

UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

Faculdade de Ciências

Programa de Pós-Graduação em Ciência e Tecnologia de Materiais

Desenvolvimento de superfícies biomultifuncionais em ligas de alta entropia por oxidação por micro-arco.

Tiago dos Santos Pereira de Sousa

Orientador: Prof. Dr. Carlos Roberto Grandini, FBSE

Bauru

2024

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Tese apresentada como requisito para a obtenção do título de Doutor em Ciência e Tecnologia de Materiais ao Programa de Pós-graduação em Ciência e Tecnologia de Materiais (POSMAT) da Universidade Estadual Paulista “Júlio de Mesquita Filho”, área de concentração Biomateriais.

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Bauru

2024

S725

Sousa, tiago dos Santos Pereira de Sousa

Desenvolvimento de superfícies biomultifuncionais em ligas de altaentropia por oxidação por micro-arco / tiago dos Santos Pereira de Sousa Sousa. -- Bauru, 2024

73 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências, Bauru

Orientador: Carlos Roberto Grandini

1. Ciência dos Materiais. 2. Ligas de alta entropia. 3. Oxidação por micro arco. 4. Biomateriais. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE TIAGO DOS SANTOS PEREIRA DE SOUSA, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE MATERIAIS, DA FACULDADE DE CIÊNCIAS - CÂMPUS DE BAURU.

Aos 23 dias do mês de outubro do ano de 2024, às 09:00 horas, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de TIAGO DOS SANTOS PEREIRA DE SOUSA, intitulada "**Desenvolvimento de superfícies biomultifuncionais em ligas de alta entropia por oxidação por micro-arco**". A Comissão Examinadora foi constituída pelos seguintes membros: Prof. Dr. CARLOS ROBERTO GRANDINI (Orientador(a) - Participação Virtual) do(a) Departamento de Física e Meteorologia / Faculdade de Ciências - Unesp/Câmpus de Bauru, Prof. Dr. ULISSES FERREIRA KANEKO (Participação Virtual) do(a) Departamento de Física / Câmpus de Rio Claro, Profa. Dra. KATIA REGINA CARDOSO (Participação Virtual) do(a) Instituto de Ciência e Tecnologia / UNIVERSIDADE FEDERAL DE SÃO PAULO - UNIFESP, Profa. Dra. ANDERSAN DOS SANTOS PAULA (Participação Virtual) do(a) Instituto Militar de Engenharia - IME, Profa. Dra. SINARA BORBOREMA (Participação Virtual) do(a) Centro de Tecnologia / UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma virtual, o discente recebeu o conceito final **APROVADO**. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo Presidente da Comissão Examinadora.

Prof. Dr. CARLOS ROBERTO GRANDINI


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Agradecimentos

Gostaria de iniciar agradecendo, primeiramente, à minha família pelo apoio e dedicação ao longo de toda a minha vida, especialmente à minha companheira Jéssica, aos meus pais e ao meu irmão. Sem eles, não teria alcançado este ponto. Agradeço ao meu orientador, Prof. Dr. Carlos Roberto Grandini, e ao Prof. Dr. Diego Rafael Nespeque Corrêa pelo exemplo de profissionalismo na pesquisa e no ensino, por me proporcionarem a oportunidade de cursar o doutorado, e por todos os ensinamentos que foram muito além da pesquisa. Obrigado pelos incentivos, pelas críticas construtivas e por acreditarem que este caminho era possível. Agradeço também aos membros da banca por disponibilizarem seu tempo para corrigir meu trabalho e por compartilharem seus conhecimentos e experiências, contribuindo para o aprimoramento deste estudo. Sou grato aos professores por todos os ensinamentos compartilhados e ao Departamento de Física e Meteorologia por me conceder a primeira oportunidade de lecionar. Agradeço a todos os colegas do laboratório, não menciono nomes para evitar o equívoco de esquecer alguém, desde os alunos de iniciação científica até os de pós-doutorado, pela ajuda e pelas produtivas conversas. Agradeço ao LNNano e ao CNPEM pela disponibilidade dos equipamentos de espectroscopia de fotoelétrons de raios X e de difração de raios X para medidas em condições extremas. À CAPES, pelo suporte financeiro, ao POSMAT e à UNESP, por fornecerem toda a infraestrutura necessária para a conclusão deste trabalho.

Resumo:

Ligas biomédicas de titânio (Ti) têm sido desenvolvidas para utilização principalmente em implantes ortopédicos e odontológicos, combinando propriedades mecânicas, eletroquímicas e biocompatibilidade mais adequadas que outros biomateriais comerciais, como o aço inoxidável austenítico 316L e as ligas de Co-Cr-Mo. Contudo, as recentes ligas de titânio não eliminam completamente os problemas que ocorrem frequentemente em implantes, como o efeito de blindagem óssea, resistência aos esforços biomecânicos e a toxicidade pela liberação de íons metálicos e partículas de desgaste em longo prazo. Adicionalmente, métodos de modificação de superfície, dentre eles a oxidação por micro-arco (MAO), têm sido amplamente utilizados para acelerar o processo de osseointegração e evitar infecção bacteriana na interface entre o tecido ósseo e implante. Nos últimos anos, ligas com alta entropia, compostas por 5 ou mais elementos em proporção próxima à equimolar, têm sido desenvolvidas para aplicação aeronáutica e estrutural. Estes materiais têm sido reconhecidos pela sua elevada resistência mecânica, ductilidade e resistência à corrosão, o que têm atraído a atenção para utilização na área biomédica. Contudo, as ligas de alta entropia desenvolvidas atualmente não possuem propriedades adequadas para a utilização como biomateriais. Além disso, há ainda pouca compreensão sobre os mecanismos de cristalização e solidificação da solução sólida e da interação de sua superfície com células e tecidos do corpo humano. Este estudo objetivou desenvolver superfícies biomultifuncionais por MAO em novas ligas de alta entropia, compostas por elementos não tóxicos (Ti, Nb, Zr, Mo, Ta, Fe e Mn) em proporção quase equiatômica, formando as ligas TiZrNbTaMo, TiZrNbFeMo e TiZrNbTaMn visando aplicações como implantes biomédicos de baixo módulo de elasticidade. As ligas foram caracterizadas em função da microestrutura, características mecânicas, físico químicas., em De forma geral, os resultados mostraram que após a superfície das ligas serem modificadas por MAO, foi possível de se obter materiais com melhor biocompatibilidade mecânica, biocompatibilidade e propriedades biofuncionais.

Palavras-chave: biomateriais metálicos; ligas de alta entropia; metais não tóxicos; MAO; superfícies bio-multifuncionais.

Abstract:

Bio Biomedical titanium (Ti) alloys have been developed primarily for use in orthopedic and dental implants, offering mechanical, electrochemical, and biocompatibility properties that are more suitable than other commercial biomaterials, such as 316L austenitic stainless steel and Co-Cr-Mo alloys. However, recent titanium alloys do not completely eliminate common issues in implants, such as stress shielding, resistance to biomechanical stresses, and long-term toxicity caused by the release of metallic ions and wear particles. Additionally, surface modification methods, including micro-arc oxidation (MAO), have been widely used to accelerate the osseointegration process and prevent bacterial infection at the bone-implant interface. In recent years, high-entropy alloys (HEAs), composed of five or more elements in near-equimolar proportions, have been developed for aerospace and structural applications. These materials are recognized for their high mechanical strength, ductility, and corrosion resistance, which have attracted attention for potential biomedical applications. However, currently developed high-entropy alloys lack the properties required for use as biomaterials. Furthermore, there is still limited understanding of the crystallization and solidification mechanisms of solid solutions and the interaction of their surfaces with human cells and tissues. This study aimed to develop bio-multifunctional surfaces through MAO on novel high-entropy alloys composed of non-toxic elements (Ti, Nb, Zr, Mo, Ta, Fe, and Mn) in near-equiatomic proportions, forming the TiZrNbTaMo, TiZrNbFeMo, and TiZrNbTaMn alloys for applications as biomedical implants with low elastic modulus. The alloys were characterized in terms of their microstructure, mechanical, and physicochemical properties. Overall, the results demonstrated that after surface modification by MAO, it was possible to obtain materials with improved mechanical biocompatibility, biocompatibility, and biofunctional properties.

Keywords: metallic biomaterials; high entropy alloys; non-toxic metals; MAO; bio-multifunctional surfaces.

Sumário

1. Introdução	1
1.1. Biomateriais Metálicos	1
1.2. Ligas de Alta Entropia	2
1.3. Oxidação por Micro Arco (MAO) em ligas biomédicas	6
2. Objetivos.....	8
3. Materiais e métodos.....	8
3.1. Processamento e composição química das amostras	8
3.2. Oxidação por Micro Arco (MAO)	9
3.3. Caracterização estrutural e microestrutural	10
3.4. Caracterização Mecânica.....	11
3.5. Caracterização Físico-Química.....	12
4. Resultados.....	12
4.1. Artigo 1: Microstructure and selected mechanical properties of novel biomedical high-entropy alloys composed of some strategic non-refractory alloying elements.....	12
Abstract	13
4.1.1. Introdução	13
4.1.2. Materials and Methods:	15
4.1.2.1. Sample processing:	15
4.1.2.2. Sample characterization	15
4.1.2.3. Sample testing:	16
4.1.2.4. Results and discussion	16
4.1.3. Conclusion.....	23
4.1.4. Acknowledgement:	24
4.1.5. Funding:	24
4.1.6. Data availability	24
4.1.7. Declaration of Competing Interest	24
4.1.8. References	24
4.2. Artigo 2: Surface aspects of novel Bio-HEAs MAO-treated in a Ca-, P-, and Mg-rich electrolyte	26
Abstract.....	27

4.2.1.	Introduction.....	28
4.2.2.	Materials and methods	30
4.2.2.1.	Sample processing	30
4.2.2.2.	Surface treatment	31
4.2.2.3.	Sample characterization	31
4.2.3.	Results and discussion.....	32
4.2.4.	Conclusions.....	46
4.2.5.	Declaration of Competing Interest	47
4.2.6.	Acknowledgments	47
4.2.7.	References	48
5.	Considerações finais.....	52
6.	Produção Científica: Artigos Publicados.....	53
7.	Referências:.....	54

Lista de Figuras

Figure 1: Calculated phase diagram of the samples.	18
Figure 2: XRD profiles (a) and corresponding phase composition (b) of the samples acquired by the Rietveld method.	19
Figure 3: Optical and SEM imaging of the samples.....	20
Figure 4: Selected mechanical properties of the samples	22
Figure 5: Preliminary cytotoxicity evaluation of the samples	23
Fig 1: XRD profile and morphology of the MAO-treated TaMo sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.	33
Fig 2: XRD profile and morphology of the MAO-treated TaMn sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.	35
Fig 3: XRD profile and morphology of the MAO-treated FeMo sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.	36
Fig 4: Cross-sectional view of the MAO-treated samples.....	37
Fig 5: Thickness values of the MAO-treated samples.	38
Fig 6: TEM results of the MAO-treated TaMn sample at 300 V: (a) selected area of the surface for FIB milling (b) cross-section view of the FIB lamella with selected analyzed regions (red and blue circles); (c) STEM image of the outer amorphous layer and SAED pattern of the yellow circle; (d) selected area and (e) elemental EDS mapping; (f) STEM image of the intermediary nano-crystalline layer and SAED pattern of the yellow circle; (g) selected area and (h) elemental EDS mapping; (i) STEM image of the bulk and SAED pattern of the yellow circle; (d) selected area and (e) elemental EDS mapping.	39
Fig 7: Survey spectrums of the MAO-treated samples: (left) full spectrum and (right) zoomed view	40
Fig 8: Semi-quantitative results of the XPS survey spectrum for the MAO-treated TaMo/TaMn/FeMo sample: (left) overall content, (center) bioactive elements, and (right) alloying metals.	41
Fig 9: HR spectrums of the MAO-treated TaMo samples.....	42
Fig 10: HR spectrums of the MAO-treated TaMn samples.....	43

Fig 11: HR spectrums of the MAO-treated FeMo samples.....	44
Fig 12: Roughness values of the MAO-treated samples	45
Fig 13: Contact angle values of the MAO-treated samples.	46
Fig 14: Surface energy of the MAO-treated samples.	46

1. Introdução

1.1. Biomateriais Metálicos

No universo dos implantes biomédicos, as ligas à base de titânio (Ti) emergem como promissores candidatos, especialmente para aplicações odontológicas e ortopédicas (KIRMANIDOU, Y.; SIDIRA, M.; DROSOU, M.-E.; BENNANI, V. *et al.*, 2016). O titânio comercialmente puro (Ti-CP) brilha com uma combinação imbatível de resistência específica, módulo de elasticidade, resistência à corrosão e biocompatibilidade, superando biomateriais metálicos tradicionais como aço inoxidável austenítico 316L e ligas de Co-Cr-Mo (CORDEIRO; BARÃO, 2017; RONO; MWEMA; DABEES; SOBOLA, 2022). Apesar de suas excelentes propriedades mecânicas e biocompatibilidade, o titânio ainda enfrenta limitações quando se trata de replicar todas as características necessárias para um implante de longo prazo, como a osseointegração ideal e a resistência ao desgaste em ambientes corporais complexos. Essas limitações podem resultar em falhas a longo prazo, como a soltura do implante ou reações inflamatórias. Portanto, há uma necessidade urgente de desenvolver novos materiais que possam superar essas deficiências e oferecer uma solução mais duradoura e confiável para implantes biomédicos. Segundo estudos recentes, a pesquisa em biomateriais avançados, como ligas de titânio modificadas ou compósitos bioativos, tem se mostrado promissora nesse sentido (SARRAF; REZVANI GHOMI; ALIPOUR; RAMAKRISHNA *et al.*, 2022).

