.58MPa and 18.12MPa (Fig. 4), respectively. Zendura FLX presented mean flexural strength of 127.70, which was higher and statistically different from the other groups. Although TC-85 and S1 have lower load, flexural strenght values and are not as resistant as other materials, they have greater elastic deformation shown by the flat stress-strength curve (Fig. 2) and therefore are less likely to deform or fracture.

Shirey at al[18], also observed lower ultimate strength in the 3D printed aligner material, compared to the thermoformed, but this result was attributed to the 99% isopropyl alcohol used to wash aligners after 3D printed. The 3D printed aligners in this study were centrifuged and no alcohol was used in the post-processing, as instructed by the manufacturers. The exposure of 3D printed polymethyl methacrylate (PMMA), which is one of the most commonly used polymers in 3D printing, to isopropyl alcohol can result in a degradation of mechanical properties, especially in the peak stress[20].

One of the biggest advantages of the 3D printed aligner is the possibility of customization. The custom virtual aligner design allows the application of different thicknesses, different attachments and pressure points in any stage of the orthodontic treatment[7]. Despite the advantage of being customizable, thickness is still a challenge for 3D printed aligners, because after printed they are thicker than their intended print design[18]. Edelman et al[9] observed 0.274mm maximum thickness increase in 3D printed aligners, however, the resins used in the study are not suitable for 3D printed aligners since they are neither flexible nor transparent. In this study, the means values of experimental groups' thickness were at least 0.105mm above the ideal 0.75mm planned thickness. The increase in thickness for these materials ranged from 0.105mm (TC-85 resin) to 0.277mm (S2 resin). A recent study[18] showed similar results with other two experimental resins for 3D printed aligners, the materials measured 0.075mm and 0.18 mm thicker, but there was no comparison to the TC-85 resin, which is the FDA approved and scientifically validated material to date for direct 3D printing of aligners. This same study[18] measured four minimum thickness on the facial aspect of each tooth. Similarly, our study also measured the thickness at three different points, as previously reported[13], mainly because it was a specimen and not an aligner and there was a concern about distortion at the edges.

The 3D printing workflow and postprocessing failures are the probable sources for the thickness deviations in 3D printed aligners[9]. In this study, after the specimens were printed (DLP 3D printer) the postprocessing involved removal of the residual liquid resin of the specimen's surface through a centrifuge, as recommended by Tera Harz (TC-85) (Graphy Inc, Korea) technical data sheet, followed by the post-curing N_2 with UV light chamber (CureM, Graphy Inc., Korea). Any residual resin that remains is polymerized during the post-curing, which could contribute to a thicker specimen[13]. The DLP 3D printer's speed and accuracy is superior to the SLA[21], but as the DLP cures the liquid resin in layers using a high-definition projector as a light source, the resin can be polymerized thicker than the programmed layer thickness. In this case, using the SLA printer could reduce this overbuilding[13], as it prints each part with a laser source to selectively cure the liquid resin, and not an entire layer as the DLP. Despite that, SLA takes a longer time to print the aligner, and therefore the DLP is still more popular in clinical practice. Once the average of overbuilding in 3D printed aligners is known for each resin, offset setting and printing calibration can be performed in advance, to obtain more accurate thickness.

In contrast with the 3D printed aligners, that allow different designs and thickness, the thermoformed ones are fabricated depending on the thickness of the available polymers in the market and cannot be altered. It is possible to choose different thickness for different aligners in the same treatment, but different thickness in the same aligner is a perk only for the 3D printed aligner. Koenig et al[10] reported that the thermoforming process generated aligners 0.125mm up to 0.30mm thinner than the original thermoforming sheet thickness. Min et al[22] observed even greater thickness deviations after thermoforming, with a decrease range of 37.9% to 48.3% of the initial thickness. Samples from Invisalign (Align Technology Inc, San Jose, Calif) aligners were tested in previous studies[18,23] and minimum thickness ranged from 0.42mm-0.50mm, especially in the canine and molar areas. These findings are in agreement with the decreased thickness in Control Group (Zendura FLX), with minimum range of 0.48-0.54mm after the thermoforming process.

3D printed aligners are the present and the future, but like all new technology, it needs research with existing materials and with new materials being developed, for validation. Standardization of mechanical tests parameters and specimens are needed to allow scientific comparison[16]. One limitation of this study is that the mechanical tests were performed in dry conditions at room temperature, and not oral environment-like conditions. Thermoplastic aligner materials, 3D printed or thermoformed, are susceptible to temperature-related alterations, especially above 37°C[13,15,24], and 3D printed aligners can recover its' original printed shape at 37°C[13]. In addition, studies focused on the cytotoxicity of 3D printed aligners and the interactions of 3D printed aligners with oral microorganisms are encouraged, to increasingly validate the safety of these new 3D printing materials,

CONCLUSIONS

- The mechanical properties of thermoformed aligners are different from printed aligners. 3D printed aligners have flatter stress-strain curves, and therefore, a better elastic range than thermoformed aligners.
- It is important to consider material thickness when calculating mechanical properties. TC-85, S1, S2 and S3 resins have at least 75% less flexural strength/rupture stress than Zendura FLX.
- Zendura FLX has the smallest thickness among all groups, as its thickness decreases after thermoforming. All resins for direct 3D printing of aligners increased in thickness after printing. TC-85 and S1 had less increase in thickness.
- Tc-85 and S1 were very similar in all mechanical properties tested.
- Future comparison studies between thermoformed and 3D printed aligners are encouraged, especially considering the thickness changes of each material in order to better compare them.

FIGURES AND TABLES

Table 1: Sample characterization.

	Product	Thickness	Manufacturer	Material
Control Group	Zendura FLX	0.76mm	Zendura	Thermoplastic polyurethane and polyester
Experimental Groups	TC-85 resin	0.75mm	It Graphy	Photo-polymerizable polyurethane based
	S1 resin	0.75mm	Smart Dent	Photo-polymerizable polyurethane based
	S2 resin	0.75mm	Smart Dent	Photo-polymerizable polyurethane based
	S3 resin	0.75mm	Smart Dent	Photo-polymerizable polyurethane based

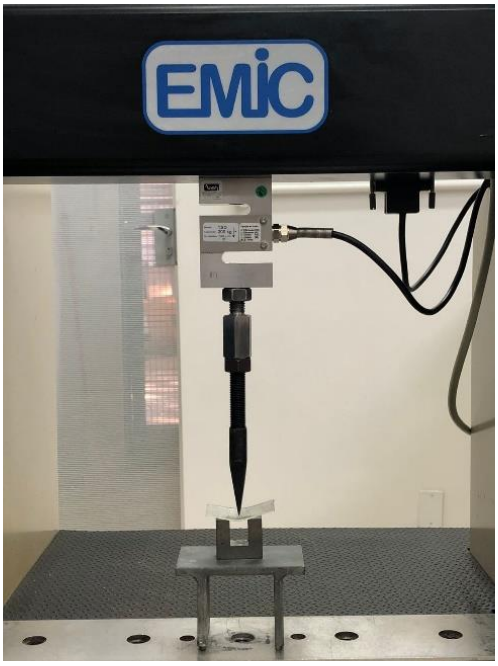

Figure 1: Three-point flexural strength test using universal testing machine (EMIC) with specimen.

