

UNIVERSIDADE DE SÃO PAULO  
FACULDADE DE ODONTOLOGIA DE BAURU

KARLA DRUZIAN OLIVEIRA

**Impact of the incorporation of 3Y-TZP nanoparticles and different sintering methods in bovine hydroxyapatite: development, microstructural characterization, and mechanical properties**

**Impacto da incorporação de nanopartículas de 3Y-TZP e métodos distintos de sinterização em hidroxiapatita bovina: desenvolvimento, caracterização microestrutural e mecânica**

BAURU  
2024

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Dissertação constituída por artigo apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Reabilitação Oral.

Orientador: Prof. Dr. José Henrique Rubo  
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BAURU  
2024

Oliveira, Karla Druzian

Impact of the incorporation of 3Y-TZP nanoparticles and different sintering methods in bovine hydroxyapatite: development, microstructural characterization, and mechanical properties/ Karla Druzian Oliveira. -- Bauru, 2024.

44 p. : il. ; 31 cm.

Dissertação (mestrado) -- Faculdade de Odontologia de Bauru, Universidade de São Paulo, 2024.

Orientador: Prof. Dr. José Henrique Rubo

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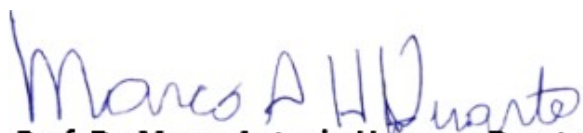
Dissertação apresentada e defendida por  
**KARLA DRUZIAN OLIVEIRA**  
e aprovada pela Comissão Julgadora  
em 21 de março de 2024.

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## DEDICATÓRIA

Aos meus pais, **Rosimeire Henrique Druzian Oliveira** e **Marco Aurélio Oliveira** (*in memoriam*), que fizeram dos meus sonhos os deles, lembrando sempre de acreditar e pensar em coisas boas. Dedico a vocês não somente essa dissertação, mas todas as conquistas da minha vida.

## AGRADECIMENTOS

Agradeço primeiramente a **Deus**, pelo cuidado e presença em cada etapa da minha vida. Com seu amor incondicional colocou pessoas incríveis e me deu suporte para ultrapassar cada desafio. Que minhas decisões sejam sempre alinhadas a Ele e não medidas pelas minhas adversidades.

À minha mãe, **Rosimeire Henrique Druzian Oliveira**, pelo zelo, apoio, dedicação e amor. Sinônimo de resiliência, a senhora sempre foi minha maior incentivadora e mostrou com exemplos a correr atrás dos meus sonhos independentemente do quão grandes e impossíveis eles pareciam ser. Não esqueço do que sempre me dizia, que não preciso ser a melhor, basta apenas dar o meu melhor. Aprendi organização, persistência, cuidado, postura e foco, que me fizeram chegar aonde cheguei. Me faz valorizar o principal da vida, que são momentos. Espero que consiga expressar o meu amor e gratidão pela senhora, na mesma intensidade que os sinto.

Ao meu pai e melhor amigo, **Marco Aurélio Oliveira** (*in memoriam*), que foi e sempre será meu exemplo de empatia, humildade e gratidão, foram inúmeros desafios enfrentados juntos. Hoje sei o quanto foi importante a presença de um pai verdadeiramente presente na minha vida. Tive muita sorte, porque aprendi com o senhor o significado de doação por amor e fé. Conviver com meu pai, era viver na prática como levar caráter, coragem, alegria e pensamento positivo para as pessoas que o rodeavam. Jamais imaginei chegar nesse momento, em que tanto lutamos juntos, sem compartilhar meus dias com o senhor. Te perder durante essa trajetória do mestrado foi o desafio mais difícil da minha vida. Mas sei o quanto finalizar essa etapa era importante para nós. Continuarei firme e vou espalhar o amor que um dia tive a sorte de receber.

À minha coorientadora, **Profa. Dra. Ana Flávia Sanches Borges**, que desde o início me aceitou como orientanda, acreditou e me apoiou na realização desse sonho. Agradeço a senhora pelas oportunidades que me concedeu, pela paciência, por me motivar, auxiliar e confiar no meu trabalho. Admiro sua ética, empatia, postura inclusiva, compreensão e humildade, são exemplos para mim. A senhora fez com que eu visse a pós-graduação com leveza e descobrisse o amor por materiais odontológicos, foi uma honra ser sua orientanda.

Ao meu orientador, **Prof. Dr. José Henrique Rubo**. Obrigada pelo privilégio de ser uma das últimas orientanda do senhor, participar da finalização de um ciclo bem-sucedido e de tamanha relevância é algo que me deixa extremamente honrada e feliz. Sempre disponível, com competência, tranquilidade e generosidade admiráveis, jamais esquecerei o “Jig sem estresse”. Sou muito grata pela oportunidade de conviver e aprender com o senhor.

À **Profa. Dra. Brunna Mota Ferrairo** e ao **Dr. Lucas José de Azevedo Silva**. Vocês são exemplos para mim, tanto como pessoas quanto profissionais, que me inspiram a ser apaixonada pela pesquisa, a dar o meu melhor, ajudar os outros sempre que puder, fazer tudo com muita dedicação e competência. Aprendi e aprendo muito com vocês, obrigada por partilharem tanto conhecimento comigo e pela paciência. Espero um dia ser como vocês.

Ao meu namorado, **Maycon Zanoni Bianchi** pelo carinho, companheirismo e suporte em todos os momentos que enfrentamos juntos. Você trouxe leveza, amor e alegria. Obrigada pela nossa sintonia e por me inspirar com sua força, persistência e cuidado com os que ama. Que continuemos nos apoiando e tornando o mundo um do outro, mais leve.

Aos meus primos/irmãos, **Eder Pereira Druzian** e **Gleica Pereira Druzian**. Obrigada por sempre estarem comigo, comemorando todas as conquistas. A parceria e união que temos é única. Vocês são sinônimo de suporte, diversão e amor. Que sigamos crescendo e evoluindo juntos. E que nosso companheirismo permaneça intacto.

Aos meus tios **Silvio Henrique Druzian**, **Ricardo Neri de Oliveira**, **Manuel Francisco de Oliveira** e **Carlos Alberto de Oliveira**. Vocês são exemplos de perseverança e dedicação para mim, cada um com suas diferenças e individualidades me transmitem carinho, atenção e cuidado. Em especial, meu padrinho Silvio que se faz sempre presente em cada momento da minha vida.

Aos meus avós, **Leonice Henrique Ferri Druzian**, **Maria Aparecida Gelo de Oliveira** e **Manuel Neri de Oliveira** (*in memoriam*), que são exemplos de força. Obrigada pelo cuidado e atenção que sempre dispensaram a mim. Sinto o carinho de vocês no pedaço de bolo, nas mudas feitas de plantas que eu gosto, nas orações e bençãos todos os dias. Aprendi com vocês postura, caráter e amor.

Aos meus amigos e colegas de profissão **Mayara Hana Narimatsu**, **Priscila Rossi Lemes Narimatsu** e **Gilberto Gallo Esteves** por despertarem em mim o amor

pela odontologia. E através do exemplo, me incentivarem a cada dia mais, me superar e dar o meu melhor para a profissão.

A todos do grupo de pesquisa **TRATEBIO** (Translational Team for Biomaterials), **Pedro Rodrigues Minim, Raphaele Santos Monteiro, Thaís Ferreira Rodrigues Mota** e **Paulo Henrique Martins Fernandes**, em especial ao **Prof. Dr. Carlos Alberto Fortulan** e **Prof. Dr. Paulo Noronha Lisboa Filho**, por toda paciência em ajudar e os muitos ensinamentos compartilhados.

Ao **Dr. Celso Antonio Goulart**, que em todos os momentos foi muito generoso, comprometido e atencioso, sempre à disposição para ajudar e sanar minhas dúvidas.

À **Faculdade de Odontologia de Bauru da Universidade de São Paulo (FOB-USP)**. A minha gratidão e orgulho por essa instituição, que construiu toda minha base profissional, são imensuráveis. Desde 2016, tive acesso ao melhor que a odontologia pode oferecer, com uma infraestrutura excelente, oportunidades de desenvolvimento e professores reconhecidos mundialmente. Neste lugar, não só conheci pessoas, como também vivi momento incríveis.