A incorporação de elementos específicos é essencial para reduzir o módulo de elasticidade e aumentar a resistência mecânica e ao desgaste, garantindo sua durabilidade em aplicações biomédicas ((NIINOMI; LIU; NAKAI; LIU *et al.*, 2016a; WONG; HSU; WU; HO, 2023). A liga Ti-6Al-4V, amplamente utilizada em implantes, oferece excelente desempenho mecânico e resistência à corrosão. No entanto, a liberação de íons potencialmente tóxicos de Al e V a longo prazo levanta preocupações sobre sua viabilidade como biomaterial (CHEN; THOUAS, 2015; SILVA; ARCOS; MONTERO; GUERRA *et al.*, 2021)

O desenvolvimento de novas ligas de Ti tem focado na adição de elementos com baixa toxicidade, como por exemplo, os metais zircônio (Zr) (CORREA; VICENTE; DONATO; ARANA-CHAVEZ *et al.*, 2014; KURODA; QUADROS; NASCIMENTO; GRANDINI, 2021), nióbio (Nb) (BERTRAND; CASTANY; YANG; MENOU *et al.*, 2016;

QUADROS; KURODA; ZAMBUZZI; FERNANDES *et al.*, 2024), ferro (Fe) (ABDEL-RHMAN; GEPREEL; ABDEL-MONIEM; KOBAYASHI, 2016; BIESIEKIERSKI; LIN; LI; PING *et al.*, 2016; UEKI; HIDA; NAKAI, 2024), tântalo (Ta) (SARAIVA; DE ALMEIDA; ZAMBUZZI; KURODA *et al.*, 2024; XIONG; YAO; YUAN; QU *et al.*, 2016) e molibdênio (Mo) (LOURENÇO; CORREA; DE ALMEIDA; KURODA *et al.*, 2024; ZHENG; BANERJEE; FRASER, 2016). Concomitantemente, tem-se buscado a manufatura de materiais com módulo de elasticidade próximo ao de tecidos ósseos duros (~30 GPa), com o intuito de evitar o efeito de blindagem óssea (NIINOMI; LIU; NAKAI; LIU *et al.*, 2016b). Para este fim, ligas de Ti tipo β (estrutura cristalina cúbica de corpo centrado - ccc) têm recebido grande atenção, visto que apresentam o menor módulo de elasticidade dentre as fases cristalinas do Ti (GEETHA; SINGH; ASOKAMANI; GOGIA, 2009a; WONG; HSU; WU; HO, 2023). Apesar disso, a combinação entre composição, processamento e propriedades destas ligas ainda não reproduzem o complexo sistema fisiológico do corpo humano (CORDEIRO; BARÃO, 2016).

1.2. Ligas de Alta Entropia

Nos últimos anos, uma nova classe de ligas, denominadas ligas de alta entropia (High Entropy Alloys - HEAs), tem despertado grande interesse nas indústrias aeronáutica e da construção civil. Essas ligas são caracterizadas pela presença de cinco ou mais elementos em proporções quase equiatômicas, geralmente variando entre 5 e 35 %at (DE OLIVEIRA; FAGUNDES; CAPELLATO; SACHS *et al.*, 2022; GAO; YEH; LIAW; ZHANG, 2016; SENKOV; MIRACLE, 2016). Esse tipo de composição aumenta a entropia de mistura, o que dificulta a formação de fases intermetálicas complexas e torna solutos e solvente praticamente indistinguíveis. Essa característica é fundamental para promover o endurecimento por solução sólida e contribuir para as propriedades mecânicas superiores dessas ligas (GAO; YEH; LIAW; ZHANG, 2016; NENE; SINHA; YADAV; DUTTA, 2024).

Mesmo com a adição de vários elementos, as ligas de alta entropia costumam formar estruturas cristalinas simples, como cúbica de corpo centrado (ccc) e cúbica de face centrada (cfc) (SENKOV; WILKS; MIRACLE; CHUANG *et al.*, 2010; WONG; HSU; WU; HO, 2023). Além disso, essas ligas apresentam uma combinação notável de alta resistência mecânica, boa ductilidade, excelente resistência à corrosão e ao

desgaste (LIU; LV; WU; ZHANG *et al.*, 2024; SHI; YANG; LIAW, 2017; V; M, 2024). Esses atributos tornam as HEAs candidatas promissoras para aplicações que exigem materiais com desempenho extremo em condições adversas, como altas temperaturas e ambientes corrosivos.

Os elementos de liga comumente utilizados nas HEAs incluem metais de transição e refratários, como ferro (Fe), zircônio (Zr), háfnio (Hf), cromo (Cr), tântalo (Ta), alumínio (Al), cobre (Cu), silício (Si), nióbio (Nb), manganês (Mn), molibdênio (Mo), cobalto (Co), níquel (Ni) e tungstênio (W) (SENKOV; WILKS; MIRACLE; CHUANG *et al.*, 2010; V; M, 2024). A combinação desses elementos em diferentes proporções oferece flexibilidade para ajustar as propriedades físicas e químicas, tornando possível a otimização das ligas para diversas aplicações tecnológicas avançadas. Além disso, o potencial dessas ligas para substituir materiais convencionais em condições extremas continua a ser um campo ativo de pesquisa, com foco em melhorar ainda mais suas propriedades térmicas e eletroquímicas.

O estabelecimento da composição química de ligas com alta entropia é baseado em parâmetros termodinâmicos e na regra de Hume-Rothery para a formação de soluções sólidas. Em geral, busca-se ligas com estruturas cristalinas simples, sem a precipitação de fases intermetálicas, pois podem comprometer as propriedades mecânicas do material. Do ponto de vista termodinâmico, a adição de uma determinada concentração atômica de um elemento promove mudanças nos valores de entropia (ΔS) e entalpia (ΔH) da mistura, as quais influenciam na redução da energia livre de Gibbs (ΔG). Entretanto, a formação de fases específicas, como soluções sólidas com estruturas ccc ou cfc, não é garantida, pois depende da combinação entre os valores de entalpia de mistura e as condições termodinâmicas do sistema. Em alguns casos, a redução da energia livre pode favorecer a formação de compostos intermetálicos ao invés de soluções sólidas com estruturas simples (KUMAR; SEETHARAM; PONAPPA, 2024; SENKOV; MIRACLE, 2016). As regras de Hume-Rothery determinam as condições para uma completa mistura dos elementos de liga, a partir da diferença de raios atômicos (δr), eletronegatividade ($\Delta \chi$), razão elétron-átomo (e/a) e valência eletrônica (VEC) (ARUN; RADHIKA; SALEH, 2024; DE OLIVEIRA; FAGUNDES; CAPELLATO; SACHS *et al.*, 2022; LILENSTEN; COUZINIÉ; BOURGON; PERRIÈRE *et al.*, 2016; POLETTI; FIORE; SZOST; BATTEZZATI, 2015). De forma geral, valores de $-22 \leq \Delta H_{\text{mistura}} \leq 7$ (kJ/mol), $11 \leq \Delta S_{\text{mistura}} \leq 19,5$ (kJ/mol), $\delta r \leq 8,5$ (%) e $\Delta \chi \leq 10$ (%), têm resultado em formação de soluções sólidas (ccc ou

cfc) sem a presença de intermetálicos. Contudo, ainda não há um consenso se qualquer mistura de elementos metálicos segue esta faixa de valores (TSAI; YEH, 2014).

Nos últimos anos, o interesse pelo uso de ligas de alta entropia (HEAs) para aplicações biomédicas tem crescido substancialmente, particularmente em relação ao desenvolvimento de implantes ortopédicos, como os utilizados em ombros, quadris e joelhos, que demandam alta resistência mecânica e durabilidade. Torrento et. al estudaram a liga TiZrNbTaMo não equiatômica e identificaram a presença de duas fases cristalinas distintas, CCC e hexagonal compacta (HC) (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022). Wang e Xu examinaram a microestrutura e as propriedades mecânicas da liga equiatômica TiZrNbTaMo (20%at) no estado bruto de fusão. Os resultados revelaram a presença de duas fases cristalinas cúbicas de corpo centrado (ccc), com parâmetros de rede ($a_1 = 0,3310$ nm e $a_2 = 0,3379$ nm), acompanhadas de elevados valores de microdureza (4,9 GPa) (WANG; XU, 2017). Essa liga apresentou um comportamento mecânico caracterizado por um limite de resistência à tração de 1390 MPa e uma deformação até a fratura de 6%. Ensaio de corrosão indicaram que a resistência eletroquímica do material foi superior à do aço 316L, bem como às ligas Co-Cr-Mo e Ti-6Al-4V, amplamente utilizadas em implantes ortopédicos. Entretanto, o módulo de elasticidade da liga foi registrado em cerca de 153 GPa, valor superior ao do titânio comercialmente puro (Ti-CP, ~100 GPa) e da liga Ti-6Al-4V (~110 GPa), o que pode representar uma limitação no contexto biomédico devido à incompatibilidade de módulo com o osso humano. Torrento et al. (2022) realizaram estudos adicionais sobre a liga TiZrNbTaMo, investigando suas propriedades após tratamentos térmicos e com intuito de ajustar a microestrutura e, potencialmente, aprimorar ainda mais as propriedades mecânicas e de resistência à corrosão da liga, tornando-a uma opção promissora para a fabricação de dispositivos biomédicos duráveis e biocompatíveis. Os resultados mostraram uma diminuição significativa do módulo de elasticidade para o tratamento à 500 °C por 6 horas (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022). (TODAI; NAGASE; HORI; MATSUGAKI *et al.*, 2017) já haviam estudado o efeito do tratamento térmico nesta liga observaram também uma melhor viabilidade celular de osteoblastos na superfície das ligas em comparação com o Ti-CP e o aço 316L. Apesar disso, os resultados não permitiram compreender os mecanismos de interação celular com a solução sólida.

E por fim, Lilensten e colaboradores (LILENSTEN; COUZINIE; BOURGON; PERRIÈRE *et al.*, 2016) utilizaram a teoria do orbital molecular, desenvolvido por Morinaga e Abdel-Hady (ABDEL-HADY; HINOSHITA; MORINAGA, 2006; MORINAGA; YUKAWA, 1997), para a formação de ligas de titânio com baixo módulo de elasticidade e com alta entropia. A partir da análise dos parâmetros eletrônicos (B_o e M_d), juntamente com a regra de Hume-Rothery, foi desenvolvida a liga tipo β metaestável $Ti_{35}Zr_{27,5}Hf_{27,5}Nb_5Ta_5$ (%at.), com elevada capacidade de endurecimento por trabalho a frio. Apesar disso, o estudo não procurou se aprofundar na relação da composição com suas propriedades para uma futura aplicação como biomaterial.

Apesar das várias ligas de alta entropia desenvolvidas para aplicação industrial ($HfNbTaTiZr$, $MoNbTaVW$ e $CrFeMnNiTi$, por exemplo), sua utilização na área biomédica ainda é um campo pouco explorado (SENKOV; MIRACLE, 2016). Como a utilização de implantes biomédicos envolve interação do material com tecidos e células do corpo humano, há a necessidade de se obter um conjunto de propriedades distintas das aplicações anteriores (WONG; HSU; WU; HO, 2023). O efeito conjunto dos elementos de liga em suas propriedades com foco em aplicação biológica é um tema de pesquisa ainda a ser melhor explorado. Além disso, ainda se faz necessário a melhor compreensão sobre os mecanismos de formação de solução sólida e precipitação de fase intermetálica nestas ligas (TSAI; YEH, 2014; WONG; HSU; WU; HO, 2023). Adicionalmente, não foi encontrado amplos estudos a respeito da modificação de superfície destas ligas visando aplicação biomédica, sendo crucial para aplicação, uma vez que é a primeira região de contato do material com o meio fisiológico do corpo humano (CORDEIRO; BARÃO, 2016).

Neste estudo, a liga equiatômica $TiZrNbTaMo$ foi selecionada como material de base devido ao seu promissor desempenho em aplicações biomédicas, conforme demonstrado em pesquisas anteriores (WANG; XU, 2018; WANG; XU, 2017). Com o objetivo de otimizar as propriedades da liga, duas novas composições foram desenvolvidas. A primeira, $TiZrNbTaMn$, foi obtida pela substituição do molibdênio (Mo) por manganês (Mn), visando explorar uma série de vantagens do Mn em ligas biomédicas. O Mn não só apresenta excelente biocompatibilidade e um baixo ponto de fusão, o que facilita o processamento da liga, como também desempenha um papel crucial na melhoria da resistência à corrosão, especialmente em ambientes fisiológicos ricos em cloretos, onde a degradação de materiais metálicos é um desafio constante (LOURENÇO; CORREA; DE ALMEIDA; KURODA *et al.*, 2024;

TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022). Adicionalmente, o Mn contribui para a redução do módulo elástico, ajudando a minimizar o efeito de stress shielding, uma vez que o módulo de elasticidade mais próximo ao do osso humano diminui o risco de reabsorção óssea ao redor de implantes (WU; TAN; AN; ZHANG *et al.*, 2022). O Mn também possui efeitos antioxidantes em ambientes biológicos, o que pode prolongar a vida útil dos implantes e reduzir a inflamação pós-cirúrgica, promovendo assim uma melhor integração com o tecido ósseo (TASKOZHINA; BATYROVA; UMAROVA; ISSANGUZHINA *et al.*, 2024).