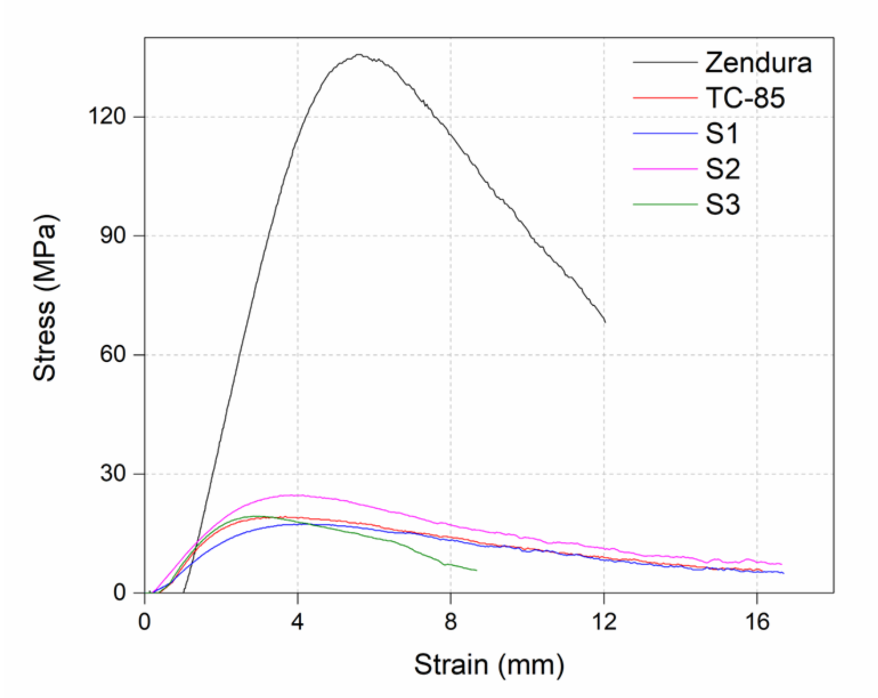

Figure 2: Stress-strain curve for each specimen of each group.

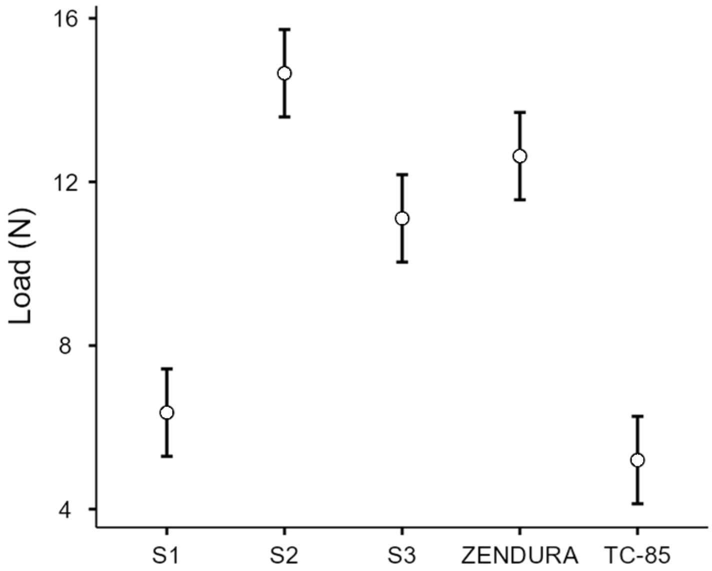

Figure 3: Comparison values of the maximum load between the groups, before starting the plastic deformation.

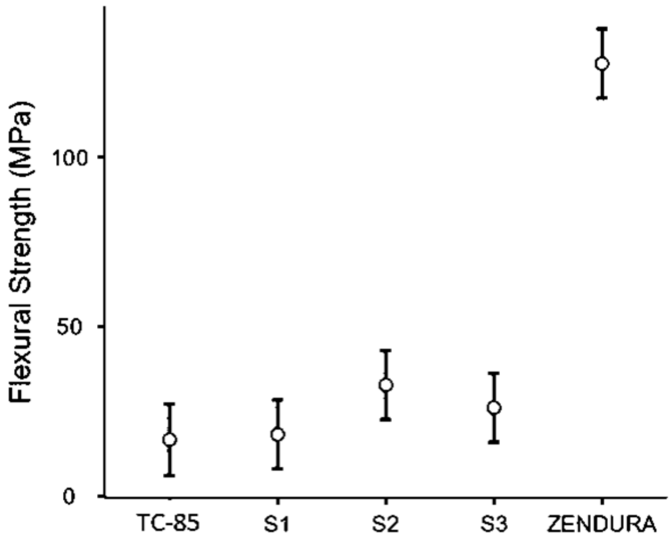

Figure 4: Comparison values of the flexural strength between the groups.

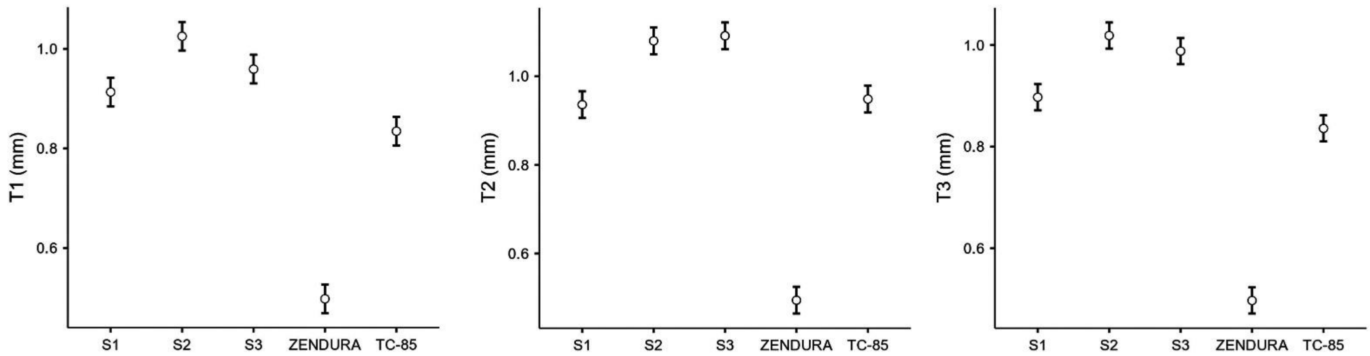

Figure 5: Thickness values (mm) measured in 3 points in each specimen. A) T1 at one end of the specimen, B) T2 in the middle of the specimen and C) T3 at the other end of the specimen.

