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UNESP - Universidade Estadual Paulista

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Faculdade de Odontologia de Araraquara

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Unveiling physical and biological properties of 3D-printed denture base resins: polishing, disinfection, and the development of a bioprinted model for biocompatibility testing

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Thesis presented to São Paulo State University (Unesp), School of Dentistry, Araraquara, as a requirement for the Doctoral degree in Dentistry area of Oral Rehabilitation.

Supervisor: Janaina Habib Jorge

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POTENTIAL IMPACT OF THIS RESEARCH

This research is expected to generate scientific, technical, and clinical impact. It improves the understanding of how 3D-printed denture base resins behave physically and biologically when subjected to routine clinical procedures such as surface polishing and disinfectant immersion. In parallel, the development of a bioprinted oral mucosa model for biocompatibility testing offers an innovative methodological contribution, advancing more physiologically relevant in vitro platforms for the evaluation of denture base materials. From a clinical and social perspective, these findings may inform safer material selection, support optimized denture hygiene protocols, and help reduce the risk of denture-associated infections such as denture stomatitis. Regarding the tissue engineering component, this work contributes to the dissemination of bioprinting technologies, particularly in national context, where their application to oral tissues is still limited. By establishing a scalable three-dimensional model for toxicity testing, it may support future academic and translational initiatives and strengthen technology transfer in this field. In addition, the use of biomimetic in vitro models aligns with the 3Rs principle by helping to reduce reliance on animal testing, consistent with current ethical standards and the sustainable development goals.

IMPACTO POTENCIAL DESTA PESQUISA

Esta pesquisa tem potencial para gerar impacto científico, técnico e clínico. Do ponto de vista científico, contribui para a compreensão do comportamento físico e biológico de resinas de base de prótese produzidas por impressão 3D quando submetidas a procedimentos clínicos de rotina, como o polimento de superfície e a imersão em soluções desinfetantes. Em paralelo, o desenvolvimento de um modelo de mucosa oral bioimpressa para avaliação de biocompatibilidade representa uma contribuição metodológica inovadora, avançando em direção a plataformas in vitro mais representativas para o estudo de materiais protéticos. Do ponto de vista clínico e social, os resultados podem orientar a seleção mais segura de materiais, subsidiar a otimização de protocolos de higienização de próteses e contribuir para a redução do risco de infecções associadas ao uso de próteses, como a estomatite protética. No que diz respeito ao componente de engenharia de tecidos, este trabalho contribui para

a disseminação das tecnologias de bioimpressão, especialmente no contexto nacional, onde sua aplicação a tecidos orais ainda é incipiente. O estabelecimento de um modelo tridimensional escalável para testes de toxicidade poderá apoiar iniciativas acadêmicas e translacionais futuras, além de fortalecer a transferência de tecnologia nessa área. Por fim, o uso de modelos biomiméticos in vitro está alinhado ao princípio dos 3Rs, contribuindo para a redução do uso de modelos animais em pesquisa, em consonância com os padrões éticos vigentes e com os objetivos do desenvolvimento sustentável.

Amanda Costa Ferro

Unveiling physical and biological properties of 3D-printed denture base resins: polishing, disinfection, and the development of a bioprinted model for biocompatibility testing

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I dedicate this thesis to the memory of my grandfather, Cleber Vale de Lima Ferro. He would have been proud to see that his constant encouragement to pursue education has borne fruit, shaping who we have become and changing our lives forever.

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“Até parece que foi ontem minha mocidade
Com diploma de sofrer de outra universidade
Minha fala nordestina
Quero esquecer o francês
E vou viver as coisas novas que também são boas
O amor, humor das praças cheias de pessoas
Agora eu quero tudo
Tudo outra vez.”
Belchior*

*Belchior. Tudo outra vez. In: Belchior. Era uma vez um homem e seu tempo. Rio de Janeiro: Warner Bros. Records, 1979.