A segunda liga, TiZrNbFeMo, foi projetada com a substituição do tântalo (Ta) por ferro (Fe), com o objetivo de diminuir o ponto de fusão, reduzir os custos de produção e agregar benefícios específicos do Fe. O Fe, além de ser um elemento amplamente disponível e econômico, Biesiekierski *et al* mostrou que as liga Ti–(Nb,Ta)–Fe têm mostrado um desempenho biológico promissor, com boa biocompatibilidade e potencial para melhorar a eficácia dos implantes biomédicos, desde que a quantidade de Fe seja otimizada para equilibrar todos os aspectos da resposta biológica (BIESIEKIERSKI; LIN; LI; PING *et al.*, 2016). Sua inclusão em ligas metálicas também melhora significativamente a resistência mecânica, promovendo uma maior durabilidade e resistência à fadiga, o que é crucial em implantes sujeitos a cargas cíclicas (BIESIEKIERSKI; LIN; LI; PING *et al.*, 2016; RABADIA; LIU; JAWED; WANG *et al.*, 2018). Por fim, o Fe favorece a produção de óxidos protetores que aumentam a resistência à corrosão, prolongando a vida útil do implante em condições fisiológicas adversas (FELLAH; HEZIL; HAMADI; IQBAL *et al.*, 2024; UEKI; HIDA; NAKAI, 2024).

1.3. Oxidação por Micro Arco (MAO) em ligas biomédicas

Técnicas de modificação de superfície são corriqueiramente aplicadas em implantes biomédicos, com intuito de melhorar, dentre outros aspectos, a biocompatibilidade, acelerar o processo de osseointegração e regeneração óssea, além de incluir uma ação antibacteriana (CARDOSO; GRANDINI; RAU, 2024; KIRMANIDOU, Y.; SIDIRA, M.; DROSOU, M. E.; BENNANI, V. *et al.*, 2016; SOUSA; COSTA; CORREA; ROCHA *et al.*, 2019). A biocompatibilidade de um implante metálico está intrinsecamente ligada à estabilidade da camada de óxido passivo que se forma em sua superfície. Essa camada, além de proteger o material contra a

corrosão, influencia a interação do implante com o tecido biológico. A composição química da liga desempenha um papel crucial na formação e estabilidade dessa camada, elementos como Zr, Nb Ta e Mo podem aumentar esta estabilidade(WONG; HSU; WU; HO, 2023). Ligas de Elementos de Alta Entropia (HEAs) oferecem uma flexibilidade única para o design de biomateriais com propriedades otimizadas. A inclusão de elementos como nióbio, conhecidos por promover a formação de óxidos estáveis, pode melhorar significativamente a biocompatibilidade dessas ligas (WONG; HSU; WU; HO, 2023). Yu e seus colaboradores (YU; BRODRICK; RYAN; SCULLY, 1999) demonstraram que o nióbio é eficaz na prevenção da ativação e despassivação da camada de óxido, o que resulta em uma maior resistência à corrosão e em uma interação mais previsível com o tecido ósseo. Entre essas técnicas de modificação de superfície, o Processo de Oxidação Anódica (MAO, do inglês *Micro-Arc Oxidation*) destaca-se como uma abordagem promissora (CARDOSO; GRANDINI; RAU, 2024).

No processo de Micro-Oxidação por Arco (MAO), a oxidação e a incorporação de elementos do eletrólito ocorrem por meio de reações eletroquímicas que são intensificadas pela presença de descargas elétricas e plasma na superfície do metal. O processo começa com a aplicação de uma alta tensão a um metal submerso em um eletrólito. O metal funciona como o ânodo, e o eletrólito é uma solução aquosa que pode conter íons metálicos, sais e aditivos. Quando a tensão aplicada atinge um nível crítico, ocorre uma quebra dielétrica da camada de óxido que se forma inicialmente, gerando microdescargas elétricas e estabelecendo um estado de plasma na superfície do metal (CARDOSO; GRANDINI; RAU, 2024; SIMCHEN; SIEBER; KOPP; LAMPKE, 2020).

Nesse ambiente de alta energia, o material da superfície do substrato (metal ou liga) reage com o oxigênio gerado pela eletrólise da água presente no eletrólito, resultando na formação de óxidos metálicos, principalmente na forma de uma camada de óxido. Simultaneamente, o plasma, que é composto de partículas ionizadas do eletrólito, promove a fusão e a incorporação de outros elementos do eletrólito na estrutura do revestimento (KURODA; DE MATTOS; GRANDINI; AFONSO, 2024). Dependendo da composição do eletrólito, esses elementos (como fósforo, silício, cálcio, entre outros) podem ser incorporados diretamente na matriz de óxido que está se formando (SOUSA; COSTA; CORREA; ROCHA *et al.*, 2019).

A técnica MAO oferece uma série de vantagens significativas para ligas biomédicas de Ti. Primeiramente, a camada de óxido formada é altamente aderente

ao substrato metálico (KURODA; ROSSI; GRANDINI; AFONSO, 2023), proporcionando uma proteção eficaz contra a corrosão e a degradação em ambientes biológicos (CARDOSO; GRANDINI; RAU, 2024). Além disso, a estrutura porosa da camada de óxido melhora a adesão de células e a integração com os tecidos ósseos, o que é crucial para a eficácia de implantes ortopédicos e dentários (OLIVEIRA; RIBEIRO; PEREZ; ARCHANJO *et al.*, 2015). O controle dos parâmetros do processo, eletrólito, tensão aplicada, limite de corrente temperatura entre outros permite a modificação das propriedades da superfície, como rugosidade, porosidade e composição química, adequando-as às necessidades específicas de cada aplicação biomédica (XUE; ATTARILAR; LIU; LIU *et al.*, 2020). Portanto, o MAO representa uma técnica valiosa para aprimorar as propriedades das ligas de titânio em aplicações biomédicas, contribuindo para a criação de implantes mais duráveis e biocompatíveis. A compreensão aprofundada dos mecanismos do MAO e das características das camadas de óxido formadas é essencial para otimizar o desempenho desses materiais e expandir suas aplicações na engenharia biomédica.

2. Objetivos

Este trabalho teve como objetivo desenvolver e caracterizar novas ligas de alta entropia, bem como superfícies biomultifuncionais utilizando a técnica de oxidação por micro-arco (MAO), com foco em aplicações biomédicas. Foram analisadas as propriedades mecânicas das ligas para obter as primeiras evidências sobre seu potencial como biomateriais. Também foram investigados os efeitos da fração volumétrica de fase, da microestrutura das ligas e da tensão aplicada durante os tratamentos de MAO na morfologia e na absorção de íons de Ca, P e Mg na superfície. Dessa forma, buscou-se produzir ligas de alta entropia com superfícies osseointegráveis, bioativas e biocompatíveis.

3. Materiais e métodos

3.1. Processamento e composição química das amostras

As ligas de alta entropia (HEA) não equiatômicas **TiZrNbTaMo**, **TiZrNbTaMn** e **TiNbZrFeMo**, denominadas **TaMo**, **TaMn** e **FeMo**, respectivamente, com uma massa de 60 g cada, foram produzidas a partir de metais comercialmente puros. Os metais precursores foram separados e decapados em um ataque químico com solução

aquosa de HNO_3 a 15% em volume por 15 segundos e limpos em banho ultrassônico em acetona por 10 minutos. Os lingotes então foram produzidos em um forno de fusão a arco-voltaico, com atmosfera de argônio, cadinho de cobre e eletrodo não consumível de tungstênio, ambos refrigerados em água. Para evitar a contaminação por impurezas, principalmente o oxigênio na liga, foi realizada a limpeza da câmara, através de um processo de purga, que consiste em criar um vácuo e, em seguida, inserir argônio na câmara. Esse processo foi repetido 5 vezes, com intervalos de 10 minutos entre cada etapa. Para garantir a completa mistura e homogeneidade da liga, os lingotes foram refundidos por no mínimo 10 vezes. Posteriormente, os lingotes foram submetidos a um tratamento térmico para homogeneização da microestrutura, com o auxílio de um forno túnel EDG, em vácuo de 10^{-7} Torr por 12 horas, em um patamar de 1000 °C, aquecimento de 10 °C/min, e posterior resfriamento lento. Os processos de fusão e tratamento térmico foram realizados no Laboratório de Anelasticidade e Biomateriais da Faculdade de Ciências, UNESP, Bauru (SP).

A composição química dos lingotes produzidos foi analisada por espectroscopia de energia dispersiva de raios X (EDX) com um espectômetro da marca bruker, modelo XFlash 6|30 acoplado à um Microscópio Eletrônico de Varredura da marca Tescan e modelo Mira pertencente ao Laboratório de Caracterização Estrutural (LCE)/DEMa na UFSCAR em São Carlos (SP).

3.2. Oxidação por Micro Arco (MAO)

A preparação das amostras para o processo de MAO seguiu os mesmos passos de lixamento, polimento ataque químico descritos o subtópico seguinte de caracterização microestrutural. O processo de modificação de superfície foi realizado utilizando o método MAO, sendo utilizada uma fonte de tensão contínua da Keysight, modelo N5751A, aplicando um potencial de 300 V e limitando a corrente em 2,5 A. A medição da corrente foi realizada com um multímetro digital de bancada da marca Agilent, conectado por cabos flexíveis a um computador para registrar os dados. O processo foi realizado em uma célula eletrolítica de 200 mL. A amostra (ânodo) foi praticamente submersa na solução, enquanto uma folha de platina atuou como cátodo. A distância entre o cátodo e o ânodo na célula eletrolítica foi mantida em aproximadamente 80 mm. O volume total de solução rica em Ca, P e Mg utilizado foi de 200 mL para cada tratamento.

Todos os experimentos foram conduzidos com agitação magnética da solução, assegurando um fluxo contínuo de íons por todo o volume da solução. O tempo de exposição da amostra ao tratamento foi de 3 minutos. Após esse período, a fonte de tensão foi desligada e as amostras foram lavadas minuciosamente com água destilada. O processo de MAO foi realizado no Laboratório de Anelasticidade e Biomateriais da Faculdade de Ciências, UNESP, Bauru (SP).

3.3. Caracterização estrutural e microestrutural

A caracterizações aqui descritas foram realizadas nas ligas após tratamento térmico de homogeneização, as ligas bruta de fusão não foram caracterizadas. Os lingotes foram cortados na sua seção transversal por um disco de corte diamantado em uma cortadora de precisão IsoMet 1000 (Buehler). As lâminas produzidas foram então submetidas a um processo de lixamento e polimento. O lixamento superficial das amostras foi realizado com lixas d'água nas granulações de 400 a 1500, utilizando uma politriz da Arotec. O polimento foi feito com panos auto-adesivos e soluções de alumina com granulometria de 0,25 μm , por 5 minutos. Após o polimento, as amostras foram colocadas em acetona por 5 minutos no banho ultrassônico. Para revelar a microestrutura das ligas, foi utilizada uma solução química composta por 15% de ácido nítrico (HNO_3), 5% de ácido fluorídrico (HF) e 80% de água destilada. A solução foi aplicada por 15 minutos. Em seguida, as amostras foram lavadas com álcool isopropílico por 15 minutos, acetona por 15 minutos e água destilada por 15 minutos, e por fim secas utilizando ar quente de um secador.

Foram adquiridas micrografias para análise microestrutural utilizando um microscópio óptico Olympus BX51M, o qual estava equipado com uma câmera conectada a um computador. Além disso, empregou-se também um microscópio eletrônico de varredura (MEV) do tipo EVO LS15 da Carl Zeiss, com filamento de tungstênio, ambos disponíveis na Faculdade de Ciências da UNESP/Bauru. O MEV desempenhou também a função de capturar micrografias do aspecto topográfico da superfície das amostras submetidas à oxidação por micro arco. As análises de seção transversal das amostras foram realizados por um microscópio eletrônico de transmissão (MET; JEOL JEM-2100F, 200 kV, modo STEM e TEM), com amostras preparadas por FIB (Feixe de íon focalizado, Thermo Fischer Scientific Helios Nanolab 660) no Laboratório Nacional de Nanotecnologia (LNNano), no Centro Nacional de

Pesquisa em Energia e Materiais (CNPEM), Campinas (SP). Por meio do programa CrysTBox foi possível analisar os padrões de difração de elétrons de área selecionada (SAED).

Os difratogramas de difração de raios X foram adquiridos por meio do difratômetro multiusuário RIGAKU DMAX ULTIMA, equipamento multiusuário pertencente ao Departamento de Física e Meteorologia, Faculdade de Ciências da UNESP – Campus Bauru. Para as medições, os parâmetros empregados foram os seguintes: modo Bragg-Brentano, com radiação $\text{CuK}\alpha$ ($\lambda = 1.544 \text{ \AA}$), corrente de 40 mA, voltagem de 40 kV, faixa de varredura em 2θ de 20° a 100° , tempo de exposição de 3,0 segundos por ponto e intervalo de varredura de $0,025^\circ$, no modo de tempo fixo. Os mesmos padrões foram utilizados para as medidas da amostra antes e após o tratamento de superfície. A estrutura cristalina e fração volumétrica de fases da microestrutura foram analisadas pelo método de Rietveld, usando o programa (LARSON; DREELE, 2004) e a interface EXPGUI (TOBY, 2001). As previsões teóricas de composição e quantificação de fases foram realizadas por meio do software ThermoCalc® software (SGTE PURE5 database).

3.4. Caracterização Mecânica

O ensaio de microdureza Vickers das ligas sem recobrimento foi realizado no Laboratório de Anelasticidade de biomaterias utilizando um microdurômetro da marca Shimadzu, modelo HMV-G. No presente estudo, as medidas de microdureza Vickers foram realizadas utilizando uma carga aplicada de 0,3 kgf. Em conformidade com as normas técnicas ASTM E92-16 e E384-16, todas as cargas foram aplicadas por um período de 15 segundos em todas as medições realizadas.