Aos **Departamentos de Engenharia Mecânica e de Materiais (USP-São Carlos)** e de **Física da Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP Bauru)**, por todo apoio, conhecimento e infraestrutura compartilhados, que foram essenciais durante a pesquisa.

Ao Departamento de Prótese da FOB-USP, representado pelos professores doutores: **Ana Lúcia Pompéia Fraga de Almeida, Estevam Augusto Bonfante, José Henrique Rubo, Karin Hermana Neppelenbroek, Paulo César Conti, Pedro César Garcia de Oliveira, Renato de Freitas, Simone Soares** e **Vinícius Carvalho Porto**. Agradeço por compartilharem seus conhecimentos, sempre serei grata por tudo que me ensinaram.

Aos funcionários da Faculdade de Odontologia de Bauru, em especial **Alcides Urias da Costa, Elizio Afonso Cardoso de Menezes, Hebe Joselina de Freitas Pereira, Déborah Andrea Riêra Blasca, Cleide Vital Martins, Reivanildo F. Viana, Valquíria F. Nogueira** e **Ziley Mara Calepso de Castro** por toda dedicação, convivência, apoio e paciência.

Aos meus colegas que ingressaram comigo no Mestrado, **Bianca Tavares Rangel, Edisa de Oliveira Sousa, Luis Fernando Basílio, Manoela Medola Marun, Mateus Zamora Galli, Pedro Henrique Amorim** e **Rafael Oliveira**. Vocês contribuíram para meu crescimento profissional e pessoal. Conhecer melhor cada um,



foi um grande presente. Obrigada pelo companheirismo e por tornar esses anos mais leves, torço pelo sucesso de todos.

Às minhas amigas **Beatriz Quevedo, Beatriz Andrade, Larissa Santi, Isadora Messias** e **Jade Zago**. Obrigada pelo companheirismo, apoio, incentivo e por se fazerem presentes, mesmo à distância. Tenho muito orgulho de todas vocês, e não importa o quanto o tempo passe, nossa amizade continuará a mesma. Seguiremos crescendo e evoluindo juntas.

Aos **pacientes** que pude conhecer durante este período. Obrigada pela compreensão, colaboração e confiança.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Código de Financiamento 001** – pela bolsa de Mestrado, auxílio indispensável para a realização deste trabalho.

Aos **professores integrantes da banca examinadora**, por terem dedicado tempo analisando e contribuindo para o aperfeiçoamento deste trabalho.

*“O mais importante é nunca parar de questionar.  
A curiosidade tem uma razão para existir. Nunca  
perca sua sagrada curiosidade.”*

***Albert Einstein***

## RESUMO

O objetivo deste estudo foi caracterizar microestruturalmente e mecanicamente uma cerâmica experimental densa de hidroxiapatita bovina na sua forma pura (HA) e com adição de 10% de 3Y-TZP (HA+10%3 Y-TZP), sinterizada por diferentes metodologias, visando sua aplicação em restaurações e próteses dentárias fixas. Discos ( $\varnothing 14 \times \pm 1.2\text{mm}$ , ISO 6872) foram obtidos por prensagem uniaxial e isostática, seguidos de sinterização em curvas convencionais e em dois passos. Foram estabelecidos dez grupos experimentais, incluindo HA pura (1300C, 1450C, 1500C, 95%2S e 105%2S) e HA+10% 3Y-TZP (1300C, 1450C, 1500C, 95%2S e 105%2S). As amostras foram submetidas a análises de dureza Vickers (DV), resistência à flexão biaxial (RFB), difração de raios X (DRX) e microscopia eletrônica de varredura (MEV). Para DV, os dados foram submetidos a ANOVA, seguido de pós teste Tukey ( $p < 0.05$ ) e para RFB, Kruskal-Wallis seguido de Dwass-Steel-Critchlow-Fligner ( $p < 0.05$ ). Os grupos 1300C ( $409 \pm 29.9$ ) de HA pura e 105%2S ( $98.6 \pm 8.72$ ) de HA+10% 3Y-TZP apresentaram melhores resultados de DV. Em RFB, os resultados da mediana, primeiro e terceiro quartis que indicaram valores mais elevados foram, respectivamente, para os grupos de HA pura 1300C (112; 106, 120 MPa) e a 105%2S (103; 90.4, 118 MPa), estatisticamente semelhantes, e para o grupo de HA+10% 3Y-TZP a 105%2S (46.2; 43.9, 47.0). Na análise de DRX, foi identificada formação de fases secundárias em praticamente todos os grupos, com exceção de 1300C e 95%2S, ambos de HA pura. Os grupos com adição de nanopartículas mostraram formação de maior quantidade de poros ao longo das superfícies, conforme imagens em MEV. Assim, a modificação da metodologia convencional de sinterização (105%2S) foi importante para a otimização das propriedades mecânicas. No entanto, a hidroxiapatita pura, sem adição de nanopartículas, apresentou melhores resultados.

Palavras-chave: apatitas; cerâmica; nanotecnologia.

## ABSTRACT

### **Impact of the incorporation of 3Y-TZP nanoparticles and different sintering methods in bovine hydroxyapatite: development, microstructural characterization, and mechanical properties**

The purpose of this study was to characterize microstructurally and mechanically an experimental dense ceramic of bovine hydroxyapatite (HA) in its pure form and with the addition of 10% 3Y-TZP (HA+10%3 Y-TZP), sintered by different methodologies, aiming at its application in fixed dental restorations and prostheses. Discs ( $\varnothing 14 \times \pm 1.2\text{mm}$ , ISO 6872) were obtained by uniaxial and isostatic pressing, followed by sintering in conventional and two-step curves. Ten experimental groups were established, including pure HA (1300C, 1450C, 1500C, 95%2S and 105%2S) and HA+10% 3Y-TZP (1300C, 1450C, 1500C, 95%2S and 105%2S). The samples underwent Vickers Hardness (DV), biaxial flexural strength (BFS), X-ray diffraction (XRD), and scanning electron microscopy (SEM) analyses. For DV, the data were submitted to ANOVA, followed by Tukey's post-test ( $p < 0.05$ ), and for RFB, Kruskal-Wallis followed by Dwass-Steel-Critchlow-Fligner ( $p < 0.05$ ). The 1300C ( $409 \pm 29.9$ ) pure HA and 105%2S ( $98.6 \pm 8.72$ ) HA+10% 3Y-TZP groups showed better microhardness results. In RFB, the results of the median, first, and third quartiles indicating higher values were, respectively, for the pure HA groups at 1300C (112; 106, 120 MPa) and 105%2S (103; 90.4, 118 MPa), statistically similar, and for the HA+10% 3Y-TZP group at 105%2S (46.2; 43.9, 47.0). Secondary phase formation was identified in practically all groups, except for 1300C and 95%2S, both of pure HA, in DRX analysis. Groups with nanoparticle addition showed a greater amount of pore formation along the surfaces, as observed in SEM images. Thus, modification of the conventional sintering methodology (105%2S) was important for optimizing mechanical properties. However, pure hydroxyapatite, without nanoparticle addition, showed better results.

Keywords: apatites; ceramics; nanotechnology.

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## 1 INTRODUÇÃO

O aumento da demanda por estética está impulsionando o desenvolvimento de novas tecnologias como alternativas para reabilitações, onde a cerâmica emerge como a base para materiais restauradores indiretos<sup>1</sup>. Isso se deve às suas características de biocompatibilidade, alta estabilidade química e apelo estético<sup>2</sup>. A biocompatibilidade é uma qualidade essencial dos materiais cerâmicos, garantindo que desempenhem suas funções sem causar danos ao indivíduo beneficiário<sup>3-5</sup>.

As cerâmicas começaram a ganhar mais destaque a partir da década de 1970 e podem ser divididas em bioinertes e bioativas. Um exemplo de cerâmica bioativa é a hidroxiapatita, um composto de fosfato de cálcio encontrado nos tecidos ósseos, proteínas e dentes, que possibilita ampla gama de aplicações na área médica e odontológica<sup>6-11</sup>, graças às suas propriedades de osteocondução, biocompatibilidade, bioatividade e ausência de toxicidade<sup>12-16</sup>.