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3.3 Artigo 3[*]

Biofilm formation and cytotoxicity of different resins for 3D printed aligners

ABSTRACT

Introduction: 3D printed aligners are a newly developed technology to overcome the current the disadvantages in the production of thermoformed aligners. However, suitable materials are not widely available. Cytotoxicity and biocompatibility of 3D printable resins are important for patient safety since aligners are worn for extended periods in the oral environment, making them susceptible to bacterial adhesion and biofilm formation. Establishing acceptable levels of cytotoxicity and clinical tolerance for 3D printed clear aligners is necessary. The aim of this study was to assess the adhesion and biofilm formation of *Candida Albicans (C. albicans)* and *Streptococcus mutans (S. mutans)* and analyze cytotoxicity in four different 3D printing resins. Methods: Four different newly developed 3D printing clear aligner resins (S1, S2, S3 and TC-85) were evaluated comparing with Zendura FLX (Control group). For the biological properties, the colony-forming unit and Alamar Blue assays were performed in 2, 7 and 14 days after specimen were 3D printed. The data were submitted to 2-way ANOVA (biological properties) and Tukey's post-test with a significance level of 5% (α= 0.05). For the cytotoxicity assay, the Kruskal-Wallis test followed by Dunn post-test were carried out (α = 0.05). Results: 3D printing resins (S1, S2, S3 and TC-85) showed lower biofilm formation and cell metabolism compared to the Control Group after 2 and 7 days. The cytotoxicity analysis showed that most groups had acceptable cell viability, but the TC-85 group had a higher variability in results. Conclusions: 3D printed aligners show less biofilm formation with *C. albicans* and *S. mutans.* The time between the 3D printing of the aligner and its use is important and increases the mixed biofilm adhesion. All groups showed similar cytotoxicity, within acceptable clinical limits.

Keywords: Removable Orthodontic Appliances; Biofilm; Cytotoxicity; Three-Dimensional Printing.

[*] Artigo escrito conforme as normas bibliográficas da revista *American Journal of Orthodontics and Orthodontics and Dentofacial Orthopedics* para a qual será submetido.

INTRODUCTION

Clear aligners have become increasingly popular in Orthodontics in recent years due to their advantages in aesthetics, comfort, and the convenience of removal for dental hygiene and eating. Additionally, clinicians benefit from faster appointments and more precise digital planning purposes. However, the production of aligners requires a significant amount of raw materials, including resin for printing the models and indirect printing of the aligners[1]. Acrylic resins are commonly used for orthodontic model production for clear aligners[2], but the potential side effects of these materials on patients and personnel should be considered[1], since residual resin monomer has the potential to be toxic if transferred with the appliance[1,3]. The current workflow has the disadvantage of high production costs and environmental issues, as plastic, a non-biodegradable material, decomposes slowly[4].

A possible solution of this dilemma probably involves direct 3D printing of the aligners. This approach would eliminate the need for model printing and thermoforming steps in the current workflow, resulting in a significant increase in efficiency and reduction in the environmental impact of clear aligner production[1]. Nevertheless, the primary obstacle to the direct printing of aligners is the current global unavailability of a material that is suitable for the application. So far, the only clear aligner resin with Food and Drugs Administration (FDA), Korean's Food and Drugs Administration (KFDA) and Conformite Europeenne (CE) approval is the Tera Harz TC-85 (Graphy, Seoul, South Korea), but there are still many places where it is not FDA approved and there are few studies with this new material. For direct printing of clear aligners, the resin must fulfill several design requirements, such as compatibility with 3D printing, aesthetic appeal, durability, stability, biocompatibility, cost-effectiveness, and appropriate mechanical properties. Although the number of 3D printable resins cleared for intraoral use on the market continues to be developed and studied[5], scientific information is still limited because clinical studies haven't yet been conducted, especially for clear aligners.

Attention should be given to the cytotoxicity of printable resins[6], including the ones for orthodontic aligners as they are a daily and sometimes long-term use appliance. A recent study found no difference between the viability of human gingival fibroblasts of 3D-printed-aligners' and its negative controls[7]. Estrogenic activity eluted by 3D-printed aligners was also not observed[7]. The cytotoxicity of thermoplastic materials used commonly for clear aligners on human gingival fibroblasts may have slight cytotoxic effects, especially after thermoforming, but this amount of cytotoxicity was not considered clinically insignificant[8].

Considering that orthodontic aligners should remain in the intraoral environment for at least 22 hours a day and is replaced by a new one every 10 to 15 days, intraoral appliances present potential target for bacterial adhesion and biofilm formation by bacteria[9] (such as *Streptococcus mutans*) and yeasts like *Candida* inhabiting the oral cavity[10]. The accumulation of thick layers of plaque on oral appliances can potentially cause gum irritation and increase the risk of developing oral diseases. In addition, most preventive oral health care protocol should be developed for clear aligners[11], 3D printed or thermoformed.

Furthermore, there is a need to establish the acceptable level of cytotoxicity and clinical tolerance for 3D printed clear aligners. The direct production of clear aligners through 3D printing is currently a challenging task, but significant advancements in 3D printing and other technologies have been made. The current study aims to evaluate the adhesion and biofilm formation of *Candida Albicans* and *Streptococcus mutans* and to analyze the cytotoxicity in four different newly developed 3D printing clear aligner resins, including Tera Harz TC-85 (Graphy, Seoul, South Korea), and comparing with the current thermoforming method with Zendura FLX®.

MATERIALS AND METHODS

Preparation of the specimens

A total of 243 discshaped samples (10 mm × 0.75 mm) of each material were obtained (n=1.215). The Zendura® FLX (thermoplastic polyurethane) samples were obtained through the thermoforming process and the S1, S2, S3 and TC-85 samples were obtained through 3D printing with the Miicraft 125 Ultra printer (Miicraft, Kempten, Germany), a digital lighting process high resolution 3D printer. After printed, the samples were removed from the printer's platform and positioned in a centrifuge machine for 4 minutes. After that, the samples were positioned in the Cure M curing machine (Graphy, Seoul, South Korea) and cured for 5 minutes in both sides.

Biofilm formation capacity

The biofilm was formed on the specimens and the biofilm formation's capacity was evaluated by the cell proliferation analysis (colony-forming unit assay) and cell viability analysis (Alamar Blue assay).

Colony-forming unit assay

The cells of *Candida albicans* (ATCC 5043) and *Streptococcus mutans* (UA 159 ATCC 700610) were reactivated individually in Petri dishes containing culture medium Sabouraud dextrose agar (SDA) and incubated at 37ºC for 48 hours for *C. Albicans* and and Brain Heart Infusion (BHI) Agar for *S. mutans* (incubation in a 5% CO2 oven at 37ºC during the same period of time). Then, five to seven *C. Albicans* colonies were inoculated in 10 mL of yeast nitrogen base (YNB) and ten to twelve *S. mutans* colonies were inoculated in 10mL of Brain Heart Infusion (BHI) broth supplemented with 1% glucose. *C.albicans* grown aerobically for 16 hours and *S. mutans* grown in a 5% CO2 oven at 37ºC for 18 hours.

After that, 0.5 mL of the starter cultures was transferred individually to 9.5 mL of fresh YNB, grown aerobically (*C. albicans*) for 8 hours and in a 5% CO^2 oven (*S. mutans*) at 37ºC for 4 hours to obtain the inoculum. The optical density measurement was made with 540nm filter for the fungus and 600 nm for the bacteria to confirm the concentration of 10^6 (*C. albicans*) and 10^8 (*S. mutans)* colony-forming unit per milliliter (CFU/mL). Each culture was harvested after centrifugation at 10,000 rpm for 5 minutes and washed twice with Phosphate-Buffered Saline (PBS) and re-suspended in Roswell Park Memorial Institute (RPMI)[12–14].