As medidas de módulo de elasticidade foram realizadas no sistema Sonelastic, desenvolvido pela empresa ATPC Physical Engineering, seguindo a norma E1876-15, da ASTM. Trata-se de um método de ensaio não destrutivo que utiliza as frequências naturais de oscilação para obter dinamicamente o módulo de elasticidade. Nesse procedimento, cada amostra é fixada nos pontos nodais para permitir sua vibração livre, sendo então sujeita a um leve impulso mecânico aplicado por um atuador. O decaimento das vibrações livres é monitorado por um microfone, revelando a resposta acústica característica da amostra. Esse decaimento é uma medida direta das propriedades elásticas da amostra, seu módulo de elasticidade, que é determinado

com base no som emitido após o impulso mecânico, sendo o processamento dos dados realizado pelo software do equipamento.

3.5. Caracterização Físico-Química

A composição química da camada externa dos filmes produzidos por MAO foi analisada por meio de espectroscopia de fotoelétrons excitados por raios X (XPS). As medidas de XPS foram realizadas com um espectrômetro XPS Thermo K-Alpha (Thermo Scientific, Inc.). A fonte de raios X era monocromática, com ânodo de alumínio ($AlK\alpha = 1486$ eV). O tamanho do *spot* do canhão de raios X foi de 400 μ m. O passo de energia foi de 200 eV para espectros em varredura de ampla energia e de 50 eV para espectros de alta resolução. A resolução usada foi de 1 eV e 0,01 eV, com um intervalo de energia de 0 a 1300 eV. Estas medidas de XPS foram realizadas no Laboratório Nacional de Nanotecnologia (LNNano), do Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas (SP).

As medidas de ângulo de contato foram realizadas em um goniômetro Ramé-Hart, com o uso de água destilada. Os valores médios foram obtidos de um total de 10 medidas aleatórias sobre as amostras. Estas medidas foram realizadas no Laboratório de Plasmas Tecnológicos, Instituto de Ciência e Tecnologia da UNESP - Campus Sorocaba (SP).

4. Resultados

O resultado apresentado no item 4.1 corresponde a um artigo preliminar, atualmente em fase de revisão para futura submissão. É importante destacar que os dados e análises contidos neste material ainda estão sujeitos a ajustes e validações adicionais, de modo a garantir a conformidade científica e atender aos padrões de publicação. Por essa razão, eventuais interpretações devem considerar o caráter provisório desta versão, que poderá sofrer modificações até sua forma final.

4.1. Artigo 1: Microstructure and selected mechanical properties of novel biomedical high-entropy alloys composed of some strategic non-refractory alloying elements

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Abstract

In this study, the low-cost alloying elements Mn and Fe were used as replacements for some refractory metals in the High Entropy Alloy (HEA) TiNbZrTaMo for potential use as biomaterials. The TiNbZrTaMo (TaMo), TiNbZrTaMn (TaMn), and TiNbZrFeMo (FeMo) alloys were produced by argon arc-melting from commercially pure raw metals. Afterward, a homogenization heat treatment was performed at 1273 K for 720 minutes and subsequent slow cooling. Then, the structural, microstructural, mechanical, and cytotoxic tests were performed to assess the viability of usage as biomedical implants. The TaMo sample depicted a major body-centered cubic (BCC) phase and minor hexagonal close packed (HCP), changing the phase composition with the addition of Mn and Fe, which have a dual HCP + BCC in coexistence with a cBCC (cluster-based body-centered cubic superlattice) phase. The identified phases were correlated with the theoretical predictions, depicting the typical dendritic microstructure of HEAs. The studied HEAs samples possessed higher Vickers microhardness and closed elastic modulus than the commercial Ti-6Al-4V alloy. The cytotoxicity assay confirmed the positive effect of the HEAs on cell viability and adhesion, with the FeMo sample depicting the most promising properties to be employed as biomaterials.

Keywords: biomaterial; high entropy alloys; crystalline structure; microstructure; mechanical properties; cytotoxicity.

4.1.1. Introdução

High-entropy alloys (HEAs) have gained attention in recent years due to their remarkable mechanical and thermal properties, resulting from combining multiple elements in equimolar or near-equimolar proportions (NENE; SINHA; YADAV; DUTTA, 2024; V; M, 2024). These alloys offer a promising alternative for advanced applications, especially in biomedical implants, where mechanical strength, biocompatibility, and thermal stability are crucial (CHANG; JING; LIU; QIU *et al.*, 2024). One of the major challenges in materials engineering for implants is the development of alloys that balance high strength with an appropriate elastic modulus, minimizing the effect of stress shielding. This phenomenon occurs when the implant is significantly stiffer than the surrounding bone, leading to bone resorption and long-term implant failure. (NIINOMI, 2002; SARRAF; REZVANI GHOMI; ALIPOUR; RAMAKRISHNA *et al.*, 2022). Titanium-based alloys, such as Ti-6Al-4V (Ti64), have been widely used in implants due to their high corrosion resistance and biocompatibility. However, their high elastic modulus compared to natural bone remains a limitation. High entropy alloys, on the other hand, present an opportunity to optimize mechanical properties such as hardness, wear resistance, and elastic modulus by adjusting the composition of transition elements like Ti, Zr, Nb, Ta, Mo, Mn, and Fe, which are known to significantly influence the crystallographic and mechanical properties of these alloys (RABADIA; LIU; JAWED; WANG *et al.*, 2018; WONG; HSU; WU; HO, 2023). Recent studies have shown that intermetallic phases, such as the Laves C14 phase, can significantly improve high-entropy alloys' microhardness and mechanical strength, increasing the resistance to dislocation movement (NENE; SINHA; YADAV; DUTTA, 2024; RYLTSEV; GAVIKO; ESTEMIROVA; STERKHOV *et al.*, 2021). Additionally, parameters such as the valence electron concentration (VEC) and the electron-to-atom ratio (e/a) play a significant role in the formation of crystalline phases such as body-centered cubic (BCC) and hexagonal close-packed (HCP), which directly affect the mechanical performance of the alloys (V; M, 2024; WONG; HSU; WU; HO, 2023).

Considering this, this study aims to explore the effect of selected metals (Mn and Fe) as replacements for some refractory alloying elements in the non-equiatomic HEA TiZrNbTaMo in the phase composition, microstructure and selected mechanical properties. The corresponding phase composition and microstructure were later correlated with some theoretical predictions commonly used in developing HEAs. After this, the potential for use as biomaterials was further evaluated by preliminary cytotoxicity assays with osteoblastic-like cells.

4.1.2. Materials and Methods:

4.1.2.1. Sample processing:

Near-equiatomic ingots of the HEAs (TiZrNb)-(TaMo), (TiZrNb)-(TaMn), and (TiZrNb)-(FeMo) were produced by argon arc-melting using commercially pure metals (purity higher than 99%). The metals were separated in an equiatomic proportion and melted in a water-cooled copper crucible by a tungsten electrode under an inert argon atmosphere. The ingots were re-melted ten times to guarantee a proper mixture of the alloying elements in the solid solution. Afterward, the ingots underwent a heat treatment for microstructural homogenization at a vacuum of 10^{-6} Torr, heating rate of $10\text{ K}\cdot\text{min}^{-1}$, temperature plateau of 1273 K for 720 minutes, and slowly cooled in a turned-off oven. For the current study, the HEAs (TiZrNb)-(TaMo), (TiZrNb)-(TaMn), and (TiZrNb)-(FeMo) samples were labeled as TaMo, TaMn, and FeMo,

4.1.2.2. Sample characterization

Theoretical predictions about the phase composition of the solid solution were obtained by the Thermo-Calc[®] version 2023b, with the TCHEA7 database (Thermo-Calc Software TCHEA7, 2023) and the HEAPS[®] (High-Entropy Alloys Predicting Software). The microstructure of the samples was analyzed by optical microscopy (OM; BX51M microscope, Olympus Inc., USA) and scanning electron microscopy (SEM; EVO LS15 microscope, Carl Zeiss Inc., USA) coupled with an energy dispersive x-ray detector (EDS; Inca X-Act, Oxford Inc., USA). The samples were previously ground in SiC water sandpapers from #360 up to #2000 mesh, polished in Al_2O_3 colloidal suspension ($0.25\text{ }\mu\text{m}$), and etched in Kroll's solution.

Crystalline structure and phase composition were evaluated by x-ray diffraction measurement (XRD; MiniFlex 600 diffractometer, Rigaku Analytical Devices Inc., USA) at 40 kV and 15 mA, with monochromatic $\text{CuK}\alpha$ radiation (0.15406 nm) in the powder method, θ - 2θ configuration, fixed time mode, step size of 0.04° , collecting time of 1.6 sec. The diffracted peaks were indexed by the comparison with crystallographical datasheets from the COD (Crystallography Open Database). The Rietveld method calculated the cell parameters and phase proportion using the GSAS[®] software (LARSON; DREELE, 2004) and EXPGUI[®] interface (TOBY, 2001), with a standard Y_2O_3 sample to remove the instrumental contribution.

4.1.2.3. Sample testing:

Selected mechanical properties were evaluated using Vickers micro-hardness and elastic modulus measurements. The Vickers micro-hardness values were obtained using an HMV-2 microhardener (Shimadzu Co., USA) with a load of 300 gf (2.942 N) and a dwell time of 15 seconds. The elastic modulus was measured in rectangular samples (100 × 10 × 1.5 mm) using the impulse excitation method and the Sonelastic® software (ATCP Physical Engineering Co., Brazil). Ten measurements were taken on each sample to calculate the average and standard deviation values.

Preliminary in vitro cytocompatibility was assessed using the MTT assay with MC3T3E1 (subclone 4) mouse osteoblastic cells. Before testing, the cells were cultured in α -MEM with 10% FBS (fetal bovine serum) in a 5% CO₂ environment at 37 °C. The samples were added to the culture medium at a proportion of 0.1 g·mL⁻¹ and kept for 24 hours. The conditioned medium was collected and placed in a 96-well dish plate, the cells were seeded at 5 × 10⁴ cells per mL, and cultured for 24 hours in contact with the conditioned medium. Cell viability was assessed using the MTT assay, and the absorbance was measured at 570 nm using a microplate reader (Synergy-HTX multi-mode reader, Biotek Inc., USA).

For statistical analysis, GraphPad Prism® software was used with one-way ANOVA and Tukey post-test, with $p < 0.05$ considered statistically significant and <0.0001 highly significant. Additional experimental details can be found in the study of *Torrento et al* (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA et al., 2022).

4.1.2.4. Results and discussion

Table 1 shows the corresponding chemical composition of the ingots produced for this study. The selected alloying elements presented in the TaMo, TaMn, and FeMo samples are in considerable amounts.

Table 1: Chemical composition of the ingots taken by EDS.

Sample	TiZrNbTaMo	TiZrNbTaMn	TiZrNbFeMo
Designation	TaMo	TaMn	FeMo
Ti (wt.%)	13.3 ± 0.9	10.4 ± 1.1	12.7 ± 0.6
Zr (wt.%)	32.4 ± 1.9	11.0 ± 2.9	14.2 ± 0.9
Nb (wt.%)	19.4 ± 2.0	43.0 ± 2.2	25.9 ± 0.5

Ta (wt.%)	25.8 ± 0.8	22.1 ± 0.5	-
Mo (wt.%)	9.1 ± 1.2	-	29.0 ± 0.7
Mn (wt.%)	-	13.6 ± 1.3	-
Fe (wt.%)	-	-	18.3 ± 1.2

Based on the experimental chemical composition, some theoretical parameters were calculated for the samples using the HEAPS[®] software. This free tool enables the exploration and design of high-entropy alloys by calculating physical and semi-empirical parameters and assessing multiple criteria to predict phase formation and mechanical properties (MARTIN; MADRID-CORTES; CÁCERES; ARAYA *et al.*, 2022). The calculated values for the valence electron concentration (VEC), electron-atom ratio (e/a), average atomic radii difference (δr), electronegativity ($\Delta\chi$), mixing enthalpy (ΔH^m), and mixing entropy (ΔS^m) are shown in **Table 2**. Considering the Hume-Rothery rules, it is possible to predict complete miscibility between the alloying elements, with the thermodynamic parameters defining the samples as high entropic materials.

Table 2: Theoretical parameters of the studied HEAs

Sample	VEC	e/a	δr (%)	$\Delta\chi$	ΔH^m (kJ·mol⁻¹)	ΔS^m (J·mol⁻¹·K⁻¹)
TaMo	4.63	1.72	5.76	0.224	0.56	12.63
TaMn	5.06	1.57	4.46	0.082	-1.27	12.01
FeMo	5.57	1.45	7.64	0.297	-10.15	12.95

The results from **Table 1** were also used to calculate the samples' phase diagrams using ThermoCalc[®] software, as shown in **Figure 1**. The results indicated a melting point below 2700 K with the addition of Mn and Fe, having a major BCC crystalline structure at room temperature and in thermodynamic equilibrium. A secondary HCP phase was predicted to be present in all samples and could be related to the C14 Laves phase. Lastly, the addition of Fe and Mn also permitted the formation of a third phase designated as cBCC (cluster-based body-centered cubic superlattice), which can significantly impact these properties.