A hidroxiapatita (HA) pode ser obtida para a fabricação de cerâmicas densas de várias maneiras e a partir de diversas fontes biológicas<sup>17-19</sup>. Uma dessas abordagens é a extração a partir de ossos de origem bovina, que se destaca como uma metodologia promissora devido à sua capacidade de reduzir custos e impactos ambientais, aproveitando resíduos da indústria pecuária como matéria-prima<sup>20,21</sup>. Durante o processo de calcinação, ocorre a eliminação de patógenos e componentes orgânicos, mitigando o risco de transmissão de doenças, como a Encefalopatia Espongiforme Bovina<sup>22-27</sup>.

Na Odontologia, pode ser utilizada para substituição e regeneração óssea guiada, na configuração densa ou porosa<sup>28-30</sup>. No entanto, apresenta resistência mecânica reduzida, a qual limita seu uso em aplicações que envolvem suporte de carga<sup>31,32</sup>. Portanto, é contraindicada em situações em que são expostas a alta incidência de forças<sup>33,34</sup> pois apresenta como fator limitante a baixa resistência à flexão e baixa tenacidade à fratura<sup>35</sup>, em comparação com outros materiais restauradores.

Visando reduzir a tensão de trincas, impedindo a propagação, otimizando a durabilidade e comportamento mecânico do material, a adição de partículas e nanopartículas têm sido empregadas, proporcionando reforço utilizando processos



como crack bridging, crack deflection e crack bridging sliding<sup>36-39</sup>. Alumina, titânio, biovidro, mulita e zircônia são alguns exemplos de agentes que podem ser combinados com a HA para esse fim<sup>40-48</sup>.

A zircônia apresenta propriedades superiores de resistência ao desgaste, flexão e tenacidade à fratura em comparação às vitrocerâmicas<sup>49,50</sup>. Além disso, ela pode ocorrer em diferentes formas cristalinas em faixas de temperatura distintas: (1) monoclinica, estável em temperatura ambiente até 1170°C, (2) tetragonal, de 1170°C a 2370°C, e (3) cúbica, a partir de 2370°C. A adição de estabilizantes permite a manutenção da fase tetragonal em temperatura ambiente, uma vez que essa fase alotrópica apresenta propriedades mecânicas superiores às outras.

Entre os óxidos estabilizantes disponíveis, o óxido de ítrio tem demonstrado resultados excelentes<sup>51-53</sup>. A melhoria nas propriedades mecânicas decorre do mecanismo de tenacificação por transformação, que induz compressão nas trincas. Quando sob estresse, os grãos ao redor da ponta da trinca passam por uma transformação de fase da tetragonal para a monoclinica, resultando em um aumento de aproximadamente 3–5% no volume e reduzindo a propagação da trinca, o que melhora a tenacidade à fratura do material<sup>52-55</sup>.

Cerâmicas policristalinas são materiais cerâmicos inorgânicos não metálicos que não possuem fase vítrea. Constituídas por grãos separados por contornos de grão, esses materiais exibem uma disposição aleatória dos cristais. A zircônia policristalina tetragonal estabilizada por ítria (Y-TZP) pode ser classificada em gerações. A primeira geração, com a adição de 3% mol de óxido de ítrio (3Y-TZP), é reconhecida por suas excelentes propriedades mecânicas e alta opacidade, devido à birrefringência natural da fase tetragonal. Esses materiais foram adotados como alternativas às cerâmicas metálicas<sup>55</sup>. Portanto, a combinação desses dois materiais, HA e 3Y-TZP, apresenta potencial significativo, uma vez que a bioatividade e biocompatibilidade da HA, combinadas à tenacidade à fratura da 3Y-TZP, oferecem características adequadas para bioaplicações<sup>56-61</sup>.

A sinterização desempenha um papel crucial na densificação do material, afetando diretamente suas propriedades mecânicas finais, incluindo o tamanho dos grãos<sup>40,62</sup>. Para aprimorar essas propriedades, novos métodos foram desenvolvidos, visando alcançar uma densificação completa da amostra e a manutenção de grãos menores.

Uma dessas técnicas, introduzida no início da década de 1990 <sup>63</sup>, é a sinterização em duas etapas, na qual o aquecimento das amostras é realizado em dois estágios. Essa abordagem, que faz uso de fornos convencionais, promove a formação de grãos uniformes, evitando seu crescimento acelerado e resultando em uma HA totalmente densa, com propriedades mecânicas aprimoradas <sup>64-67</sup>.

Na primeira etapa da sinterização, o material é aquecido até uma temperatura mais alta (T1) por um curto período, geralmente cerca de 1 minuto. Esse tempo é suficiente para atingir uma densidade intermediária, correspondente a pelo menos 75% da densidade teórica, seguido de um resfriamento rápido. Na segunda etapa, a temperatura é reduzida para um nível mais baixo (T2) e mantida por um período mais longo, permitindo a densificação total do material. Esse processo fornece a energia necessária para manter a difusão do contorno do grão sem promover a migração do mesmo <sup>66,69</sup>. Como resultado, ocorre uma estabilização da microestrutura inicialmente desenvolvida, impedindo o crescimento dos grãos ao mesmo tempo em que preserva os mecanismos de difusão envolvidos no processo de densificação.

A sinterização em duas etapas, com a primeira temperatura mais elevada, pode ser empregada na fabricação de materiais cerâmicos com propriedades aprimoradas, resultando em melhorias significativas nas propriedades mecânicas. Essa abordagem tem sido associada a maior resistência, inclusive ao envelhecimento hidrotérmico, quando comparada à sinterização convencional de cerâmicas 3Y-TZP <sup>74</sup>. Além disso, alguns estudos têm demonstrado melhorias em algumas características da HA, como dureza e tenacidade à fratura, por meio dessa metodologia <sup>75</sup>.

## 2 ARTIGO

The article presented in this Dissertation was written according to the *Dental Materials* instructions and guidelines for article submission.

### **Impact of the incorporation of 3Y-TZP nanoparticles and different sintering methods in bovine hydroxyapatite: development, microstructural characterization, and mechanical properties**

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'Declarations of interest: none'.

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## ABSTRACT

*Objectives.* Characterize microstructurally and mechanically an experimental dense ceramic bovine hydroxyapatite (HA) in its pure form and with the addition of 3Y-TZP (HA+10%3 Y-TZP), sintered by different methodologies, aiming at its application in fixed dental restorations and prostheses.

*Methods.* Discs ( $\varnothing 14 \times \pm 1.2$ mm, ISO 6872) were obtained by uniaxial and isostatic pressing, followed by sintering in conventional and two-step curves. Ten experimental groups were established, including pure HA (1300C, 1450C, 1500C, 95%2S and 105%2S) and HA+10% 3Y-TZP (1300C, 1450C, 1500C, 95%2S and 105%2S). The samples underwent Vickers Hardness (DV), biaxial flexural strength (BFS), X-ray diffraction (XRD), and scanning electron microscopy (SEM) analyses. For DV, the data were submitted to ANOVA, followed by Tukey's post-test ( $p < 0.05$ ), and for RFB, Kruskal-Wallis followed by Dwass-Steel-Critchlow-Fligner ( $p < 0.05$ ).

*Results.* The 1300C ( $409 \pm 29.9$ ) pure HA and 105%2S ( $98.6 \pm 8.72$ ) HA+10% 3Y-TZP groups showed better microhardness results. In RFB, the results of the median, first, and third quartiles indicating higher values were, respectively, for the pure HA groups at 1300C (112; 106, 120 MPa) and 105%2S (103; 90.4, 118 MPa), statistically similar, and for the HA+10% 3Y-TZP group at 105%2S (46.2; 43.9, 47.0). Secondary phase formation was identified in practically all groups, except for 1300C and 95%2S, both of pure HA, in DRX analysis. Groups with nanoparticle addition showed a greater amount of pore formation along the surfaces, as observed in SEM images.

*Significance.* Thus, modification of the conventional sintering methodology (105%2S) was important for optimizing mechanical properties. However, pure hydroxyapatite, without nanoparticle addition, showed better results.

Keywords: apatites; ceramics; nanotechnology.