Before the biofilm formation, all specimens were placed under UV light for 20 minutes on each side for surface disinfection. The sample discs were placed individually in a 24-well plate containing 1500 µL RPMI medium with *C. albicans*, 1500 µL RPMI medium with *S. mutans* and 1500 µL RPMI medium with both microorganisms for each biofilm formation. Then, they were incubated for 90 minutes at 37ºC under agitation at 76 rpm for the cell adhesion. After that time, the samples were washed twice with PBS to remove unbound cells. The specimens were transferred to a new plate with fresh RPMI and incubated for 48 hours for the formation of mature biofilm (with the RPMI medium being refreshed after 24 hours). [12–14].

After the incubation period, each well was washed twice with PBS to remove the unbound cells, then 150 µL of PBS were added and the specimens were scraped with a pipette tip for 1 minute to peel off the formed biofilm. Thereafter, serial dilutions of 10^{-1} to 10^{-4} were obtained. Twenty-five microliters of the 10^{-3} dilution and the 10^{-4} dilution were plated on Petri dishes containing the SDA culture medium with 5% chloramphenicol, and the plates were incubated at 37ºC for 48 hours[12–14].

Next, the number of CFU/mL was calculated. These procedures were made in triplicate.

Alamar Blue assay

After the formation phase, the biofilm on the samples (n=9) was washed twice with PBS, placed in a 24-well plate containing 1,500 µL of RPMI and 150 µL of Alamar Blue solution and incubated under agitation at 37ºC and 76 rpm for 4 hours. Subsequently, the fluorescence reading was performed using Fluoroskan Ascentg at 560 nm (A560) and 590 nm (A590).[24,27] The assay was performed three times[14].

All these procedures were made after 2, 7 and 14 days the materials were 3D printed, to assess if there is any difference in biofilm formation depending on when the material was printed.

Cytotoxicity assay

The cytotoxicity effect of the different materials was assessed by the cell culture method. Normal oral keratinocytes (NOK) cells were used. The cells were propagated in the Dulbecco's Modified Eagle's Medium (DMEM) with 7.5% fetal bovine serum and 80 µg/mL gentamicin. The culture was maintained at 37°C in an atmosphere of 5% CO2 and 95% air. Next, 10 µL of the cells (1×10^5 cell/mL) were added to 90 µL Trypan Blue. Of this solution, 10 µL were removed and introduced into a Neubauer hemocytometer counting chamber, in which the viable cells were counted with an optic microscope (40x magnification).

After that, the cells (1×10^4 cell/mL) were seeded into 96-well culture plates and incubated for 24 hours at 37°C in the same atmosphere. Meanwhile, the samples of the aligners were incubated with DMEN medium for 24 hours. After this period, the culture medium was removed and 100 µL of culture medium with the extract of every aligner were added in each well. Subsequently, 10 µL of the Alamar Blue® solution were also added. After 4 hours, the fluorescence reading was performed. The fluorescence of the samples was measured using Fluoroskan Ascent at 560 nm (A560) and 590 nm (A590). The cytotoxicity assay was performed in triplicate on 3 different occasions[15].

Statistical analysis

Statistical analysis included initially descriptive statistics with mean and standard deviation after appropriate outlier checks. The Shapiro-Wilk test was used to assess normality and the F test to assess groups homoscedasticity.

For the evaluation of biological properties, two-way ANOVA and Tukey's multiple comparison test were performed. For the cytotoxicity assay, the Kruskal-Wallis test followed by Dunn post-test were carried out ($\alpha = 0.05$).

All statistical analyses were performed with a significance level of 5% using SPSS software.

RESULTS

Colony-forming Unit assay

Considering the simple biofilm of *C. albicans*, it was observed that for the Control Group (Zendura FLX), there was no statistically significant difference in the microorganism formation in 2, 7 and 14 days. *C. albicans* formation capacity was significantly lower in S1, S2, S3 and TC-85 groups compared to the Control Group (Zendura FLX) in 2 and 7 days after 3D printed. After 14 days of being 3D printed, groups S2, S3 and TC-85 showed similar results to Control Group (in 2, 7 and 14 days) and S1 was only similar with Control Group of 14 days (Fig. 1).

Regarding the simple biofilm of *S. mutans,* it was also observed that for the Control Group (Zendura FLX), there was no statistically significant difference in the microorganism formation in 2, 7 and 14 days. *S. mutans* formation was significantly lower in S1, S2, S3 and TC-85 groups compared to the Control Group (Zendura FLX) in 2 and 7 days after 3D printed. After 14 days, S2 group had similar values of *S. mutans* biofilm formation to the Control Group and S1. On the other hand, S3 and TC-85 groups had significantly higher values of the microorganism formation when compared to the Control Group (Fig. 2).

For the mixed biofilm of *C. albicans*, all groups, except S1, had no statistically significant difference in 2, 7 and 14 days. S1 results for 2 and 7 days were similar, the 14 days' result was statistically higher. S2 14 days' results were statistically lower compared to Control Group. S3 group had statistically significant higher values of mixed *C. albicans* formation compared to S2 group in 2, 7 and 14 days and to TC-85 group in 2 and 7 days (Fig. 3).

For the mixed biofilm of *S. mutans*, Control Group's 14 days result was statistically lower than 2 and 7 days result, which is the opposite of the 14 days result of all the other groups, that were statistically higher. Groups S2, S1 and TC-85 14 days result are similar to the Control Group's 2 and 7 days results. Mixed *S. mutans* formation was significantly lower in S1, S2, S3 and TC-85 groups compared to the

Control Group (Zendura FLX) in 2 and 7 days after 3D printed. Groups S1, S2 and S3 had similar results in 2 and 7 days, while TC-85 group presented statistically higher values of mixed *S. mutans* formation (but still lower than the Control Group) (Fig. 4).

Alarmar blue assay

When analyzing the cell metabolism of the single and mixed biofilms of *C. albicans* and *S. mutans*, a statistically significant difference (P<0.0001) in the absorbance values of cellular metabolism was observed between the S1, S2, S3, and TC-85 groups and the control group. The control group showed the highest values of cellular metabolism, indicating the highest amount of living cells in all biofilms during the 2-day analysis (Fig. 5).

For the 7-day analysis (Fig. 6), a statistically significant difference (P<0.0001) in the absorbance values of cellular metabolism was observed between the S1, S2, S3, and TC-85 groups, and the control group for biofilm formation capacity of single and mixed *C. albicans* and *S. mutans* biofilms. However, the mixed biofilm of the TC-85 group showed values similar to the mixed biofilm of the control group. The S1 group presented results similar to TC-85 for all tested biofilms. Similar to the 2-day group, the control group after 7 days exhibited the highest values of cellular metabolism. Among all groups, the S3 group had the lowest values for biofilm formation capacity, and S2 showed the highest discrepancy between *C. albicans*, with a lower value, and *S. mutans* and Mixed biofilm, which exhibited the highest values among all 3D printing resin groups.