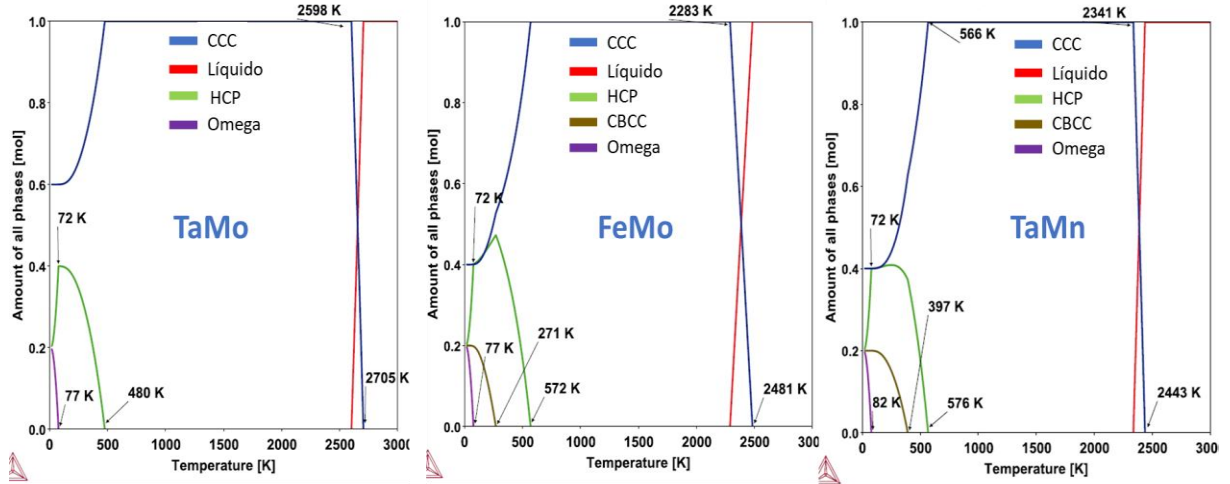


Figure 1: Calculated phase diagram of the samples.

Figure 2 shows the XRD profiles of the samples and their respective phase compositions. The TaMo sample depicted a major BCC phase (~ 90%) with a secondary HCP phase, following the predictions of **Figure 1**. However, the FeMo and TaMn samples exhibited a more complex XRD profile, with the presence of the BCC and HCP phases and some additional peaks from a third phase. The third phase was quantified jointly with the HCP one for the Rietveld refinement, resulting in a predominant phase proportion compared to the BCC phase. From this point, it is possible to classify the studied samples as major BCC (TaMo), dual HCP + BCC (FeMo), and major HCP (TaMn), respectively. Compared to the simulations performed, it is possible to identify that the simulations were accurate regarding the phases present in the material; however, the phase quantifications were somewhat distant from the actual values. This discrepancy in quantification can be attributed to the fact that the simulations were conducted under equilibrium conditions for the alloys. However, even with homogenization heat treatment, achieving equilibrium conditions in the alloys isn't easy. High entropy alloys (HEAs) consist of elements with different atomic sizes, and during the solidification process, this atomic disparity induces deformation and stress in the crystal lattice of these alloys. This lattice distortion reduces the impact of thermal effects on the properties of the alloys (ARUN; RADHIKA; SALEH, 2024). This is because the atomic mobility is lower in HEAs due to their distorted crystal lattices. This characteristic imparts greater thermal stability to HEAs (KUMAR; SEETHARAM; PONAPPA, 2024).

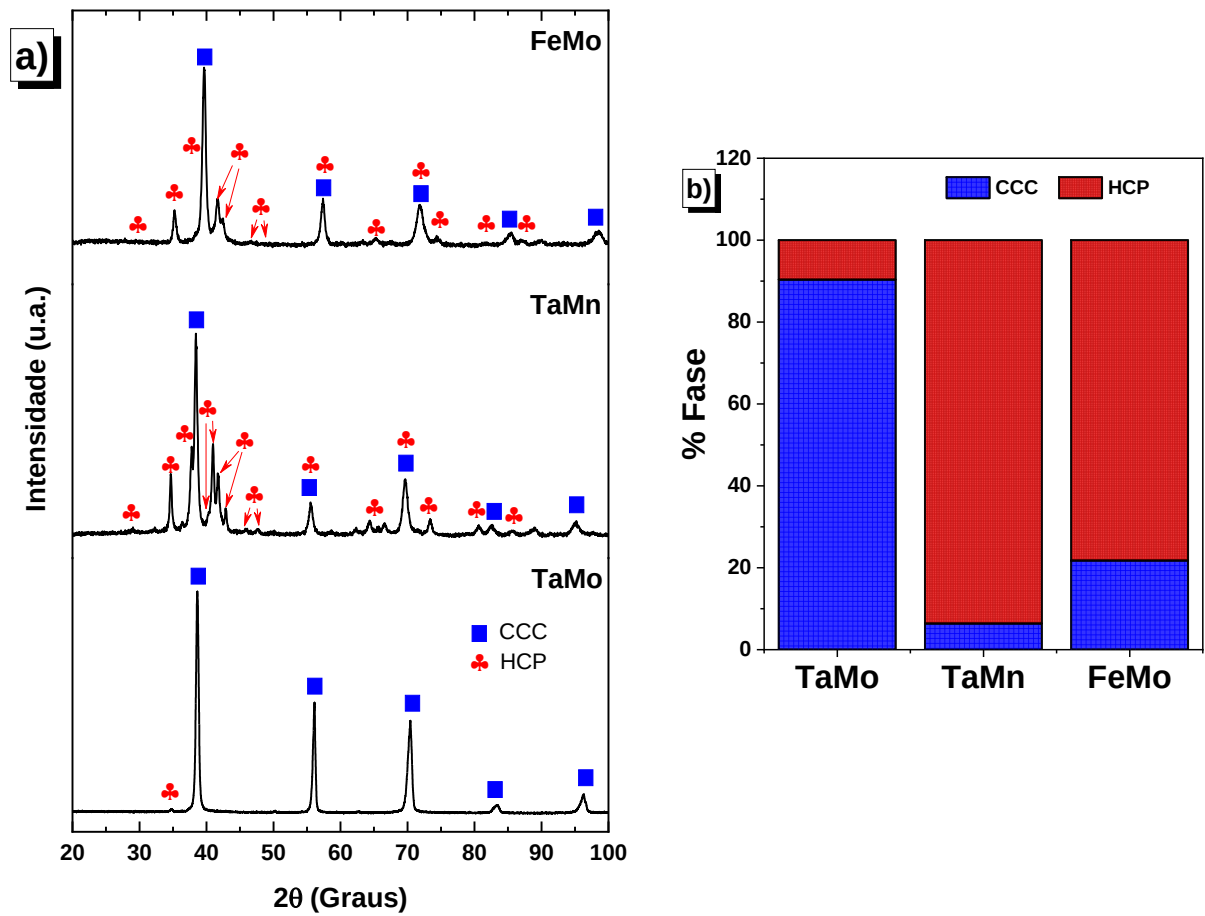


Figure 2: XRD profiles (a) and corresponding phase composition (b) of the samples acquired by the Rietveld method.

The optical and SEM imaging of the samples is illustrated in **Figure 3**, where some grain boundaries for the TaMo sample and the typical dendritic structure for the TaMn and FeMo samples can be seen. As reported in the literature (ARUN; RADHIKA; SALEH, 2024; DE OLIVEIRA; FAGUNDES; CAPELLATO; SACHS *et al.*, 2022), the melting point, atomic radius, and electronegativity can cause an impact on the atomic diffusion and chemical segregation between the grain boundaries and dendrites during solidification, resulting in complex microstructures.

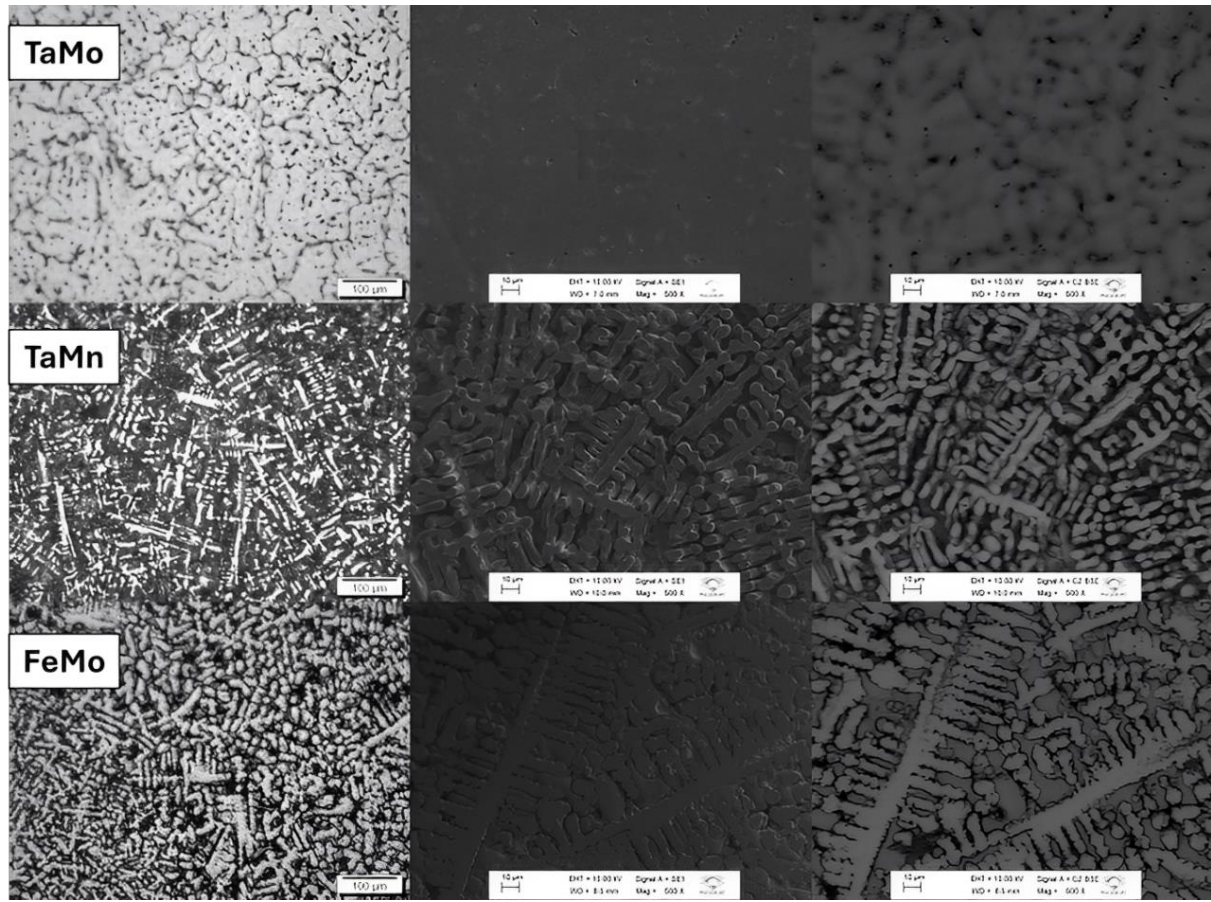


Figure 3: Optical and SEM imaging of the samples

Vickers microhardness is an essential measure in evaluating the mechanical properties of alloys used in biomedical implants, playing a crucial role in determining wear resistance, deformation, and surface integrity. This characteristic is vital to ensure the longevity and functionality of implants, especially those subjected to cyclic loads and repetitive movements, such as joint prostheses articulação (ELIAS; LIMA; VALIEV; MEYERS, 2008; WANG, 1996). Moreover, microhardness directly influences the material's biocompatibility, as inadequate hardness can lead to abrasion of surrounding tissue or structural failure of the (GEETHA; SINGH; ASOKAMANI; GOGIA, 2009b). Additionally, microhardness greatly affects the production of medical devices, as lower microhardness can facilitate the machining and shaping of materials, allowing for the manufacture of components with stricter tolerances and more refined surface finishes (CARDOSO; KURODA; GRANDINI, 2023). Therefore, accurate assessment of Vickers microhardness is indispensable in developing and quality control biomedical implants, ensuring they meet rigorous clinical safety and long-term performance requirements (NIINOMI, 2002).

Figure 4 compares the Vickers microhardness and elastic modulus values of the samples. All the samples presented Vickers microhardness higher than the commercial Ti-6Al-4V alloy (~ 400 HV), which is conventionally used to manufacture metallic implants. While the TaMo sample depicted values close to those reported by Wang et al. (WANG; XU, 2017) and Torrento et al. (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022), the TaMn and FeMo presented higher values, probably due to the presence of the C14 Laves phase. Thus, the augmented hardness of the samples can be linked with a combination of the solid solution and phase precipitation hardening mechanisms.

The valence electron concentration (VEC) plays a crucial role in determining the mechanical properties of high entropy alloys (HEAs). Recent studies have demonstrated a significant correlation between VEC and the microhardness of these alloys (GORBAN'; KRAPIVKA; FIRSTOV, 2017; STEIN; LEINEWEBER, 2020), influencing the formation of crystal phases such as BCC, FCC, and the C14 Laves phase. Alloys with higher VEC tend to favor the formation of FCC phases, which are associated with greater ductility, while lower VEC values promote BCC phases that exhibit higher hardness and mechanical strength mecânica (NENE; SINHA; YADAV; DUTTA, 2024). Furthermore, recent research indicates that controlling the VEC, in conjunction with the potential formation of the C14 Laves phase, may optimize mechanical properties such as hardness and wear resistance, thereby tailoring HEAs for specific applications (YANG; SHAO; DUAN; CHEN *et al.*, 2021).

On the other hand, the TaMo sample had elastic modulus values at the same level as those of the Ti-6Al-4V (~ 110 GPa), within the values reported by Torrento et al. (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022). The TaMn sample exhibited the highest value (134 GPa), probably associated with the cBCC phase precipitation as a third phase. Lastly, the FeMo sample depicted the lowest value, having minor BCC and cBCC phases to the HCP one.