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ABSTRACT

The fabrication of removable dentures has shifted from heat-polymerized poly(methyl methacrylate) (PMMA) toward digital workflows based on 3D-printed denture base resins. Although this transition has improved standardization and efficiency, uncertainties persist regarding the clinical behavior of these materials and their biocompatibility. This thesis investigated the physical and biological properties of 3D-printed denture base resins subjected to routine clinical procedures of surface preservation and hygiene and, in parallel, established a bioprinted in vitro model for biocompatibility evaluation, integrating five complementary studies. The first line of investigation addressed a surface polishing and the long-term effects of disinfectant immersion on these materials. The proposed surface polishing protocol reduced the roughness inherent to the printing process to a clinically acceptable threshold and decreased *Candida albicans* biofilm formation without compromising mechanical properties. Under long-term disinfectant immersion, the 3D-printed resin maintained smoother surfaces and more stable hardness than conventional heat-polymerized PMMA. Nonetheless, the 3D-printed resin was more susceptible to *C. albicans* colonization, and the tested disinfectants differed in their balance between antimicrobial efficacy, material preservation, and cytotoxicity. A critical review of the literature, conducted within this first line of investigation, corroborated these material-specific responses and highlighted the need for tailored chemical disinfection protocols and solutions for this newer class of photopolymers. The second line of investigation advanced the biological assessment of these materials through tissue engineering. Initially, a gelatin methacryloyl (GelMA) bioink was developed and characterized to support extrusion bioprinting. Then, a bioprinted oral mucosa model combining gingival fibroblasts and oral keratinocytes was assembled. Applied as a proof of concept to a 3D-printed denture base resin, the model revealed that the low degree of conversion elicited cytotoxic responses across conventional two-dimensional cultures and in the three-dimensional construct that captured these effects in a cell-type specific manner under daily leachate exposure. These findings altogether provided an integrated evaluation of 3D-printed denture base resins, linking their physical and biological properties to a more physiologically relevant platform for evaluating their biocompatibility. Beyond informing material's behavior under clinical maintenance aspects, it introduces the first preliminary bioprinted oral mucosa model developed for the biocompatibility assessment of denture base materials, offering a potential scalable and animal-free alternative for future in vitro evaluation.

Keywords: Denture bases; Printing, three-dimensional; *Candida albicans*; Surface properties; Materials testing; Bioprinting.

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RESUMO

A confecção de próteses removíveis passou dos métodos convencionais baseados em poli(metacrilato de metila) (PMMA) termopolimerizável para fluxos de trabalho digitais que utilizam resinas para base de prótese impressas em 3D. Embora essa transição tenha melhorado a padronização e a eficiência, ainda existem incertezas quanto ao comportamento clínico desses materiais, à sua biocompatibilidade e aos métodos adequados para avaliá-la. Esta tese investigou as propriedades físicas e biológicas de resinas para base de prótese impressas em 3D submetidas a procedimentos clínicos de higienização e preservação e, paralelamente, estabeleceu um modelo in vitro bioimpresso para a avaliação da biocompatibilidade, reunindo cinco estudos complementares. A primeira linha de investigação abordou uma modificação de superfície e os efeitos a longo prazo da imersão em soluções de desinfecção sobre estes materiais. O protocolo de polimento proposto reduziu a rugosidade inerente ao processo de impressão a um limite clinicamente aceitável e diminuiu a formação de biofilme de *Candida albicans*, sem comprometer as propriedades mecânicas do material. Após imersão prolongada em soluções desinfetantes, a resina para impressão 3D manteve superfícies mais lisas e valores de dureza mais estáveis do que o PMMA termopolimerizável. No entanto, a resina para impressão 3D mostrou-se mais suscetível à colonização por *C. albicans*, e as soluções testadas diferiram quanto ao equilíbrio entre eficácia antimicrobiana, preservação do material e citotoxicidade. A revisão crítica da literatura conduzida nesta linha de investigação corroborou esses comportamentos específicos de cada material e reforçou a necessidade de adaptar os protocolos de higienização química a essa nova classe de fotopolímeros. A segunda linha de investigação avançou na avaliação biológica desses materiais por meio da engenharia de tecidos. Inicialmente, uma biotinta de gelatina metacriloil (GelMA) foi desenvolvida e caracterizada para aplicação em bioimpressão por extrusão. Em seguida, foi estabelecido um modelo bioimpresso de mucosa oral composto por fibroblastos gengivais e queratinócitos orais. Este modelo foi aplicado à avaliação de uma resina para base de prótese para impressão 3D e demonstrou que o baixo grau de conversão do material induziu respostas citotóxicas tanto em culturas de monocamada convencionais quanto no constructo tridimensional, que foi capaz de reproduzir esses efeitos de maneira específica para cada tipo celular sob exposição diária aos lixiviados. Em conjunto, esses achados forneceram uma avaliação integrada das resinas para base de prótese impressas em 3D, relacionando suas propriedades físicas e biológicas a uma plataforma biomimética para o estudo da biocompatibilidade. Além de ampliar o conhecimento sobre o comportamento desses materiais frente aos protocolos de manutenção clínica, este trabalho apresenta o primeiro modelo preliminar de mucosa oral bioimpresso voltado à triagem de bases de prótese, oferecendo uma alternativa potencialmente escalável e livre de animais para futuros ensaios in vitro.