## 1. INTRODUCTION

The growing interest in aesthetics drives the search for new technologies in rehabilitation, with ceramics standing out as a restorative material due to its biocompatibility, chemical stability, and aesthetic appearance [1,2]. Biocompatibility, essential for biomedical materials, ensures that no harm is caused to the organism [3-5]. Ceramics, classified as bioinert and bioactive, have gained popularity since the 1970s, with hydroxyapatite (HA) being an example of a bioactive ceramic with broad applications in medicine and dentistry due to its characteristics [6-16].

The obtaining of HA for the fabrication of dense ceramics can be carried out in various ways, including the extraction from bovine bones, a promising approach in terms of cost and sustainability [17-27]. Although HA is employed in dentistry for bone regeneration, its limited mechanical strength makes it unsuitable for load-bearing applications [28-35]. To enhance its mechanical properties and durability, it has been associated with particles and nanoparticles, such as zirconia [36-55].

Tetragonal zirconia stabilized with yttria (3Y-TZP) is widely used due to its excellent mechanical properties, serving as an alternative to metallic ceramics restorations [49-55]. The combination of HA and 3Y-TZP shows potential for biomedical applications, harnessing the bioactivity of HA and the fracture toughness of 3Y-TZP [56-61].

Two-step sintering is a methodology involving the heating of samples in two stages. Introduced in the early 1990s, it was developed to enhance the mechanical properties of ceramic materials such as HA and 3Y-TZP, providing complete densification and grain size control [62-67]. In the first stage of sintering, the material is heated to a higher temperature ( $T_1$ ) with a dwell time of 1 minute, which is sufficient to achieve an intermediate density (at least 75% of the theoretical density), followed by rapid cooling. In the second stage, the lower temperature ( $T_2$ ) is kept for a longer period until total densification provides energy to maintain grain boundary diffusion without grain migration [66, 69].

The purpose of this study was to characterize microstructurally and mechanically the effects of different sintering parameters on experimental bovine HA ceramics, both pure and with the addition of 10% 3Y-TZP nanoparticles. The goal was

to improve mechanical properties while restraining the decomposition of HA, by comparing two-step sintering methodology with conventional methods for both pure HA and HA with a significant proportion of added nanoparticles. The null hypothesis was that the two-step sintering process would provide no difference at microstructural and mechanical properties to sintering conventional method for dense bovine hydroxyapatite ceramics with 10% 3Y-TZP addition and pure HA.

## 2. MATERIAL AND METHODS

### 2.1. Sample preparation

In this study, the dense polycrystalline ceramic of HA + 10% 3Y-TZP was fabricated at the School of Engineering of São Carlos (Composite and Tribology Laboratory) and at the Integrated Research Center of the Bauru School of Dentistry (Biomaterials Laboratory II).

After approval by the Ethics Committee for Animal Use (CEUA/FOB/USP) under registration number 006/2023, Nelore bovine metatarsals were used as the source for obtaining HA. The bone tissue underwent thermochemical processes for complete removal of organic matter. This included immersion in hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at 100 volumes for five minutes, heated to  $100^\circ\text{C}$ , followed by washing with deionized water. Subsequently, the material was placed in an oven at  $900^\circ\text{C}$  for calcination for 24 hours, with a heating rate of  $5^\circ\text{C}/\text{min}$  [16].

The remaining material underwent a reduction process using mortar and pestle during manual grinding. The bones were subjected to grinding in a rotary mill at a speed of 104 rpm for 48 hours and in a vibratory mill for 72 hours. In a polyethylene flask, with a height of 85 mm and a volume of  $300\text{ cm}^3$ , 40% volume (500 g) of 3-YTZP spheres with a diameter of 3 mm, 0.05% volume of para-aminobenzoic acid ( $\text{C}_7\text{H}_7\text{NO}_2$ ), 30% volume of HA, and 69.95% volume of isopropyl alcohol ( $\text{C}_3\text{H}_8\text{O}$ ) were added. As a result, a nanometric (40 nm) and uniform powder is obtained, which reduces the porosity of the ceramic, sintering time and temperature, and increases reactivity between particles, without altering the stoichiometry, morphology, and purity of the HA [16,68].

Nanoparticles of 3Y-TZP (Tosoh® Corporation Tokyo, Japan) were weighed at a concentration of 10% relative to the volume of HA and added to this mixture, which was then returned to the vibratory mill for 24 hours for homogenization. The flask was emptied, and the mixture was dried with a hot air blower at 80 °C and subsequently granulated into smaller particles on stainless steel meshes (#100 ≤ 150 μm; #150 mesh ≤ 106 μm; #200 mesh ≤ 75 μm) [16,68].

Following ISO 6872 [70], specimens were produced in the form of discs (Table 1). The pure HA and the mixture (HA + 10% 3Y-TZP) in powder form were weighed at 0.7 g and inserted into a cylindrical matrix, followed by uniaxial pressing at 100 MPa for 1 minute. Subsequently, using elastomeric balloons under vacuum, they were pressed by an isostatic press at 200 MPa for 1 minute [47].

Table 1 - Density and proportion of components used in specimens' preparation

Component	Density (g/cm <sup>3</sup> )	Proportion (%)	Function
HA	3.14	30%	Ceramic powder
Para-aminobenzoic acid	1.37	0.05%	Deflocculant
Isopropyl alcohol	0.78	60,95%	Solvent
3-YTZP nanoparticles	6	10%	Reinforcement

## 2.2. Sintering

The sintering process was carried out in a Lindberg/Blue M chamber furnace (Asheville, NC, USA) under atmospheric pressure. After sintering, the specimens reached final dimensions of  $14 \pm 2$  mm in diameter and  $1.2 \pm 0.2$  mm in height, following ISO 6872:2015 standards [70]. The study design is illustrated in Figure 1.



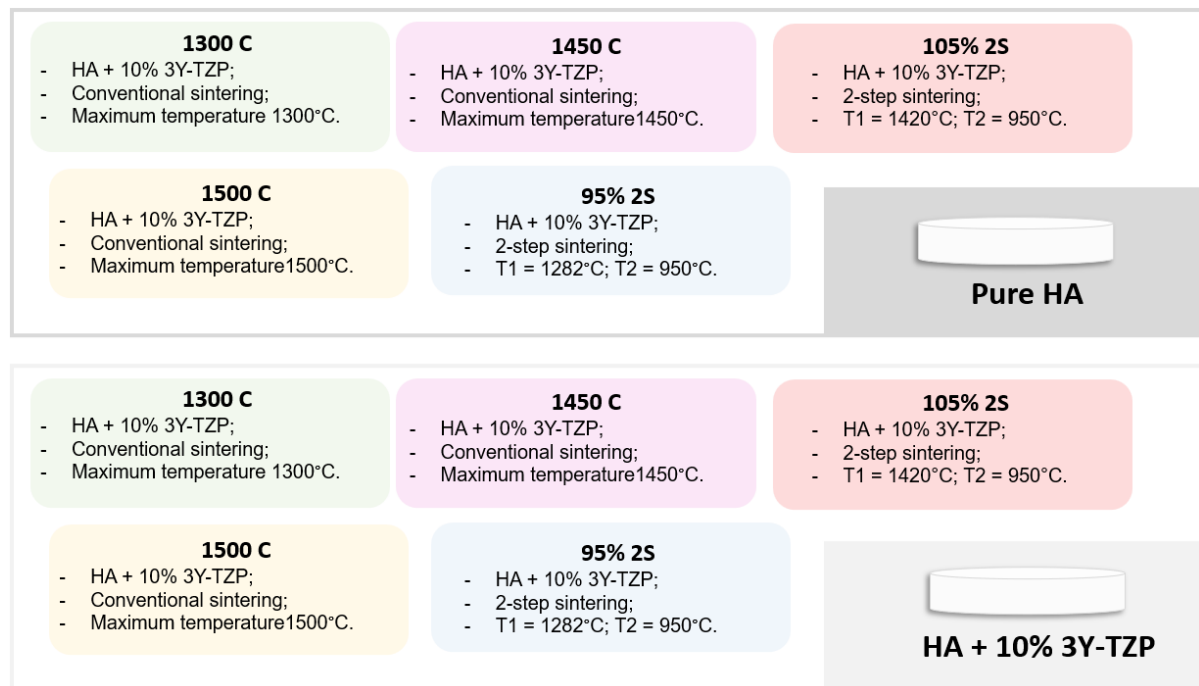


Figure 1. Study design.