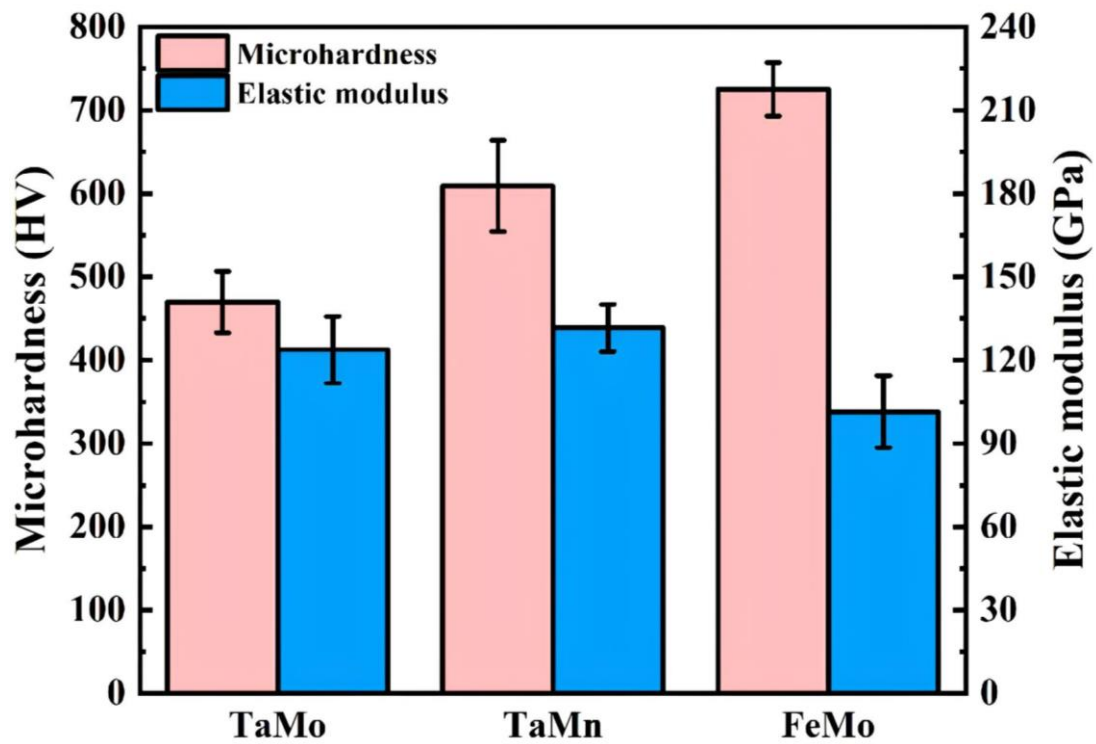


Figure 4: Selected mechanical properties of the samples

The modulus of elasticity is a crucial mechanical property of high-entropy alloys (HEAs) intended for biomedical applications, such as orthopedic implants. Having an appropriate modulus of elasticity is essential to ensure mechanical compatibility between the implant and the surrounding bone, reducing the risk of adverse effects, such as bone resorption due to disuse. This can occur when there is a significant difference in stiffness between the implant and natural bone tissue (GONÇALVES; CORRÊA; DE SOUSA; PINTÃO *et al.*, 2024; KURODA; QUADROS; NASCIMENTO; GRANDINI, 2021; NIINOMI; NAKAI, 2011). Considering the performance of the studied HEA samples compared to the commercial Ti-6Al-4V alloy, it is possible to highlight the feasibility of using them as biomaterials.

Figure 5 shows the preliminary results for the MTT 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), it is a widely used assay to evaluate cell viability and metabolic activity in cell cultures and crystal violet (CV) assays after 24h of cell culturing. It can be noted that all the samples presented cell viability and adhesion at the same level or even statistically higher than the positive control, especially for the FeMo sample, which presented the highest values. These results show that the samples did not exhibit any harmful effects on osteoblastic cell viability and adhesion,

indicating potential for use as biomaterials. Further biological tests can more profoundly verify the studied samples' biocompatibility for the intended application.

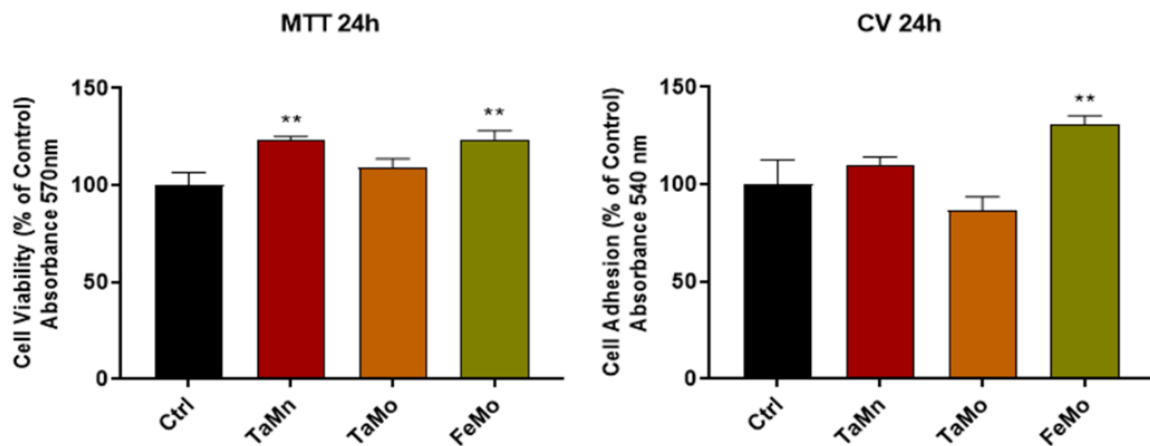


Figure 5: Preliminary cytotoxicity evaluation of the samples

4.1.3. Conclusion

From the results reported in this study, it is possible to conclude that the TiZrNbTaMo, TiZrNbTaMn, and TiZrNbFeMo HEAs had favorable characteristics to be used as biomedical implants, considering the following:

- The structural analysis revealed that the alloying elements played a role in the formation of the compact crystalline structures BCC, HCP (Laves C14 phase), and cBCC, which were further confirmed by the theoretical predictions;
- The replacement of some alloying elements by Mn and Fe resulted in dendritic structures with distinct sizes, which probably indicated chemical segregation of the alloying elements;
- The Vickers microhardness of the HEAs was affected by the alloying elements throughout the solid solution and phase precipitation hardening effects, with higher values than the Ti-6Al-4V alloy;
- The elastic modulus was also sensible to the alloying elements and phase composition, having values closer to the Ti-6Al-4V alloy;
- The HEAs did not exhibit any harmful effects on the cell viability and adhesion after 24h of culturing with osteoblastic cells;
- The studied HEA samples, composed by some non-refractory alloying elements, depicted promising properties for use as implants.

4.1.4. Acknowledgement:

The authors thank Professor F.M.L. Pontes from the Department of Chemistry (UNESP - Campus Bauru) for the XRD measurements.

4.1.5. Funding:

This study was funded by the Coordination of Superior Level Staff Improvement (CAPES; Finance Code 001), National Council for Scientific and Technological Research (CNPq; grants #407251/2018-9 and #404020/2023-2) and São Paulo Research Foundation (FAPESP; grants #2018/24931-7, #2021/13921-3, and #2024/03148-3) funding agencies.

4.1.6. Data availability

The raw-processed data required to reproduce these findings can be shared under request.

4.1.7. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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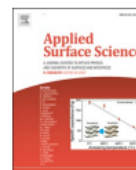
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4.2. Artigo 2: Surface aspects of novel Bio-HEAs MAO-treated in a Ca-, P-, and Mg-rich electrolyte

Este subcapítulo de resultados apresenta, na íntegra, o artigo publicado na revista *Applied Surface Science* em 2024. O artigo completo pode ser acessado através do seguinte link:

<https://www.sciencedirect.com/science/article/abs/pii/S0169433224009401>.



Full Length Article

Surface aspects of novel Bio-HEAs MAO-treated in a Ca-, P-, and Mg-rich electrolyte

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Surface aspects of novel Bio-HEAs MAO-treated in a Ca-, P-, and Mg-rich electrolyte

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Abstract

This study evaluated the effect of alloying elements, applied voltage, and bioactive species (Ca, P, and Mg) enrichment during the micro-arc oxidation (MAO) treatment of novel high entropy alloys (HEAs) targeted for biomedical applications. The non-equiatomc TiZrNbTaMo (TaMo), TiZrNbTaMn (TaMn), and TiZrNbFeMo (FeMo) samples were submitted to MAO treatment at distinct voltages (100, 200, and 300 V), then their chemical, phase, and morphological aspects were evaluated by x-ray photoelectron spectroscopy (XPS), x-ray diffraction (XRD), and scanning and transmission electron microscopy (SEM and TEM). Finally, the preliminary physicochemical behavior regarding contact angle and surface energy in distilled water

at room temperature was evaluated. The results indicated that the alloying elements played a global role in the bulk's phase composition, possessing distinct proportions of BCC and HCP phases. The MAO-treated surfaces depicted different mechanisms of anodic oxide growth and dielectric breakdown, forming the typical porous surface at distinct applied voltages. The bioactive species (Ca, P, and Mg) were gradually enriched in the oxide layer, composed of distinct nano-crystalline and amorphous layers that are typical of MAO-treated surfaces. As a result, roughness, contact angle and surface energy values of the MAO-treated samples were affected by the applied voltage and the bulk's chemical composition, indicating potential for use in the biomedical field, especially as long-term implants and bone fixation devices.

Keywords: Biomaterial; HEA; MAO; Voltage; Bioactive elements.

4.2.1. Introduction

Metallic biomaterials are mainly used as biomedical implants, surgical instruments, and bone fixation devices, being commercial products mainly composed of stainless steel, cobalt-chromium alloys, and Ti and its alloys (DAVIS; SINGH; JACKSON; COELHO *et al.*, 2022). However, the advent of high entropy alloys (HEAs) as an emerging material in the 2000s has attracted the attention for biomedical applications (LIU; YANG; LIU; TANG *et al.*, 2022). Thus, several studies have been conducted to develop Bio-HEAs with favorable properties for use in Medicine.

The equiatomic TiZrNbTaMo alloy was one of the first HEAs designed for use as orthopedic implants (Bio-HEA), due to its non-toxic and non-allergenic chemical composition and superior properties. The previous results indicated a body-centered cubic (BCC) phase in the form of equiaxed grains, which guaranteed high mechanical strength, low elastic modulus, high corrosion strength, and proper biocompatibility (HORI; NAGASE; TODAI; MATSUGAKI *et al.*, 2019; NAGASE; TODAI; HORI; NAKANO, 2018; TODAI; NAGASE; HORI; MATSUGAKI *et al.*, 2017). Further studies with non-equiatomic composition indicated that the precipitation of the secondary hexagonal close-packed (HCP) phase can also tune its properties and open new horizons for use as biomaterials (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022). In the meantime, considering that Nb, Ta, and Mo are refractory metals, producing this Bio-HEA and maintaining low processing

costs is challenging. Thus, the replacement of these alloying elements by ones with low-cost and low melting points can be useful to reduce the melting point and facilitate the manufacture of Bio-HEAs (WANG; YANG; WANG; DAI *et al.*, 2022). In this scenario, Fe and Mn can be adequate candidates to form Bio-HEAs once the elements are naturally found in the human body and play a role in critical biological reactions at the cellular level (JOMOVA; MAKOVA; ALOMAR; ALWASEL *et al.*, 2022).

Within several surface modification techniques, micro-arc oxidation (MAO) has been constantly employed to coat valve metals, for instance, Ti and its alloys (LI; MA; LIU; QI *et al.*, 2023). The MAO treatment is based on applying a high anodic voltage on the Ti surface under an electrolyte enriched with chemical species. As a result, oxidative reactions and dielectric breakdown induce micro-arc discharges throughout the oxide layer (SIMCHEN; SIEBER; KOPP; LAMPKE, 2020). Current studies have highlighted the advantages of the MAO treatment and their electrical parameters to promote biocompatibility, osseointegration, and bioactivity on the Ti surface, especially those enriched with Ca, P, and Mg (CHU; LIU; CHEN; LI, 2020; WEN; LIU; XI; ZHANG *et al.*, 2023). However, in the case of multicomponent alloys, such as Bio-HEAs, the MAO treatment can result in complex surface reactions, resulting in a diverse variety of oxides according to their chemical and phase composition.

In this scenery, more studies regarding the growth mechanisms of MAO coatings in HEAs are required to certify this potential usage as biomedical implants, once the growth mechanisms can be changed in according with the alloying elements in solid solution. Recently, Xie *et al* (XIE; WANG; CUI; HU *et al.*, 2024), applied MAO treatment in Ti-xFe ($x = 3, 5$, and $9 \text{ wt}\%$) alloys, finding a porous $\text{TiO}_2/\text{Fe}_3\text{O}_4$ coatings with proper corrosion resistance and antimicrobial activity. And, Sangur *et al.* (SONGUR; DIKICI; NIINOMI; ARSLAN, 2019) used the MAO treatment to produce a anticorrosive and bioactive coatings on the Ti-29Nb-13Ta-4.6Zr ($\text{wt}\%$) alloy, which indicated the presence of anatase and rutile TiO_2 compounds, permeated with hydroxyapatite and tricalcium phosphate. While, Cheng *et al.* (CHENG; YANG; XU; SHAO *et al.*, 2024) submitted the refractory HEA AlTiCrVZr to MAO treatment, where it was detected a competition between the oxidation mechanisms of the alloying elements, having the inner layer formed by the oxides Al_2O_3 , TiO_2 , V_2O_5 , ZrO_2 , and Cr_2O_3 , while the outer layer was enriched with SiO_2 from the electrolyte. Lastly, Zhang *et al.* (ZHANG; KHANLARI; HUANG; LI *et al.*, 2023) produced a dual coating (amorphous matrix and HFZrO_2 nanocrystals), with anticorrosive and biocompatible

properties, in the HEA TiNbTaZrHf alloy after MAO treatment at distinct applied voltages.