Observing the 14-day analysis (Fig. 7), there was no statistically significant difference (P>0.05) in the absorbance values of cellular metabolism between S1, S2, S3, TC-85 and the *C*ontrol group considering the single *S. mutans* biofilm. For all groups, the mixed biofilm presented statistically significant higher values of cell viability, and TC-85 Group had the highest values among all groups. Compared to the 7-day analysis, the 14-day presented closer values for all groups, without major discrepancies between them.

Cytotoxicity assay

Considering the cell viability in fluorescence values there was no observed statistical difference between the groups and the live cell control. S2 and S3 groups were statistically different from each other (Fig. 8). When the results were converted into percentages, Control, S1 and S2 groups were statistically different from the live cell control group and S3 and TC-85 groups showed no statistically significant difference (Fig. 9). Although no outliers were observed, the standard deviation (SD) of the TC-85 group was greater than the SD of the other groups (89.61%) (Fig. 9).

DISCUSSION

Treatment with orthodontic aligners involves changing the sequence of aligners, which are in contact with the oral microbiota for a long period of time. There are treatments with orthodontic aligners that last more than a year and although the recommended replacement is in 15 days, sometimes this period can be increased due to loss of tracking or improper use by the patient and therefore the aligner remains in the oral cavity for a longer period of time. With the development of 3D printed aligner technology, all its properties, including aligner interaction with the oral microbiota, need to be tested to assess its comparability to the thermoformed aligner that has been safely used for several years. It is known that appliances placed in the oral cavity increase the number of microorganisms species, and cause substantial alterations in the oral microbiome[16]. To the moment, there is no scientific evidence assessing biofilm adhesion in 3D printed aligners.

Biofilms are highly organized microbial communities, adhered to a biotic surface (oral mucosa) or abiotic surface (such as of orthodontic aligners, prostheses, occlusal splints) and protected by an extracellular polymeric matrix[17]. Initially the biome of the biofilm consists of mostly gram-positive cocci bacteria, such as *Streptococcus mutans*, which is cariogenic[18]. The main components of biofilms formed in long-term structures in the oral environment, such as dentures, are bacteria, with a high prevalence of *S. mutans*, while the yeasts occupy another part of the flora total microbial activity[19], highlighting *Candida* species. *Candida* has an ability to adhere in resinous materials in the oral environment[10]. One factor that increases the virulence of *C. albicans* is the interaction with oral microorganisms such as *S. mutans*[20]. As 3D printed aligners are also resinous materials, the evaluation of the adhesion of these microorganisms and mixed biofilm, as this study conducted, is pertinent.

C. albicans and *S. mutans* have a symbiotic relationship[21], which supports the importance of assessing the mixed biofilm, with both microorganisms, as reported in this study. This relationship allows the microorganisms to produce an enhanced biofilm, in vitro and in vivo. Infection by both *C. albicans* and *S. mutans* showed higher levels of infection and microbial carriage in plaque biofilms, compared to infections with either species alone. Previous studies found that this coinfection increases the virulence of the biofilm, which in more extreme cases can lead to the development of rampant caries[21,22].

Regarding the single *C. albicans* biofilm formation, results showed significantly greater adherence in the control group (Zendura FLX) than in the 3D printing resins groups (Fig. 1) for the 2 and 7-day evaluation. After 14 days of being 3D printed, all groups presented similar results to Control Group, of approximately 7.5 $\log^{10}$ of microorganism growth, and S1 had the highest value. For the mixed *C. albicans* biofilm, the results were also compatible between the groups, except for S1 that was 0.5 $\log^{10}$ increased (Fig. 3) and S3 that presented slightly higher values than S2 and Tera in the 2 and 7-day analysis. Bonafé et al[23] recently observed a biofilm growth of 9.7 $\log^{10}$ for *C. albicans* in Invisalign trays, which was approximately 2 logs higher than the amount of *C. albicans* observed for the Control Group in this study and nearly 3 logs higher in all 3D printing resins groups for the 2 and 7-day assessment. Overall, *C. albicans* adhesion appears to be greater with thermoformed aligners. Although the difference is not substantial with the 3D printed aligner group, the lower amount of *C. albicans* formation is an advantage for the new material.

S. mutans is the main pathogen responsible for dental caries when an imbalance in the microbiota occurs[24]. The amount of *S. mutans* biofilm formation on the samples observed in this study for all groups was very similar to the one reported before for thermoformed aligners, around 8 $\log^{10}$ of microorganism growth[23]. For the control group (Zendura FLX) at 2, 7 and 14-day analysis the results of single *S. mutans* biofilm formation was close, with no statistical differences between them. As reported for *C. albicans, S. mutans* showed significantly greater adherence in the control group (Zendura FLX) than in the 3D printing resins groups (Fig. 2) for the 2 and 7-day evaluation. In the 14-day analysis, the adherence values of *S. mutans* in the 3D printing resins groups were higher than the control group, differently from *C. albicans*, except for the S2 group. The results for mixed *S. mutans* cells (Fig. 4) were comparable to the single *S. mutans* cells, but in this evaluation, TC-85 group presented more biofilm formation then all other 3D printing resins for the 2 and 7-days analysis, despite that, was still below Control Group values.

The metabolic activity of a biofilm is related to its pathogenicity, as it represents cellular growth, reproduction of microorganisms and production of toxic substances[15,25]. For the 2 and 7-day evaluation in this study, control group presented increased cellular metabolism, compared to the 3D printing resins groups (Fig. 5 and

6). TC-85 and S1 groups results showed a statistically significant higher cellular metabolism for the mixed biofilm, with values close to control group. S3 group had the lowest values for single and mixed *C. albicans* and *S. mutans* biofilms among all groups on the 7-day analysis (Fig. 6). In the 14-day cell viability analysis (Fig. 7), *S. mutans* results were equal in all groups, while TC-85 group presented the highest values for mixed and single *C. albicans* biofilms. Cellular metabolism of mixed biofilm was increased for all groups, compared to single *C. albican*s and *S. mutans* biofilms.

The different results among S1, S2 and S3 groups are mostly because they are composed of different monomers in specific concentrations for each group. Resins S1 and S3 were composed of the same monomer, a more viscous one than that used for the composition of S2. The main difference between S1 and the other tested resins was the amount of this viscous monomer, a higher percentage of this monomer was used in its final composition. TC-85 also present a viscous behavior, which is great for mechanical properties[26], but there is a possibility that this feature contributed to the higher values presented for mixed and single *C. albicans* biofilm metabolism.

Despite differences in material composition, another factor that can affect biofilm formation on the surface of aligners is roughness. Although clear aligners present an apparently smooth surface, they are susceptible to cracks and microfractures with intraoral use, which may favor sites for microorganism proliferation[23,27]. The evaluation 2, 7 and 14 days after the 3D printing the sample materials was carried out to investigate alterations in the biological properties of the 3D printed aligners over time. A limitation of this study is that the roughness of the materials was not evaluated. The higher mixed biofilm values for the 3D printing resins groups in the 14-day evaluation may be related to changes in the surface of the material over time, such as roughness. As resins for 3D printing are photosensitive materials, improper storage, susceptible to light, humidity or high temperatures can alter these materials' surface and consequently their biological and mechanical properties. Control group (Zendura FLX) presented stable results through the 2, 7 and 14-day analysis because thermoformed aligners are not light sensitive materials or need specific storage conditions, differently from the 3D printed aligners. Currently, aligner companies ship all the treatment aligners at once to the orthodontist, mostly in sealed transparent packages. For 3D printed aligners to have a similar workflow, it is necessary to establish shipping and storage conditions for these aligners, such as anti-UV packaging, for example, so that their properties are not damaged on the way.