In the conventional method, temperature was gradually increased from room temperature to 160°C at a heating rate of 2.7°C/min; from 160°C to 600°C at 4°C/min; from 600°C to 1100°C at 5°C/min; and finally, from 1100°C to the determined temperature for each group (1300°C, 1450°C, or 1500°C), at 6°C/min. The specimens were held at this maximum temperature for 120 minutes, followed by furnace cooling to room temperature (Fig. 3).

In the two-stage sintering process, the temperature for the first sintering stage (T1) was determined based on the temperature at which the material reaches critical density without grain growth [63, 67]. The sintering process begins with a gradual temperature increase from room temperature to 650°C at a heating rate of 4°C/min, then up to 900°C at 5°C/min, reaching 95% (1282°C) or 105% (1420°C) of 1350°C at 10°C/min, with a 1-minute plateau at this temperature (T1), followed by cooling to 950°C (T2) at 10°C/min, with a 20-hour plateau, and then slow furnace cooling to room temperature (Figure 2).

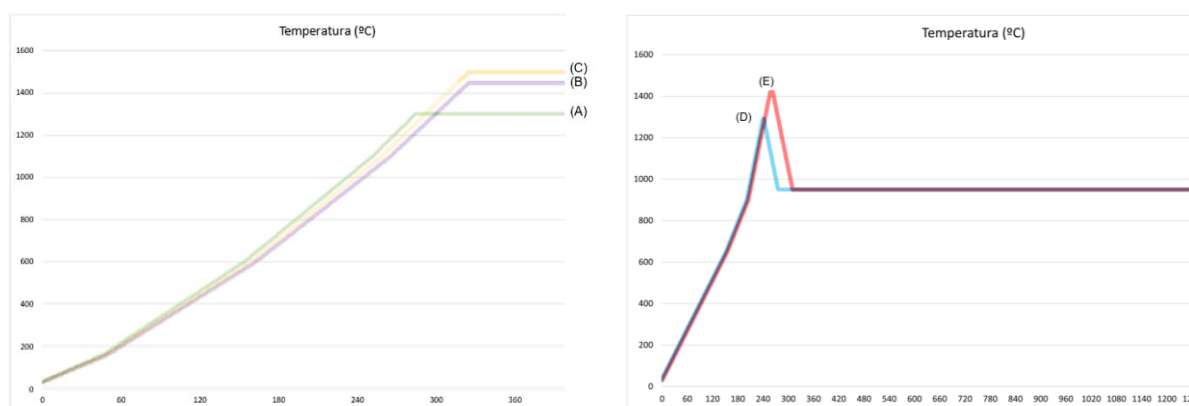


Figure 2. Sintering curves for the groups: 1300C (A); 1450C (B); 1500C (C); 95%2S (D); and 105%2S (E).

## 2.3. Mechanical properties

### 2.3.1. Vickers Microhardness

A Wilson Tukon 1202 machine from Buehler, Illinois, USA, equipped with a Vickers indenter was utilized. Five specimens from each group were evaluated, with three indentations performed on each disk. The microhardness tester was programmed to apply a load of 500 gf for 15 seconds per indentation. The Buehler Omnimet MHT software was employed for data analysis, and an average hardness value was calculated based on the obtained data.

### 2.3.2. Biaxial Flexural Strength (BFS)

The biaxial flexural strength (BFS) test was conducted using the device (OD36, Odeme Dental Research, Luzerna, Brazil) on a universal testing machine (Sintec 5G, MTS). Ten disk-shaped specimens from each group were analyzed following the recommendations of ISO 6872:2015 [69].

The specimens, cleaned with distilled water and isopropyl alcohol, were centrally positioned and supported by three steel spheres with a diameter of  $(4.5 \pm 2)$  mm, placed  $120^\circ$  apart on a circular support with a diameter of  $(11 \pm 1)$  mm. A load was applied through a flat pin with a diameter of  $(1.4 \pm 0.2)$  mm at the center of the specimen at a speed of  $(1 \pm 0.5)$  mm/min until fracture occurred. The obtained values

were collected in newtons (N). To calculate the biaxial flexural strength (MPa), the following eq.(1) was used:

$$\sigma = -0.23870 P(X-Y)/b^2 \quad (1)$$

$\sigma$  is the maximum strength (MPa),  $P$  is the total fracture load (N), and  $b$  is the sample thickness (mm).

$X$  and  $Y$  are values that were calculated from the eq.(2)(3):

$$X = (1 + \nu)\ln(r_2/r_3)^2 + [(1 - \nu)/2](r_2/r_3)^2 \quad (2)$$

$$Y = (1 + \nu) [1 + \ln(r_1/r_3)^2 + [(1 - \nu)(r_1/r_3)^2] \quad (3)$$

$\nu$  corresponds to the value of the Poisson's ratio (if the value is unknown, 0.25 was used),  $r_1$  is the radius of the support circle (mm),  $r_2$  is the radius of the load application area, and  $r_3$  is equivalent to the radius of the specimen.

## 2.4. Microstructural properties

### 2.4.1. X-Ray Diffraction (XRD)

For the identification of crystalline phases in the specimens under different sintering parameters, an X-ray diffractometer (PC Rigaku D/MAX 2500) with Cu radiation ( $k\alpha$ )  $\lambda=1.54060 \text{ \AA}$  was utilized, operating at 20 mA and 40 kV. For samples of HA + 10% 3Y-TZP, the Step Scan mode was employed with steps of  $0.02^\circ$  and a speed of 1 second per step, and an angular scan between  $20^\circ$  and  $70^\circ$ . For the samples with Pure HA, Continuous Scan mode was used with an angular scan between  $10^\circ$  and  $70^\circ$ . Rietveld refinement was performed using X'pert HighScore Plus software (Malvern Panalytical, Malvern, United Kingdom).

### 2.4.2. Scanning Electron Microscopy (SEM)

Five specimens were cleaned using an ultrasonic bath for 15 minutes immersed in distilled water, and then submerged for 15 minutes in 92.8% ethanol (Rioquímica, São José do Rio Preto, SP, Brazil). Subsequently, they were coated with

gold (Denton Vacuum, Moorestown, NJ, USA), and the analyses of the groups were performed using a Scanning Electron Microscope (ZEISS, Supra40, Jena, Germany). Measurements were taken on the fracture surfaces at magnifications of 500x, 1.000x, and 2.000x.

## 2.5. Statistical analysis

The results of Vickers Microhardness and Biaxial Flexural Strength were organized and subjected to the Shapiro-Wilk normality test. In the case of parametric distribution, the data were subjected to ANOVA followed by Tukey's post hoc test. For non-parametric distribution, they were subjected to Kruskal-Wallis followed by Dwass-Steel-Critchlow-Fligner post hoc test. In all cases, a significance level of 5% ( $p < 0.05$ ) was adopted. The datasets were analyzed using the statistical software Jamovi (Version 2.3.21).

## 3. RESULTS

### 3.1. Vickers Microhardness

The results from specimens composed of pure HA revealed no statistically significant difference between the 1300C ( $409 \pm 29.9$ ) and 105% 2S ( $403 \pm 9.29$ ) groups, which demonstrated superior performance compared to the others. However, groups characterized by conventional curves and higher maximum temperatures exhibited lower values, with the 1500C group ( $151 \pm 47.1$ ) showing the lowest result.

For groups containing HA + 10% 3Y-TZP, the microhardness values did not show statistically significant differences; however, it was observed that higher results were obtained in groups subjected to higher temperatures in both curves, namely 105% 2S ( $98.6 \pm 8.72$ ) and 1500C ( $94.1 \pm 4.92$ ). The microhardness data are presented in Table 2.

Table 2 - Vickers Microhardness divided by nanoparticle quantity and sintering methodology

	1300 C	1450 C	1500C	95% 2S	105% 2S
<b>HA Pura</b>	409± 29.9 Aa	198± 7.48 Ca	151± 47.1 Da	336± 14.6 Ba	403± 9.29 Aa
<b>HA+10% 3Y-TZP</b>	58.7± 3.66 Ab	82.5±3.20 Ab	94.1± 4.92 Ab	75.4± 3.14 Ab	98.6± 8.72 Ab

Different uppercase letters represent statistical differences compared within rows. Different lowercase letters represent statistical differences compared within columns ( $p < 0.05$ ).