This study investigated the effect of the alloying elements, applied voltage during the MAO treatment, and electrolytes' chemical composition on the oxide layer growth of novel Bio-HEAs. The applied voltage was varied to assess the distinct stages of oxidative reactions and dielectric breakdown in each sample. Then, the phase composition, morphology, and wettability were evaluated and correlated with the bulk and electrolyte aspects.

4.2.2. Materials and methods

4.2.2.1. Sample processing

Ingots of the HEA samples (60 g) were produced from commercially pure metals (Ti, Zr, Nb, Ta, Mo, Fe, and Mn), previously separated in equiatomic proportion. Afterward, the metals were etched in an aqueous 15 vol% HNO₃ solution for 15 s and cleaned ultrasonically in an acetone bath for 10 min. The metals were cast in an argon arc melting furnace under an inert argon atmosphere using a non-consumable tungsten electrode and a water-cooled copper crucible. The ingots were re-cast seven times to guarantee a complete moisture of the alloying elements. After that, the bottom-shaped ingots were subjected to homogenization heat treatment under a vacuum of 10⁻⁶ Torr, a temperature plateau of 1273 K for 43.2 ks, and posterior slow cooling. **Table 1** summarizes the samples' nomenclature and experimental chemical composition of the as-cast samples obtained by EDX (Energy Dispersive X-ray Spectroscopy, Bruker, USA).

Table 1 - Chemical composition of the samples in this study.

Sample	TiZrNbTaMo	TiZrNbTaMn	TiZrNbFeMo
Designation	TaMo (at%)	TaMn (at%)	FeMo (at%)
Ti	25 ± 1	18 ± 1	20 ± 1
Zr	31 ± 2	10 ± 3	11 ± 1
Nb	20 ± 2	39 ± 2	22 ± 1
Ta	14 ± 1	13 ± 1	-
Mo	10 ± 1	-	25 ± 1
Mn	-	20 ± 1	-
Fe	-	-	22 ± 1

4.2.2.2. Surface treatment

Before the surface treatment, the ingots were cut into lamellar-shaped samples using a diamond cutting wheel and a precision cut machine (IsoMet 1000, USA). Then, the samples were etched in an aqueous 15 vol% HNO₃ and 5 vol% HF solution for 15 s and ultrasonically cleaned in acetone for 300 s. The MAO treatment was conducted in a DC power source (Keysight, model N5751A, USA). The sample was set up as an anodic electrode, while a platinum disc was used as a cathodic one. The MAO treatment was conducted at voltages of 100, 200, and 300 V, with a limited current of 2.5 A, for 180 s. As the samples had a surface area of around 2.6 cm², the estimated current density remained around 1.0 A·cm⁻² during the galvanostatic period of the MAO treatment. The electrolyte was composed of 0.35 M β - glycerol phosphate pentahydrate (C₃H₇Na₂O₆P·5H₂O), 0.02 M calcium phosphate monohydrated (C₄H₆CaO₄·H₂O), and 0.1 M magnesium acetate tetrahydrated (C₄H₆MgO₄·4H₂O), following earlier studies from the literature (OLIVEIRA; RIBEIRO; PEREZ; ARCHANJO *et al.*, 2015; SOUSA; COSTA; CORREA; ROCHA *et al.*, 2019). The electrolyte was constantly mixed by a magnetic stirrer. The MAO treatment was done in triplicate for each condition, and the MAO-treated samples were later cleaned in distilled water and dried in a hot air stream.

4.2.2.3. Sample characterization

The microstructural and topographical characteristics were analyzed by scanning electron microscopy (SEM; Carl Zeiss microscope, model LS15 EVO, USA). For the microstructural analysis, the samples were submitted to grinding with SiC waterproof emery papers until #1500 mesh and polishing with colloidal diamond (1.0 μ m) and silica (0.25 μ m) suspensions. The mirror-like surfaces were immersed in an aqueous 10 vol% HNO₃ and 5 vol% HF solution for around 30 s to reveal the microstructure. Specific features in the cross-sectional view of the coatings were evaluated by scanning transmission electron microscopy (STEM; JEOL JEM-2100F, USA; 200 kV), performed in a lamella produced by focused ion beam (FIB; Thermo Fischer Scientific Helios Nanolab 660, USA). The selected area electron diffraction (SAED) patterns were indexed by the CrysTBox[®] software.

The crystalline structure was evaluated by x-ray diffraction (XRD; Panalytical diffractometer, X' Pert Pro model, USA), with monochromatic $\text{CuK}\alpha$ radiation source (0.1544 nm), operating at 40 kV and 40 mA, Ni filtered, in the fixed time mode, collecting time of 3.0 s, and step-size of 0.03° . The XRD profiles were compared with crystallographic data sheets from the Crystallographic Open Database (COD). Semi-quantitative chemical analysis was performed by x-ray photoelectron spectroscopy (XPS; Thermo Scientific Spectra Inc., model Thermo K-alpha, USA) operating with an $\text{AlK}\alpha$ radiation source (1486 eV), with energy correction by the C1s peak. The XPS results were analyzed using the CasaXPS[®] software version 2.3.24.

Roughness values were taken by confocal laser microscopy (DCM3D, Leica, USA). The wettability of the samples was evaluated by contact angle measurements using a Ramé-Hart goniometer (Ramé-Hart Instrument Inc., Cedar Knolls, NJ, USA), model 100-00, and distilled water and diiodomethane droplets with volume of 20 μL at room temperature. The average values were taken from 3 droplets randomly inserted in the surfaces.

4.2.3. Results and discussion

The XRD profiles and corresponding SEM images of the bulk and MAO-treated TaMo samples are depicted in **Fig. 1**. The bulk TaMo sample presented intense peaks from the BCC phase (COD number #44391) and faint peaks from the HCP phase (COD number #43416). All MAO-treated samples exhibited peaks from the bulk phase, indicating a thin oxide layer over the surface. However, it is possible to note a peak from MgCO_3 (COD number #2101057) together with a smooth halo from an amorphous phase in the zoomed view at 300 V, which follows some earlier reports with MAO treatment of β -type Ti alloys (BCC crystal structure) (KURODA; DOS SANTOS; ROSSI; CORREA *et al.*, 2023). Regarding the microstructure, the bulk TaMo sample had a granular structure, agreeing well with previous studies in the literature (TORRENTO; SOUSA; CRISTINO DA CRUZ; SANTOS DE ALMEIDA *et al.*, 2022; WANG; XU, 2018). At 100 V, it is impossible to identify any surface pore formation, indicating an absence of dielectric breakdown in the oxide layer. However, augmenting the voltage over 200 V, it was able to obtain the typical porous structure of MAO-treated metallic surfaces. The further increase of the voltage for 300 V resulted in an apparent enlargement of the pore size and the incorporation of chemical species from the

electrolyte, as exposed in the XRD profiles. Pore size enlarges with the voltage augmenting once it promotes high intensity electric arcs, forming O_2 , H_2 , and vapor bubbles. The vapor bubbles provide extreme pressure and temperature locally on the surface, favoring the formation of amorphous ceramic oxides (SIKDAR; MENEZES; MACCIONE; JACOB *et al.*). In addition, a high applied voltage results in more driving-force for incorporating the elements present in the electrolyte into the coating (FATTAH-ALHOSSEINI; MOLAEI; BABAEI, 2020). Earlier studies in the MAO treatment of multi-component Ti-based alloys, e.g. Ti-20Zr-10Nb-4Ta and Ti-25Ta-10Zr-15Nb (wt%), have found similar results in terms of phase composition and morphology, which assured proper corrosion resistance and biocompatibility (BORCHERS; PIELER, 2010).

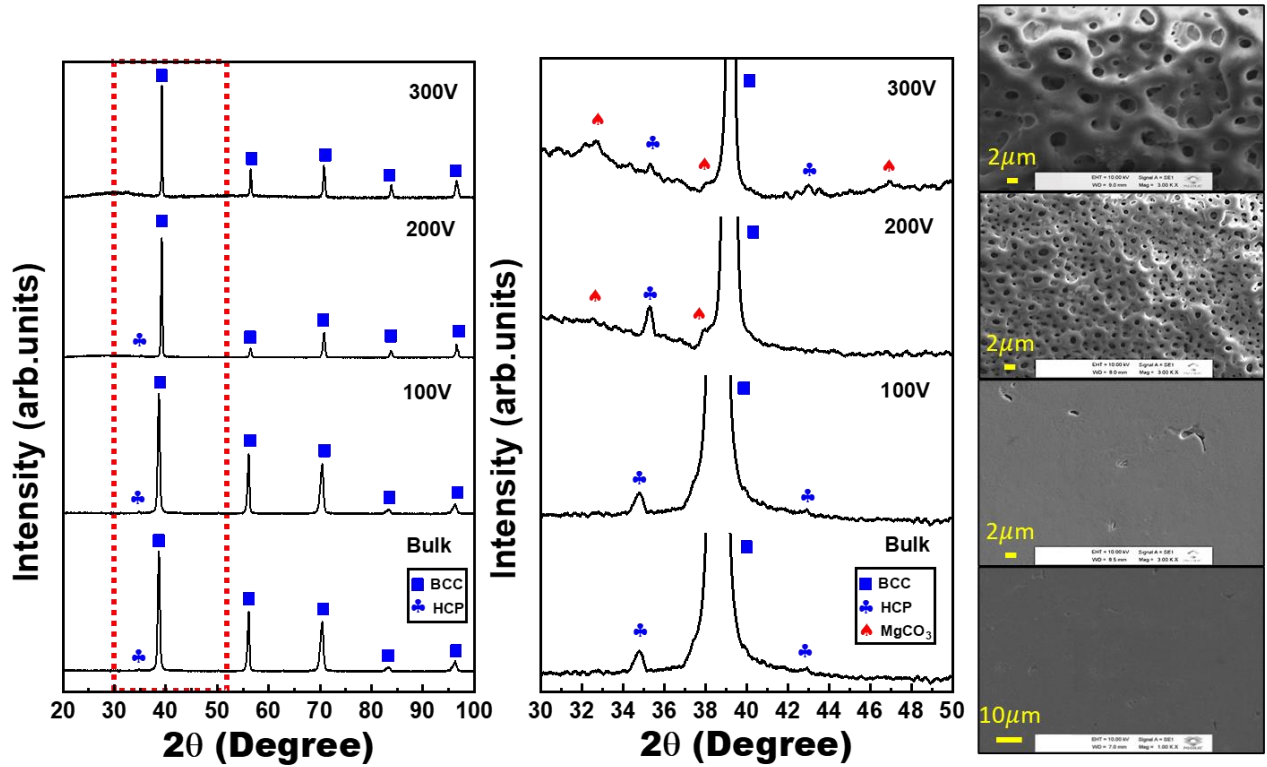


Fig 1: XRD profile and morphology of the MAO-treated TaMo sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.

For the TaMn sample (**Fig. 2**), the XRD profile exhibited a predominance of HCP in detriment to the BCC phase. The MAO-treated TaMn samples presented more pronounced oxide peaks above 200 V, indicating a distinct tendency for anodic oxidation. At 300 V, the TaMn sample also depicted new and intense peaks, which matched those from $MgCO_3$ and Ca_3PO_4 (COD number #1517238) compounds, as indicated in the zoomed view of the XRD profile. Thus, it can be concluded that the

sample incorporated vast amounts of bioactive species from the electrolyte, being positive for its bioactivity. The microstructure of the bulk exhibited dendritic structures with an elevated aspect ratio. The MAO-treated sample at 100 V and 200 V presented a rough and non-uniform surface, evidencing a distinct growth mechanism of the anodic oxide layer compared to the TaMo sample. When the voltage reached 300 V, the surface presented the typical porous layer with a non-rounded shape pore. Overall, the morphological aspects followed the steps established for the anodic oxidation and dielectric breakdown of valve metals, Ti, Al, and Mg, for instance (ALIOFKHAZRAEI; MACDONALD; MATYKINA; PARFENOV *et al.*, 2021). Cardoso *et al.* (CARDOSO; BARBARO; KURODA; IMPERATORI *et al.*, 2023) found similar results for the MAO-treated Ti-30Nb-5Mo (wt%) alloy, having the enriched surface with Ca, P, and Mg provided superior cell growth for use as biomedical implants. The high amount of MgCO_3 and Ca_3PO_4 compounds on this sample can be linked to the great affinity of Mn atoms with Ca and P, as already reported by some studies involving bioactive materials (CAVALCANTI; ALVES; SANTOS; SPANIOL *et al.*, 2023; QUEIROZ; BARRIONI; VALVERDE; GÓES *et al.*, 2023). However, further studies focusing on the real surface chemical reactivity effect of the Mn in a HEA solid solution is recommended.