Palavras – chave: Bases de dentadura; Impressão tridimensional; *Candida albicans*; Propriedades de superfície; Teste de materiais; Bioimpressão.

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1 INTRODUCTION

3D-printed dentures have gained increasing attention in recent years[1,2]. In this workflow, additive manufacturing techniques have introduced a significant shift in denture fabrication by reducing material waste, streamlining clinical and laboratory procedures, shortening treatment time, and enabling more cost-efficient and personalized rehabilitations. This approach also reduces potential sources of human error while offering predictable production costs[3–5]. Despite these advantages, there is limited clinical evidence regarding the material properties and long-term performance of 3D-printed denture base polymers under maintenance conditions[6].

The study of the physical and mechanical properties of 3D-printed denture base resins is essential because surface characteristics directly influence the adhesion of pathogenic oral microorganisms. Studies have established that a surface roughness of 0.2 µm represents the clinically acceptable threshold for minimizing microbial retention[7]. Wettability is also important, since more hydrophobic and rough surfaces tend to be more susceptible to biofilm accumulation[8,9]. Fungal infections related to *Candida albicans* may result from the adhesion and proliferation of these microorganisms, leading to inflammation of the oral mucosa adjacent to contaminated dentures[10].

The most common disease affecting denture wearers is denture stomatitis. Among its etiological components, the adhesion of *Candida* species to the oral mucosa and to the denture surface plays a central role in both occurrence and progression[11]. However, *Candida* is more frequently isolated from the internal surface of complete dentures than from the corresponding mucosa[12], likely due to the presence of pores and surface irregularities within the acrylic resin base that facilitate microbial retention. Once established, this microenvironment creates a protected niche that promotes biofilm maturation, increased pathogenicity and inflammatory responses in the adjacent mucosa[10].

Thereby, the topographical characteristics of the denture's intaglio surface significantly favor the adhesion and proliferation of *C. albicans* on the oral mucosa, representing a critical step for microbial colonization and subsequent tissue invasion[13]. This occurs because the denture-mucosa interface forms a protected ecological niche that supports microbial growth, characterized by a relatively anaerobic and acidic

environment[10]. Interestingly, recent studies have further shown that *C. albicans* adheres more to 3D-printed denture base resins than to conventional heat-polymerized PMMA, exhibiting increased pathogenicity factors and an elevated potential to induce denture stomatitis[14,15].