### 3.2. Biaxial Flexural Strength (BFS)

For pure HA, the 1300C (112; 106, 120 MPa) and 105% 2S (103; 90.4, 118 MPa) groups showed the best performance in terms of biaxial flexural strength, with no statistically significant difference between them. On the other hand, the 1500C group (26.4; 24.6, 28.0 MPa) exhibited the lowest result, differing significantly from the others ( $p < 0.05$ ).

Groups containing 10% addition of 3Y-TZP showed better results with two-stage sintering. The group with the highest value was the 105% 2S (46.2; 43.9, 47.0 MPa), followed by the 95% 2S group (38.5; 35.9, 40.4 MPa). Conventional sintering curves yielded lower results, with no statistically significant differences between them. The biaxial flexural strength data are presented in Table 3.

Table 3 - Biaxial Flexural Strength (MPa) of the different groups under analysis

	1300 C	1450 C	1500C	95% 2S	105% 2S
<b>HA Pura</b>	112(106-120) Aa	42(39.9-43.6) Ca	26.4(24.6-28.0) Da	57.5(53.0-61.3) Ba	103(90.4-118) Aa
<b>HA+10% 3Y-TZP</b>	34.9(33.3-36.5) Cb	32.4(31.3-32.6) Cb	33.5(31.2-34.4) Ca	38.5(35.9-40.4) Bb	46.2(43.9-47.0) Ab

Different uppercase letters represent statistical differences when comparing rows. Different lowercase letters represent statistical differences when comparing columns ( $p < 0.05$ ).

### 3.3. X-Ray Diffraction (XRD)

From the XRD analysis, in the groups of pure HA at 1300C and 95% 2S, it is possible to observe crystalline peaks corresponding to Hydroxyapatite (ICSD 26204). In the 105% 2S group, there is a significant amount of Hydroxyapatite (ICSD

26204) associated with a small portion of CaO (ICSD 14922). In the 1450C and 1500C groups, the predominance of HA peaks was not maintained, and the formation of secondary phases was observed. In the conventional curve with a maximum temperature of 1450°C, besides HA, peaks consistent with the crystallographic pattern of  $\beta$ -TCP (ICSD 6191) were identified (Table 4). However, in the conventional curve with a maximum temperature of 1500°C, complete HA degradation and the presence of only  $\alpha$ -TCP (ICSD 923) and  $\beta$ -TCP (ICSD 6191) were observed (Figure 3).

Table 4 - Composition of the groups after Rietveld analysis

	1300 C	1450 C	1500C	95% 2S	105% 2S
<b>HA Pura</b>	100% HA	42.3% HA 57.7% $\beta$ -TCP	54.9% $\beta$ -TCP 45.1% $\alpha$ -TCP	100%HA	99.1%HA 0.9%CaO
<b>HA + 10% 3Y-TZP</b>	79.9% $\beta$ -TCP 18.9%CaZrO <sub>3</sub> 1.2% ZrO <sub>2</sub>	80.5% $\beta$ -TCP 17.2%CaZrO <sub>3</sub> 2.3%YSZ	78.3% $\beta$ -TCP 21.7%CaZrO <sub>3</sub>	76.6% $\beta$ -TCP 20.6%CaZrO <sub>3</sub> 2.8%ZrO <sub>2</sub>	77.7% $\beta$ -TCP 21.2%CaZrO <sub>3</sub> 1.1%ZrO <sub>2</sub>

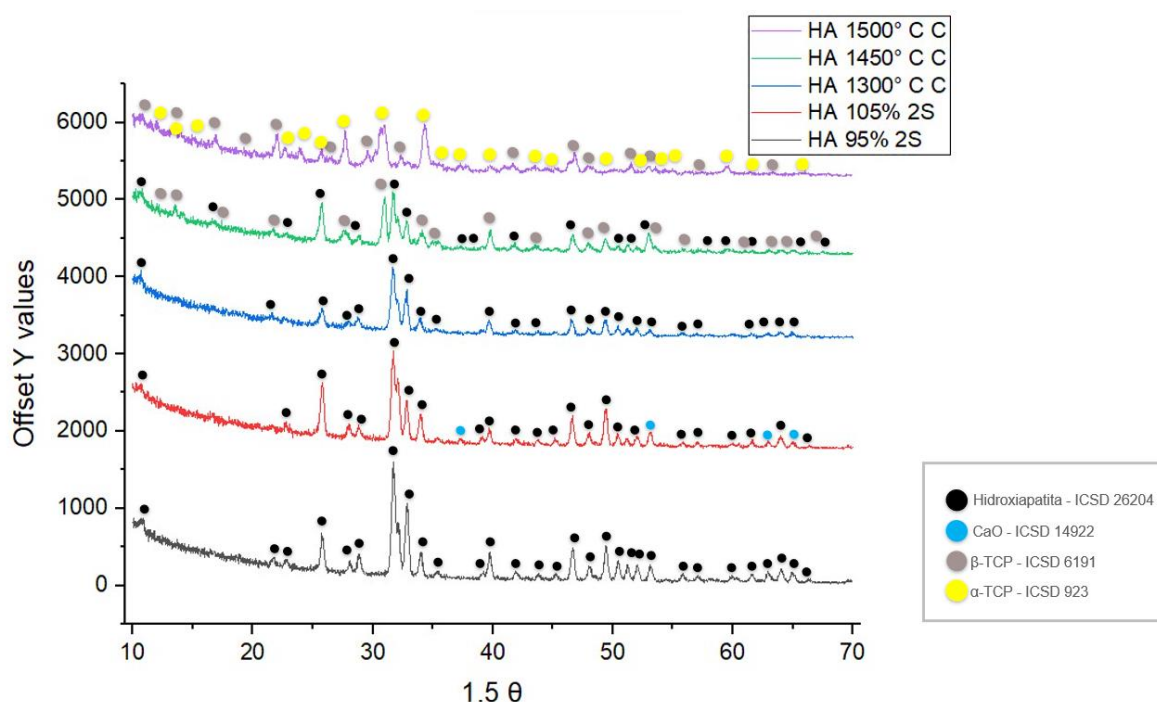


Figure 3 - X-Ray Diffraction (XRD) of samples containing pure HA.

In the HA + 10% 3Y-TZP groups, decomposition of HA, formation of secondary phases, and reaction between the components can be observed in all

groups (Table 4). The powder sample presents crystalline peaks corresponding to Hydroxyapatite (ICSD 26204) and YSZ (ICSD 75309). After sintering, regardless of the curve used, all groups showed a crystalline pattern of  $\beta$ -TCP (ICSD 6191) and  $\text{CaZrO}_3$  (ICSD 97463). In the case of  $\text{ZrO}_2$  (ICSD 165037), among the sintered groups, the corresponding crystalline peaks were not identified only in the 1500C group (Figure 4).

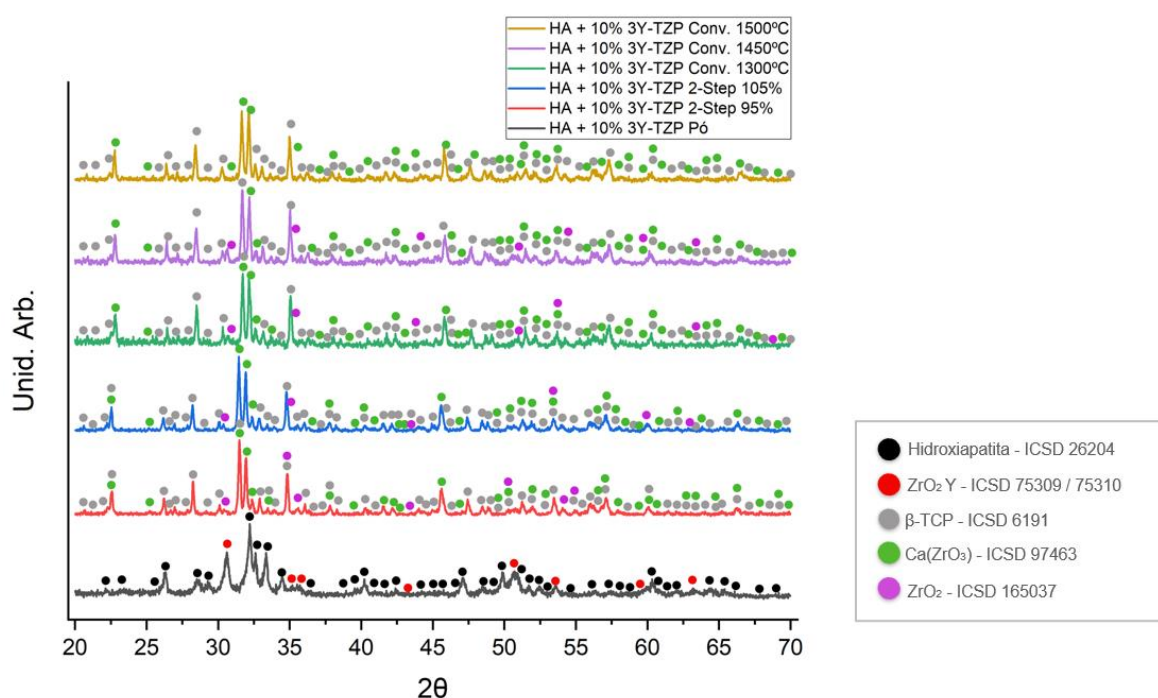


Figure 4. X-Ray Diffraction (XRD) of samples containing HA + 10%3YTZP.