There is very few scientific evidence regarding the biocompatibility and safety of 3D printed aligners. Pratsinis et al[7] recently was the first to investigate the cytotoxicity of 3D printed aligners, in this case were used only aligners printed with TC-85 resin (Graphy, Seoul, South Korea) and the comparison was made in an environment with and without the 3D printed aligner incubated (negative control). In this study, TC-85 resin was included but also new resins for 3D printed aligners were tested (S1, S2 and S3), and Zendura FLX (TPU material) for thermoformed aligners was used as a control. Since Zendura FLX it is one of the validated and standard materials used to fabricate thermoformed aligners nowadays, it is important to compare its or other thermoforming aligner material's biocompatibility with these new 3D printing resins for orthodontic aligners.

Results showed that all the tested materials were within the acceptable range of cytotoxicity for normal oral keratinocytes (NOK) cells. These results are comparable and concordant with a previous study that also concluded that 3D printed aligners are biocompatible for human gingival fibroblast[7]. Although previous reseaches[3,7,28] show positive results regarding cytotoxicity of 3D printed aligners, including this study, they don't take into account factors such as salivary enzymatic reactions, aligner surface degradation, chewing forces, dietary chemical effects and thermal changes[28].

In addition to assessing cytotoxicity through cell viability, studies on Bisphenol-A (BPA) release from orthodontic aligners are growing, as this compound is an endocrine disruptor[3,29,30]. BPA release from for thermoformed aligners can neither be confirmed nor denied[30]. For 3D printed aligners, Willi et al.[3] measured the amount of UDMA (Urethane-dimethacrylate) and BPA that leached from 3D printed aligners with TC-85 resin. No leaching of BPA was detected but varying amounts of UDMA monomer were released and may represent a health risk that require further investigation. UDMA toxic effects are concentration-dependent and can induce cytotoxicity in human pulp dental cells[31], induce toxic effects and apoptosis of macrophages[32], among other effects. In order to reduce the cytotoxicity and allergic potential of 3D printing resins, methods to increase their biocompatibility, such as association with natural polymers, are being explored[28,33,34].

One of the limitations of this study is that it is *in vitro*, and therefore cannot reproduce the entire oral environment that the aligner is subjected during treatment, such as salivary reactions, enzymatic effects, presence of attachments, temperature changes. *In vivo* and clinical studies are encouraged to overcome this limitation. 3D printed aligners proved that they are biocompatible *in vitro*, but its overall safety remains uncertain until high-quality in vivo trials corroborate with these results. Future studies on the roughness of 3D printed aligners and efficient disinfection methods are important to continue the investigation on biofilm formation.

CONCLUSIONS

- 3D printed aligners presented lower formation of *Candida Albicans* and *Streptococcus mutans* then the control group (Zendura FLX).
- After 14 days of being printed, all experimental groups showed increased biofilm formation for single and mixed *S. mutans.*
- Control group had the highest values of cellular metabolism the single and mixed biofilms of *C. albicans* and *S. mutans,* when compared to the other groups.
- For all groups, the mixed biofilm presented statistically significant higher values of cell viability.
- All groups showed similar cytotoxicity, within acceptable clinical limits.

FIGURES

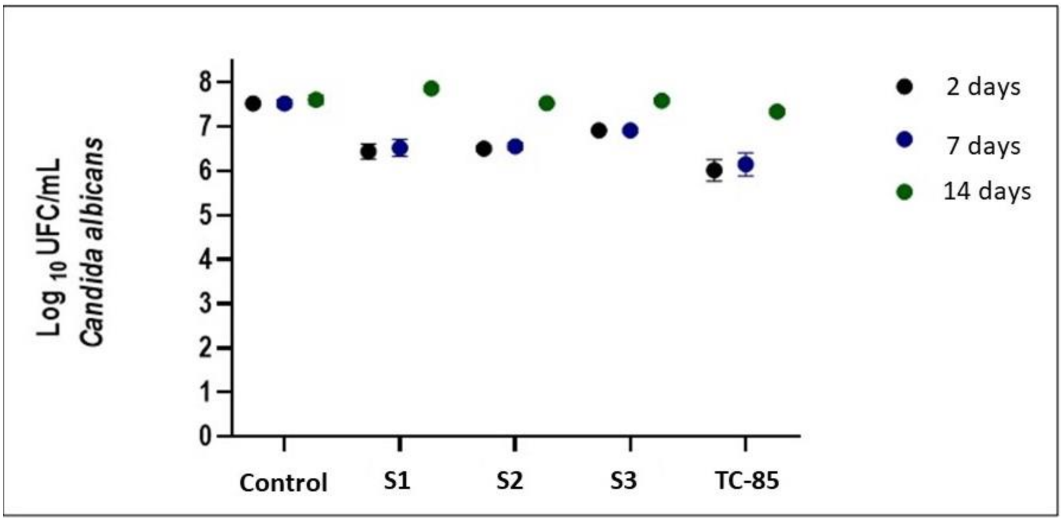

Figure 1: CFU/mL values of *C. albicans* cells referring to biofilm formation in each material 2, 7 and 14 days after the specimens were 3D printed.

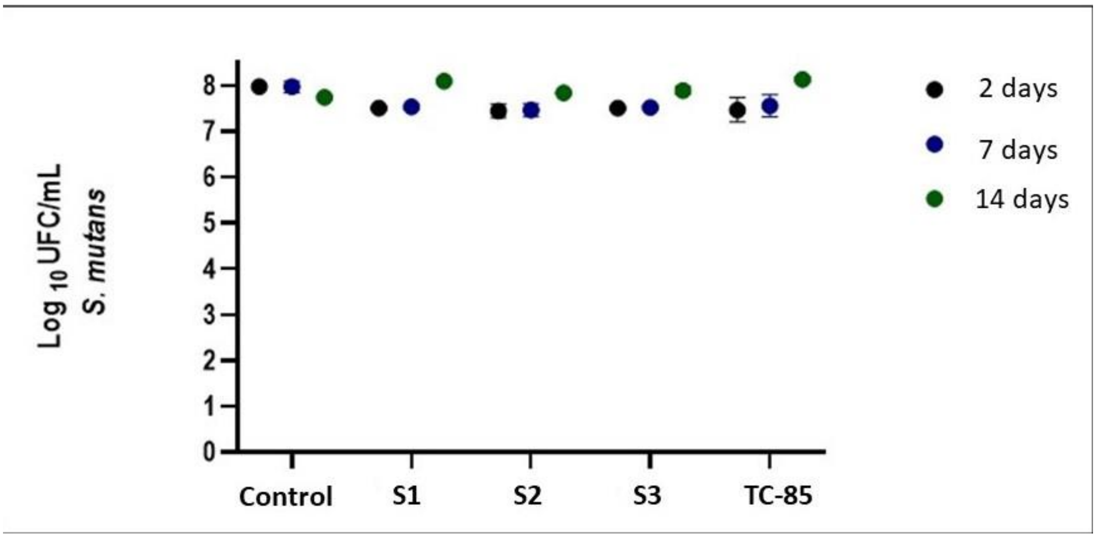

Figure 2: CFU/mL values of *S. mutans* cells referring to biofilm formation in each material 2, 7 and 14 days after the specimens were 3D printed.