Given the influence of surface irregularities and roughness on adhesion of microorganisms and debris[8,16,17], the surface finishing of denture base resins is considered important, particularly in the internal surface, which is traditionally left unpolished. In 3D-printed dentures, this issue becomes particularly relevant because printing lines may be more evident depending on the technology used, creating additional sites for microbial retention. Kraemer Fernandez et al.[18] reported significant differences in surface properties between conventional resins and printed materials after coating and polishing, highlighting the importance of surface modification strategies to enhance the quality of printed denture bases and reduce biofilm accumulation.

Another crucial method used for removing biofilm is the cleaning of the dentures, which can be performed mechanically, chemically, or through a combination of both[19,20]. Brushing with soap or dentifrice is the most common method, but its effectiveness is limited because denture base surfaces are often irregular and porous, making biofilm removal difficult. Mechanical cleaning also requires manual dexterity, which may be compromised in elderly individuals[19,21]. Thus, chemical methods such as immersion in disinfectant solutions are recommended as part of routine denture care and are widely supported due to their ability to inhibit or eliminate various microorganisms[22].

Several chemical agents are used for denture disinfection or for reducing biofilm accumulation. Sodium hypochlorite is considered effective because it inactivates bacterial biofilm and inhibits calculus formation[22,23]. However, its use may lead to moderate cytotoxic effects on oral tissues and can cause bleaching of acrylic denture bases, and may also corrode metallic components[24]. Chlorhexidine digluconate also inactivates biofilm and reduces microbial adhesion, making it an effective denture disinfectant[25]. Nonetheless, its use has been associated with staining of acrylic denture bases when chlorhexidine-based solutions are applied regularly[26].

Antiseptic soaps have been used to eliminate microorganisms from different surfaces, and their antimicrobial effectiveness is consistently demonstrated. These

products can remove approximately up to 85% of microorganisms present on human skin[27,28]. Zoccolotti et al.[29] demonstrated that disinfectant liquid soaps such as Dettol, Lifebuoy and Protex effectively reduced biofilm on acrylic resin specimens, with Dettol and Lifebuoy achieving complete removal, as well as they did not show cytotoxicity and altered surface roughness. A subsequent clinical study confirmed these results, showing that liquid soaps effectively reduce denture biofilm and may serve as an alternative method for disinfecting partial or complete removable dentures[30].

Another factor regarding clinical success of complete dentures is the biocompatibility of the base material, given its prolonged and intimate contact with the oral mucosa[31]. This property is directly linked to the polymerization process, a chemical reaction that promotes the crosslinking of unsaturated hydrocarbons and converts methyl methacrylate (MMA) into PMMA[32]. The degree of polymerization is relevant, as insufficient conversion results in higher levels of residual monomers. These compounds are the primary contributors to cytotoxic effects and have been associated with mucosal irritation, burning sensations, allergic reactions and even genotoxicity[33–37].

Recent studies suggest that 3D-printed denture base resins release lower amounts of MMA than milled PMMA dentures, which positions them as promising materials for safer and broader clinical adoption[38,39]. In addition to their mechanical, physical and aesthetic advantages, the potential biocompatibility of these materials may offer important benefits for patients. However, as additive manufacturing technologies continue to evolve, comprehensive biological investigations are still needed, including studies using advanced mimetic oral tissue models and experimental conditions that better replicate clinical patterns of daily use.

Moreover, in the 3D printing process, UV light initiates polymerization and promotes the formation of crosslinked polymer chains[40]. However, because the object is built layer by layer, light penetration may not be uniform, which can result in incomplete polymerization. Consequently, post-curing cycles are required to reduce the amount of unreacted monomers[41]. Nevertheless, residual monomer may still be present even after post-curing. For example, Aati et al.[5] reported that 20 min of post-curing were sufficient for 3D-printed resins to reach ≥75% cell viability, although reductions in fibroblast viability were still observed.