### 3.4. Scanning Electron Microscopy (SEM)

The SEM images of the fractured surface of the pure HA samples show a similarity between the 1300C, 95%2S, and 105%2S groups. It is also possible to observe apparently greater cohesion between the grains of the 1300C and 150%2S groups. The surface of the 1450C and 1500C was characterized by greater grain disorganization and a apparently higher number of pores (Figure 5).

The addition of 3Y-TZP nanoparticles to the HA matrix resulted in increased porosity and the presence of more defects, as well as influencing densification. Therefore, the SEM analysis indicated that the HA+10%3Y-TZP group exhibited a less uniform composite (Figure 6).



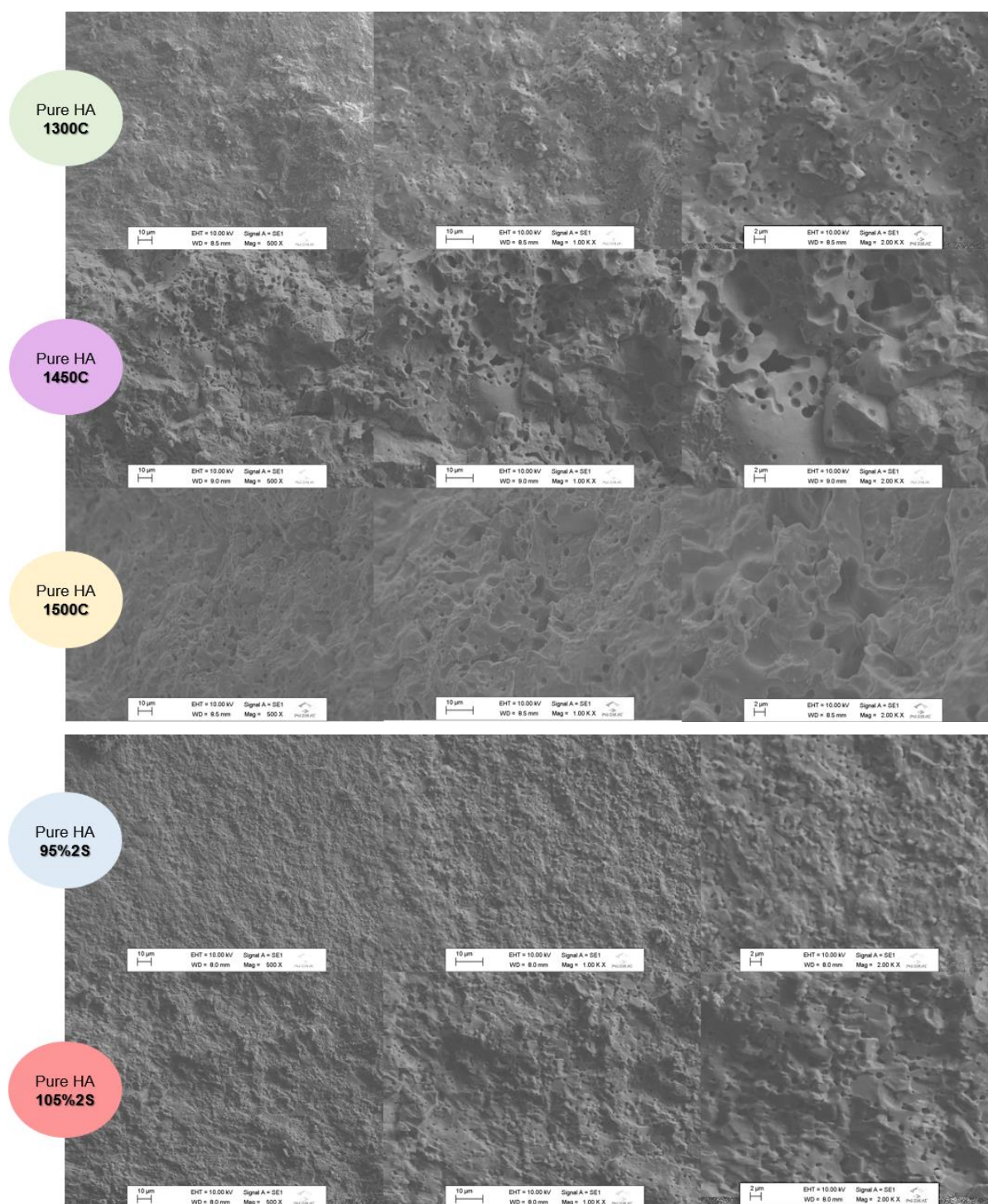


Figure 5. Micrographs of the fractured surface (bulk) of sintered specimens Pure HA: 1300C (A), 1450C (B), 1500C (C), 95%2S (D), and 105%2S (E).