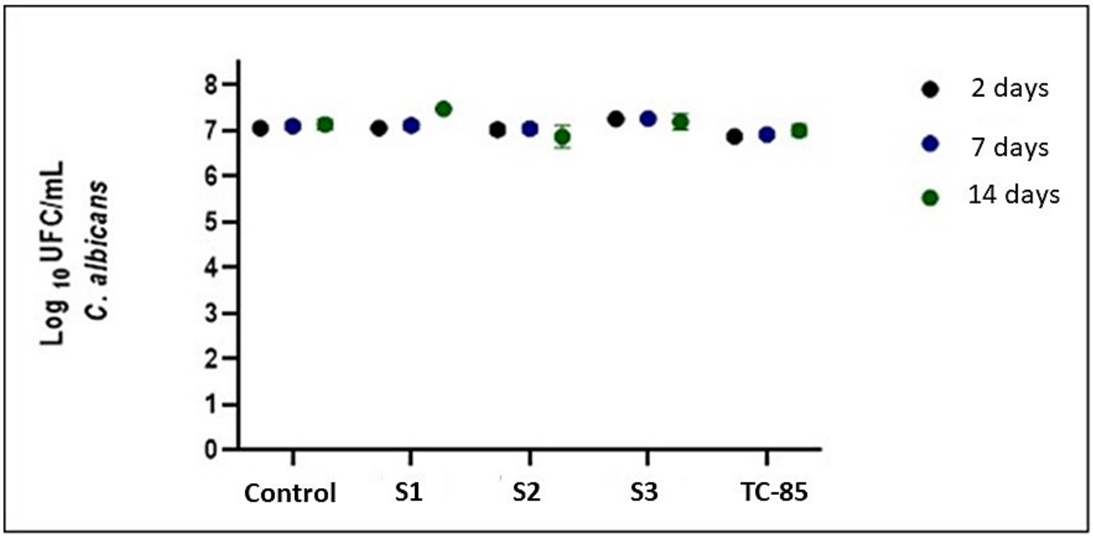

Figure 3: CFU/mL values of mixed *C. albicans* cells referring to biofilm formation in each material 2, 7 and 14 days after the specimens were 3D printed.

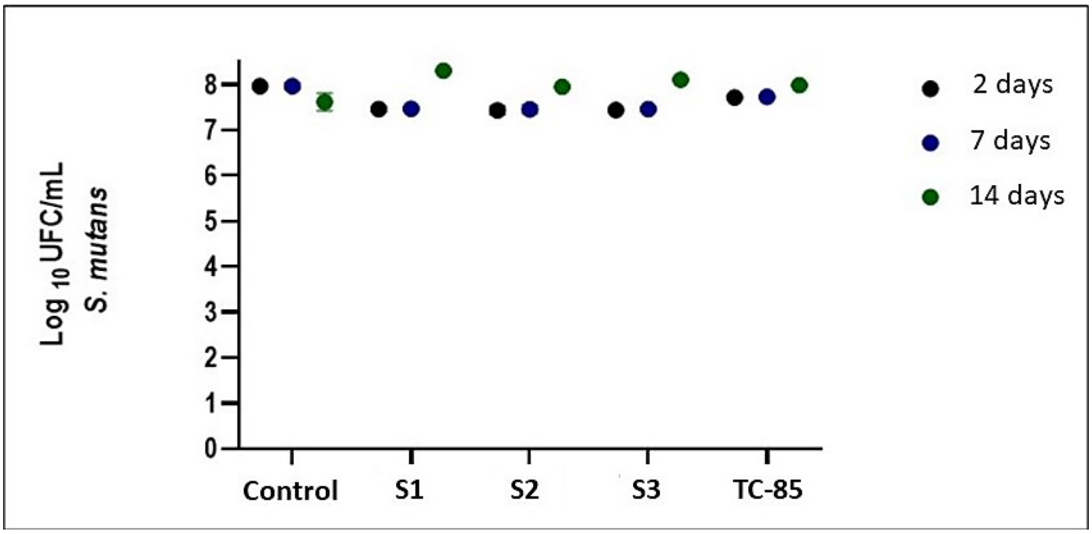

Figure 4: CFU/mL values of mixed *S. mutans* cells referring to biofilm formation in each material 2, 7 and 14 days after the specimens were 3D printed.

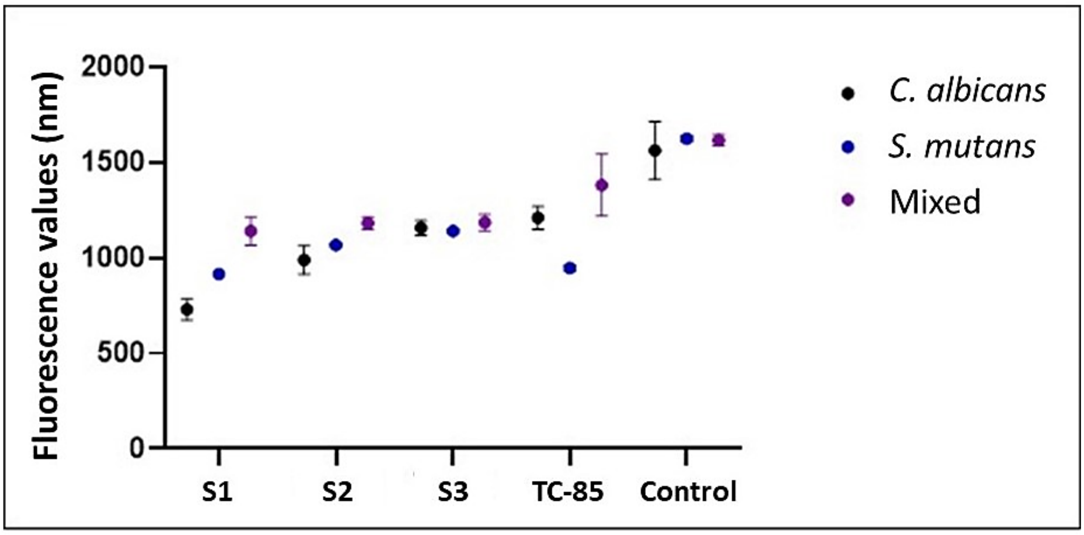

Figure 5: Fluorescence values of *C. albicans, S. mutans, C. albicans and S. mutans (mixed)* cells referring to biofilm formation 2 days after the specimens were 3D printed.