Although several 3D printing resins are now commercially available with CE or FDA certification for intraoral use, their chemical composition is not often disclosed by manufacturers. In the United States, the Food and Drug Administration (FDA) regulates medical devices for commercial approval, while in the European Union (EU), CE marking is applied to devices that comply with safety and performance requirements under standards such as ISO 13485. Most denture base resins fall under Class IIA CE certification, that typically includes low- to medium-risk medical devices intended for limited-duration use, generally between 60 min and 30 days[42]. However, this classification does not reflect the long-term clinical conditions under which dental prostheses are actually used.

Therefore, biocompatibility testing becomes essential and is commonly performed through cytotoxicity assays using cell culture methods, which are relatively simple and reproducible. Conventionally, these tests are performed using monolayer cell cultures and follow ISO 10993 standards[43,44], which guide the biological evaluation of medical devices[45]. Although human cells are preferred to better simulate clinical relevance, cell lines such as L-929 are widely used because of their consistent biological responses. To improve the physiological relevance of in vitro modelling, researchers have developed coculture systems, three-dimensional constructs and reconstituted tissue models to create more biomimetic platforms for biocompatibility or drug testing[46].

Several approaches have been explored recently to create tissue-engineered constructs, reflecting the growing demand for models that more accurately reproduce native tissue structure and function[47]. One strategy that has gained particular attention is bioprinting, a group of additive manufacturing techniques that allow the spatial organization of cells, biomaterials and signaling molecules into three-dimensional living structures with precise control over geometry and composition[48,49]. Furthermore, recent studies have focused on the use of cell-laden or spheroid-based bioinks to better support and mimic extracellular matrix production and promote more advanced stages of tissue maturation, resulting in constructs that reflect the dynamic and heterogeneous characteristics of natural biological systems[50,51].

This evolution is particularly important because monolayer cultures do not reproduce the structural or functional complexity of oral mucosa, which is characterized by multiple stratified epithelial layers supported by underlying connective tissue.

Consequently, advanced tissue models better emulate barrier functions and cell-cell interactions, addressing limitations of simpler in vitro systems[52,53]. Studies have also demonstrated the biological relevance of full-thickness oral and skin models for toxicity and cosmetic testing[54–56]. However, their application to the biocompatibility assessment of denture base materials remains poorly explored.

With the increasing adoption of 3D-printed dentures, it is essential to evaluate the physical and biological properties of these materials, as well as the influence of clinical procedures intended to reduce microbial adhesion, including surface polishing and long-term immersion in different disinfectant solutions. These assessments are necessary to guide appropriate hygiene and disinfection protocols for this new category of denture base materials. At the same time, the growing availability of advanced tissue engineering and bioprinting technologies provides an opportunity to improve the biological assessment of these materials. However, 3D-bioprinted oral tissue models have not yet been established for the biocompatibility assessment of dental materials, including 3D-printed denture base resins. Addressing these gaps may support the evidence-based clinical adoption of these materials.

5 CONCLUSION

This thesis integrated the study of surface treatment, disinfection, microbial adhesion, and biocompatibility testing to provide a comprehensive assessment of 3D-printed denture base materials. The proposed polishing protocol reduced the roughness inherent to 3D printing and the associated microbial adhesion, without compromising mechanical strength or cytocompatibility. Under long-term disinfection, the 3D-printed resin showed overall superior physical stability compared with heat-polymerized PMMA, yet greater *C. albicans* colonization, thereby requiring careful selection of maintenance protocols. Supported by a critical review of the literature, effervescent cleansers and antiseptic soaps offered the best balance between material preservation and antimicrobial action, while chlorhexidine was limited by its cytotoxic and esthetic effects. Building on these findings, an optimized GelMA formulation was developed and characterized for a bioprinted oral mucosa model which, applied to a 3D-printed resin, detected its cytotoxicity across two- and three-dimensional systems and thus demonstrated its usefulness as a proof of concept for the preclinical evaluation of denture base materials. Overall, the studies progressed from optimizing and maintaining current 3D-printed denture materials to developing an advanced in vitro platform for their safe and evidence-based clinical adoption.

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