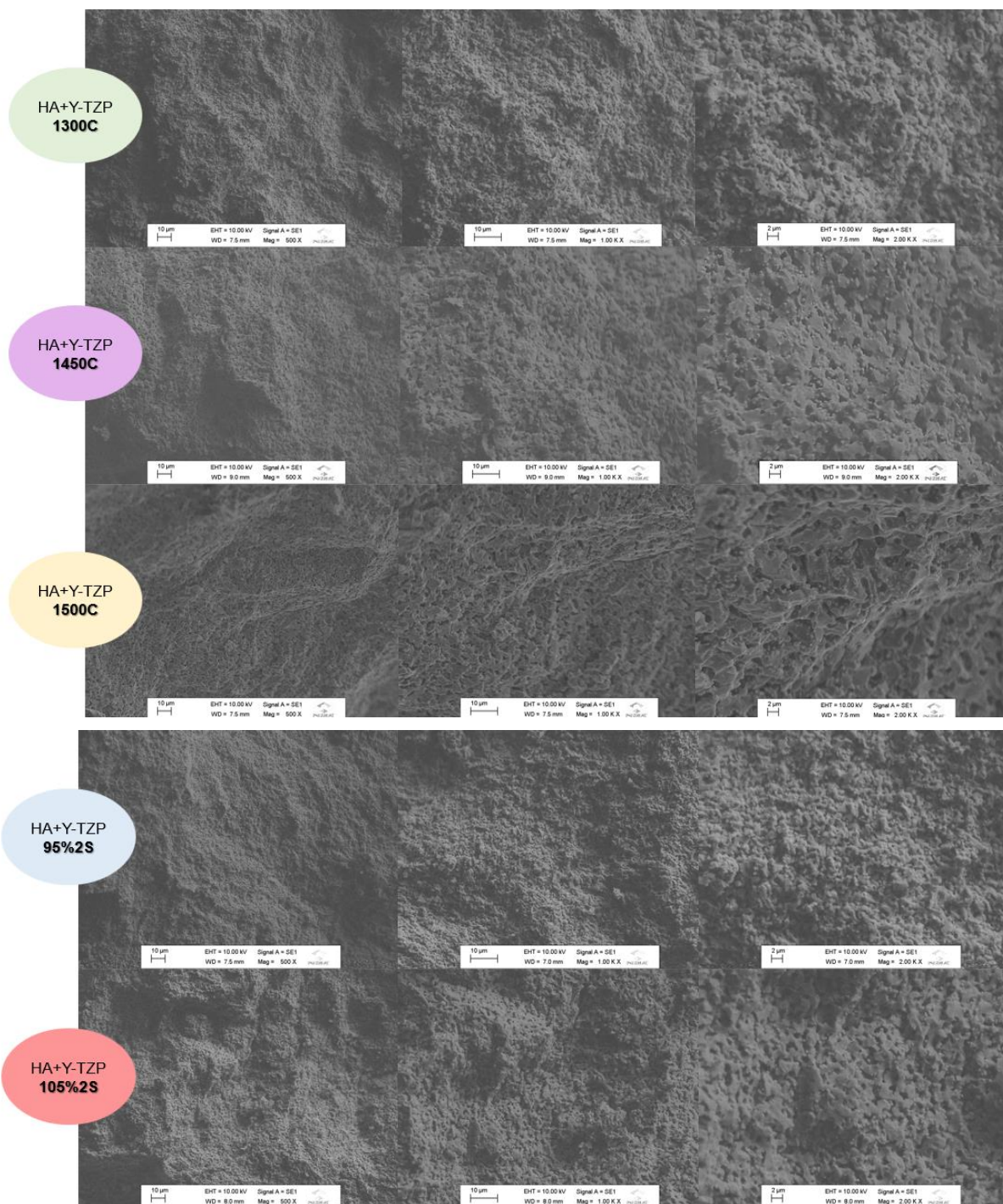


Figure 6. Micrographs of the fractured surface (bulk) of sintered specimens of HA + 10% 3Y-TZP: 1300C (A), 1450C (B), 1500C (C), 95%2S (D), and 105%2S (E).

#### 4. DISCUSSION

The null hypothesis of the study was rejected, as the two-step sintering process resulted in distinct microstructural and mechanical properties compared to conventional curves for the investigated groups. Notably, the 150%2S groups, both for Pure HA and HA+10%3Y-TZP, showed promising results compared to other groups with the same composition tested at different temperatures in both conventional and two-step curves.

The values of biaxial flexural strength (BFS) for pure HA indicated that the 105%2S group, along with the 1300C group, recorded values exceeding 100 MPa and showed no statistically significant difference, consistent with the microhardness analysis results. This finding, favoring the two-step sintering parameter, aligns with findings from other studies [66,67], but contrasts with the conclusions of Minim et al. (2023) [68]. The presence of binders, such as polyvinyl butyral (PVB), and lower temperatures in the two-step methodology may account for the discrepancies observed between the studies.

For the HA+10%3Y-TZP composite, the 105%2S group stood out with higher RFB values, statistically differing from all other groups. Conversely, the conventional curves yielded lower results, with no statistically significant difference among them. When considering these results alongside the microhardness values, which demonstrate better outcomes at higher temperatures in both sintering methods, it is evident that higher temperatures led to an improvement in mechanical properties in samples with 3Y-TZP addition. This aligns with the literature, which suggests that adding Y-TZP to an HA matrix requires the use of high temperatures to produce dense samples, as conventional sintering of commercial 3Y-TZP powder, on the nanoscale, can only achieve high densities at temperatures above 1300°C [71]. In this study, the lowest results for the groups with 10% zirconia nanoparticle addition were observed among the conventional curves, with a Vickers microhardness of  $58.7 \pm 3.66$  for the 1300C group and an RFB of 32.4; 31.3, 32.6 MPa for the 1450C group.

However, Hydroxyapatite (HA) proves to be unstable at temperatures above 1300°C, leading to its decomposition and the formation of  $\alpha$ - and  $\beta$ -TCP [ $\text{Ca}_3(\text{PO}_4)_2$ ], calcium oxide (CaO), and water. Furthermore, the nanoparticulation of HA improves the sintering process of the sample and reduces the temperature required for densification to around 1100°C [72]. Therefore, it is justified that for pure HA, the 1500C

group, with a conventional methodology and maximum temperature of 1500°C, exhibited the lowest combined result of microhardness ( $151 \pm 47.1$ ) and biaxial flexural strength (26.4; 24.6, 28.0 MPa).

From the XRD analysis, it is observed that the formation of secondary phases occurs not only with increasing temperature but also with the addition of nanoparticles in both groups. However, in the group composed of pure HA 105%2S, despite the high temperature, only 0.9% of CaO formation was observed, maintaining a significant amount (99.1%) of crystalline peaks consistent with the pattern of Hydroxyapatite (26204-ICSD). This indicates that the decomposition of HA was lower than at high temperatures with conventional curves (1450C and 1500C).

Samples with the addition of 3Y-TZP exhibited lower results compared to pure HA, a finding consistent with the SEM images presented, which show a higher presence of pores along the surfaces, reducing the material's resistance. The incorporation of ZrO<sub>2</sub> into HA, as observed by Nayak *et al.* (2008), indeed influences porosity and densification [73]. A previous study indicated that ZrO<sub>2</sub> nanoparticles, obtained through a hydrothermal method, did not contribute to the increase in the mechanical strength of HA, with the HA + 15% ZrO<sub>2</sub> composite exhibiting SEM images with a considerable number of pores compared to other tested groups [48]. According to an investigation involving YTZP/HA in proportions of 95/5, 90/10, 85/15, and 80/20, pore development can be attributed to the evolution of water vapor during the reaction between zirconia and hydroxyapatite [3].

In previous studies, the addition of 5% and 8% TiO<sub>2</sub> nanoparticles to the HA matrix [47] demonstrated the absence of secondary phase formation in a conventional sintering curve with a maximum temperature of 1300°C. However, another study using ZnO nanoparticles in the HA matrix indicated that samples with 1% ZnO nanoparticle addition did not show secondary phase formation, while those with 5% exhibited more pronounced formation [26].

When 10% of 3Y-TZP was added, the formation of secondary phases was observed in all sintering parameters. Our findings are consistent with those of Towler *et al.* (2000) [30], who identified inherent disadvantages in the combination of HA + Y-TZP (300 and 50 nm, respectively). With the increase in ZrO<sub>2</sub> content beyond 4% by weight, even at relatively low temperatures, the decomposition of HA was intensified,

resulting in an increase in the amount of  $\beta$ -TCP formed with additions of 6 and 10% by weight of  $ZrO_2$  in the HA matrix.

The addition of zirconia phases not only necessitates higher sintering temperatures but also reduces the decomposition temperature of HA to the range of 1000-1050°C due to the interaction between these materials. At 1150°C, the decomposition of HA results in the formation of other phases, including calcium-stabilized cubic zirconia and calcium zirconate ( $CaZrO_3$ ).

In the presence of zirconia (10% by volume), nearly all HA decomposes into some TCP phase at 1400°C, reaching only 78.8% relative density at 1500°C/1h [72]. Consequently, the accelerated decomposition of HA in the presence of zirconia restricts densification and impairs the mechanical properties of the composites, negating the advantage of combining a bioactive ceramic of low resistance (HA) with a bioinert ceramic of high resistance (Y-TZP) [56]. Further studies are required to reduce the reactivity between these materials and assess long-term effects, using 3Y-TZP percentages below 4% with the two-step sintering methodology under hydrothermal aging.

## 5. CONCLUSION

The samples of pure HA exhibited superior mechanical properties compared to those containing 10% of 3Y-TZP nanoparticles. However, the two-step sintering parameter with the higher temperature (105%2S) yielded the best results among the groups with nanoparticle addition (HA+10%3Y-TZP). Moreover, among the pure HA groups, it was statistically similar to conventional sintering (1300C), which achieved the best results. Therefore, the modification of the conventional sintering methodology was crucial for optimizing the studied material.

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## Anexo A – Declaração de uso exclusivo de artigo em dissertação.



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## DECLARAÇÃO DE USO EXCLUSIVO DE ARTIGO EM DISSERTAÇÃO/TESE

Declaramos estarmos cientes de que o trabalho "Impact of the incorporation of 3Y-TZP nanoparticles and different sintering methods in bovine hydroxyapatite: development, microstructural characterization, and mechanical properties" será apresentado na Dissertação/Tese do(a) aluno(a) KARLA DRUZIAN OLIVEIRA e que não foi e nem será utilizado em outra dissertação/tese dos Programas de Pós-Graduação da FOB-USP.

Bauru, 16 de fevereiro de 2024.

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