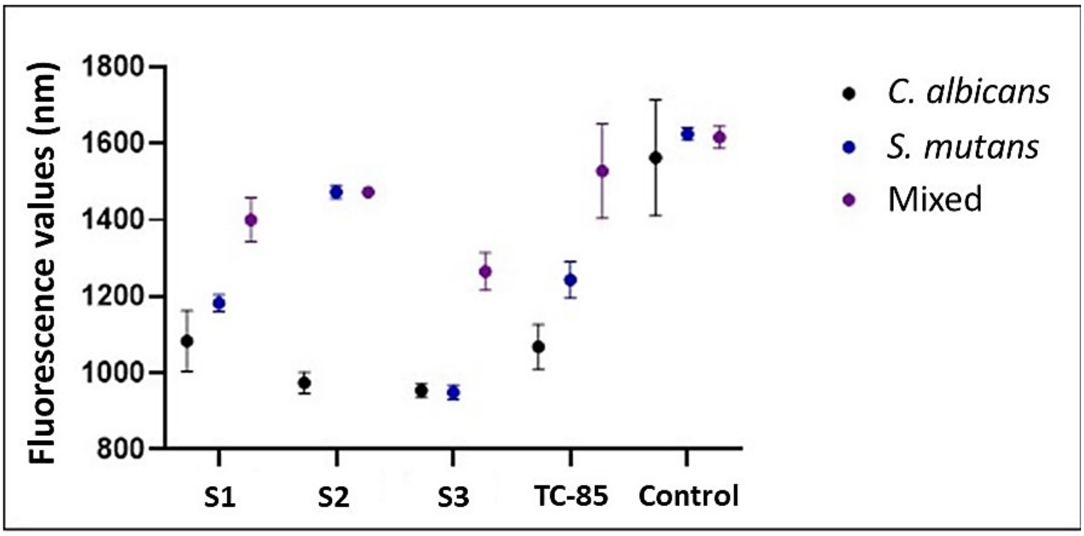

Figure 6: Fluorescence values of C. albicans, S. mutans, C. albicans and S. mutans (mixed) cells referring to biofilm formation 7 days after the specimens were 3D printed.

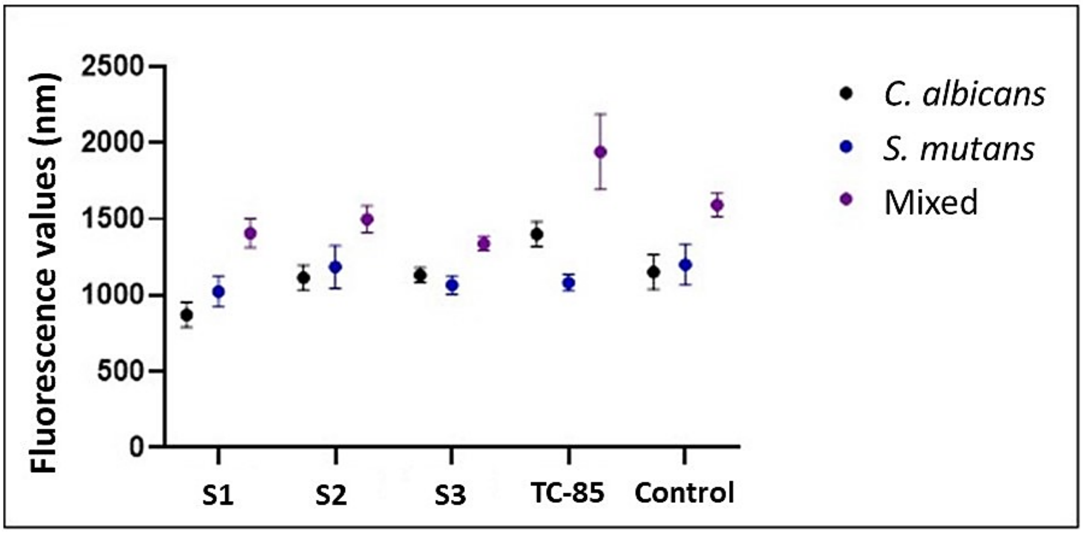

Figure 7: Fluorescence values of C. albicans, S. mutans, C. albicans and S. mutans (mixed) cells referring to biofilm formation 14 days after the specimens were 3D printed.

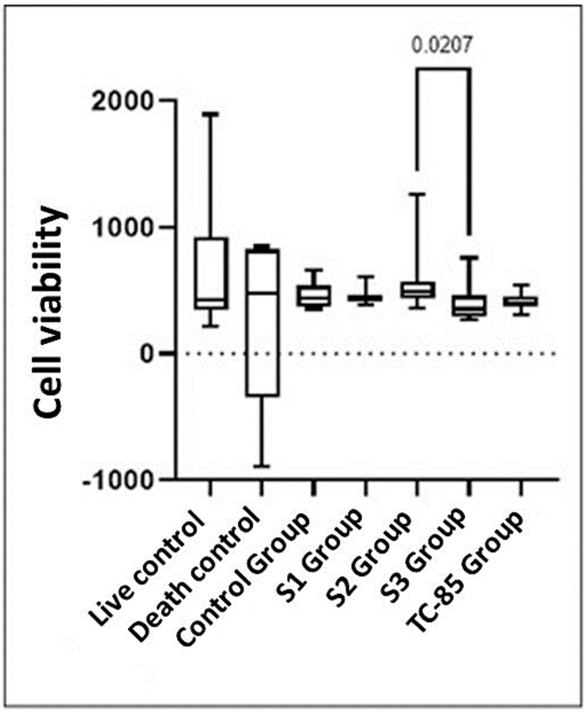

Figure 8: Cell viability in fluorescence values.

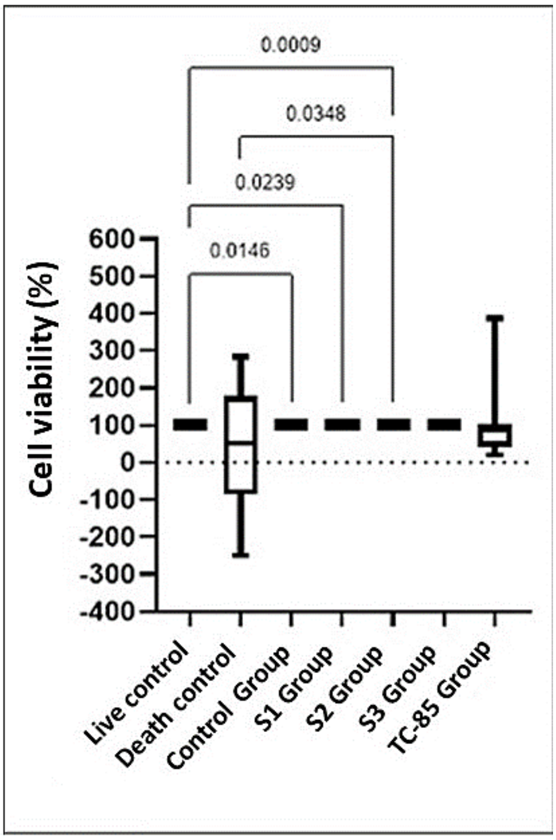

Figure 9: Percentage of viable cells compared to the live cell control group.

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4 CONSIDERAÇOES FINAIS

Os alinhadores impressos são uma nova tecnologia que ainda precisa de muitos estudos. Quando comparados aos alinhadores termoformados, eles aparentam ter melhores propriedades e elásticas e entrega de forças mais leves e contínuas. Além disso, os resultados mostraram que são biocompatíveis e possuem menor adesão de biofilme.

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Julianna de Oliveira Lima Parizotto