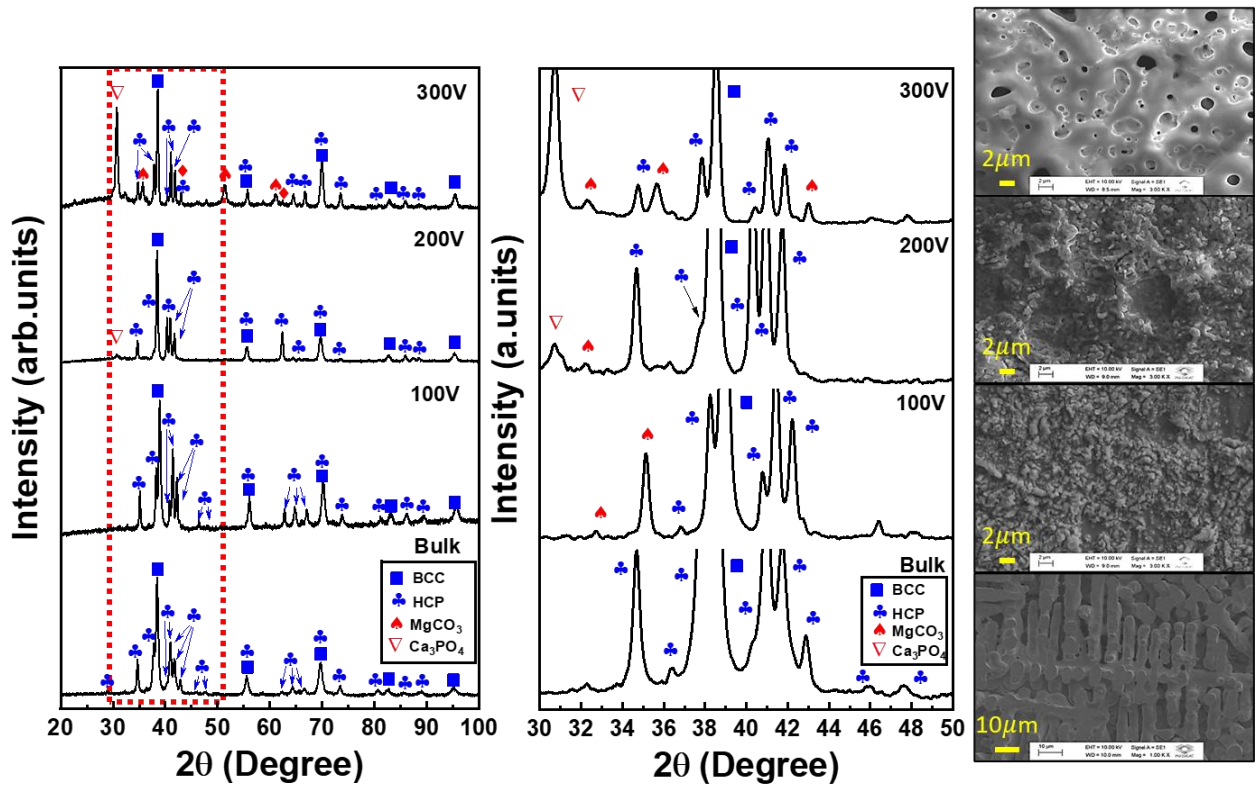


Fig 2: XRD profile and morphology of the MAO-treated TaMn sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.

The FeMo sample depicted the same biphasic HCP + BCC composition but with less intense peaks from the HCP phase. The XRD profile of the MAO-treated sample at 100 V did not present clear peaks from oxides but only smoothed peaks from the bulk's phase, indicating the growth of a thin oxide layer. However, the MAO-treated samples at 200 V and 300 V depicted some clear peaks from MgCO_3 and Ca_3PO_4 , displaying a similar incorporation of bioactive species from the electrolyte. The FeMo sample presented a similar dendritic structure, with a minor aspect ratio of the needles. As a result, the phase proportion between the BCC and HCP phases could be slightly different, supporting the results indicated in the XRD. The minor size of the dendritic needles could be provoked by the lesser mismatch in the melting point of the Fe (1538 °C) instead of Mn (1246 °C) when added as an alloying element in a refractory solid solution (CAMPARI; CASAGRANDE; COLOMBINI; GUALTIERI *et al.*, 2021). The MAO-treated sample at 100 V exhibited some plates, indicating a preferential growth on a specific phase, probably induced by the distinct chemical composition between the phases, especially that enriched by the valve metals (Ti and Zr). When the voltage moved up to 200 V and 300 V, the surface depicted the same porous structure but with micro-sized pores, indicating that the dielectric breakdown somewhat occurs distinctly

for this sample. Recently, Xie *et al.* (XIE; WANG; CUI; HU *et al.*, 2024) successfully addressed superior corrosion resistance and antimicrobial ability in Ti-Fe alloys using MAO treatment. The porous coating obtained in this study was similar to that found by the authors.

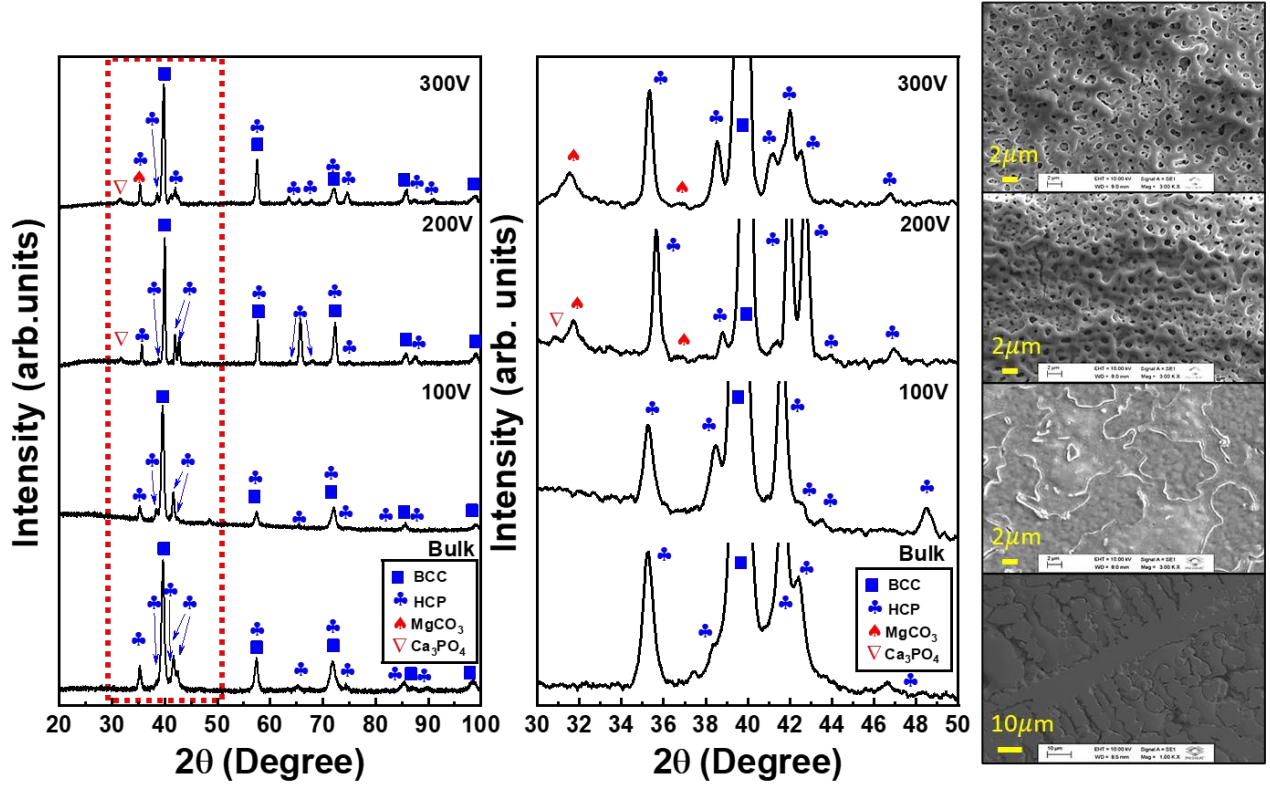


Fig 3: XRD profile and morphology of the MAO-treated FeMo sample as a function of the applied voltage: (left) full pattern, (center) zoomed view, and (right) SEM imaging.

Overall, the HEA samples had distinct oxidative reactions and species incorporation ability throughout the applied voltage, probably due to the bulk samples' different chemical and phase aspects, which can impact the dielectric breakdown and current flow. **Fig. 4** presents a cross-sectional view of the samples under different voltage conditions. The thickness of the samples was analyzed using ImageJ software, and the values found are presented in **Fig. 5**. For all samples, it was not possible to quantify the film thickness under the 100 V condition because of their sizes below 1 μm . The TaMo alloy exhibited a thickness of $8 \pm 2 \mu\text{m}$ for 300 V and $4 \pm 1 \mu\text{m}$ for 200 V. While for the TaMn alloy, the values remained around $5 \pm 1 \mu\text{m}$ and $3 \pm 1 \mu\text{m}$ for 300 V and 200 V, respectively. Lastly, the FeMo alloy showed values of $7 \pm 2 \mu\text{m}$ for 300 V and $4 \pm 1 \mu\text{m}$ for 200 V. As the high voltage intensifies the electric field between the anode and cathode, it promotes water hydrolysis in the electrolyte, and the

generation of oxidative species. Thus, the produced oxidative species react with metal ions (M) at the surface, forming metal oxides (MO_x) (KURODA; DOS SANTOS; ROSSI; CORREA *et al.*, 2023). The anodic voltage accelerates the oxide growth process, contributing to a thick oxide layer. The oxidation of metals in an electrolyte can be summarized by the following equation (SIKDAR; MENEZES; MACCIONE; JACOB *et al.*):



Where:

- M represents the metal (e.g., Ti, Zr, Nb, Ta, Mo, Mn, Fe)
- x is the number of water molecules involved in the reaction
- MO_x is the metal oxide formed (e.g., TiO₂, ZrO₂, MoO₃, MnO₂, FeO)

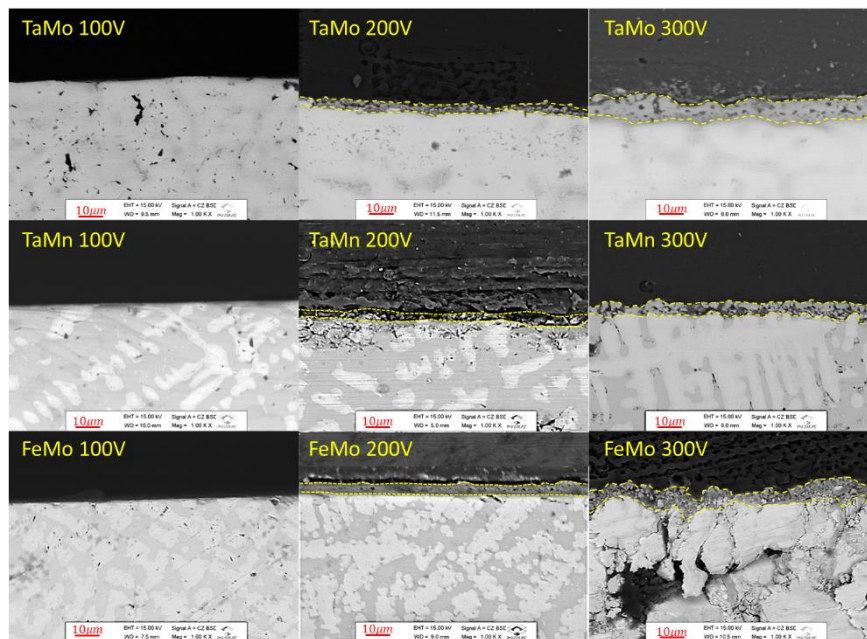


Fig 4: Cross-sectional view of the MAO-treated samples.

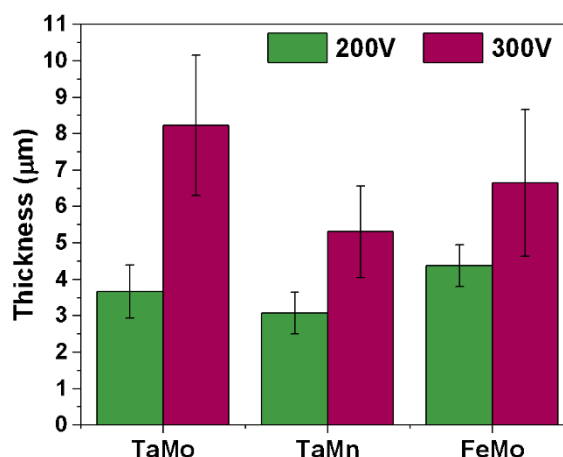


Fig 5: Thickness values of the MAO-treated samples.

The MAO-treated TaMn sample at 300 V was chosen for further examination by TEM, considering the multiple phases exhibited in the XRD profile. The nanoscale features of this sample's cross-section view are depicted in **Fig. 6**. The lamellar-shaped sample was analyzed in three distinct regions, being two regions located within the MAO coating (highlighted in blue) and one region located in the bulk (highlighted in red). **Fig. 6a** denotes the selected region in the MAO-treated surface chosen for the ion-milling, and **Fig. 6b** presents the cross-section view of the coating. Then, the top and bottom parts of the coating were selected for TEM examination, as indicated by the color circles. The top region exhibited an amorphous outer layer and a nano-crystalline inner layer, with thickness in the order of some micrometers, as depicted in Fig. 6c and 6f. The corresponding inset SAED pattern indicated an absence of diffracted spots and diffraction patterns related to the TiO_2 in the anatase form (COD number #7006075) in the $[7\bar{1}4]$ zone axis, respectively. The elemental EDS mapping of the outer layer (**Fig. 6d** and **6e**) indicated an enrichment of C and O. In contrast, in the nano-crystalline layer (**Fig. 6g** and **6h**) predominates O and some valve metals (Ti, Zr, and Ta). For the bottom region of the lamella (**Fig. 6i**), it was possible to note a clear boundary between the oxide layer and the bulk, having the SAED pattern in the inset indicating spots from the HCP phase in the $[1\bar{5}2]$ zone axis. The corresponding elemental EDS mapping (**Fig. 6f** and **6g**) indicated an uniform distribution of the alloying elements in the bulk at the nanoscale and the O enrichment in the MAO coating. The distinct layers found in the coating originated from the aggressive temperature and pressure promoted during the dielectric breakdown of the MAO treatment, following some earlier studies with multicomponent Ti alloys (CORREA;

ROCHA; RIBEIRO; GEMINI-PIPERNI *et al.*, 2018). Furthermore, the detected amorphous and nano-crystalline layers are in line with those found in MAO-treated Ti and Ti-based alloys (CORREA; ROCHA; RIBEIRO; GEMINI-PIPERNI *et al.*, 2018; OLIVEIRA; RIBEIRO; PEREZ; ARCHANJO *et al.*, 2015).

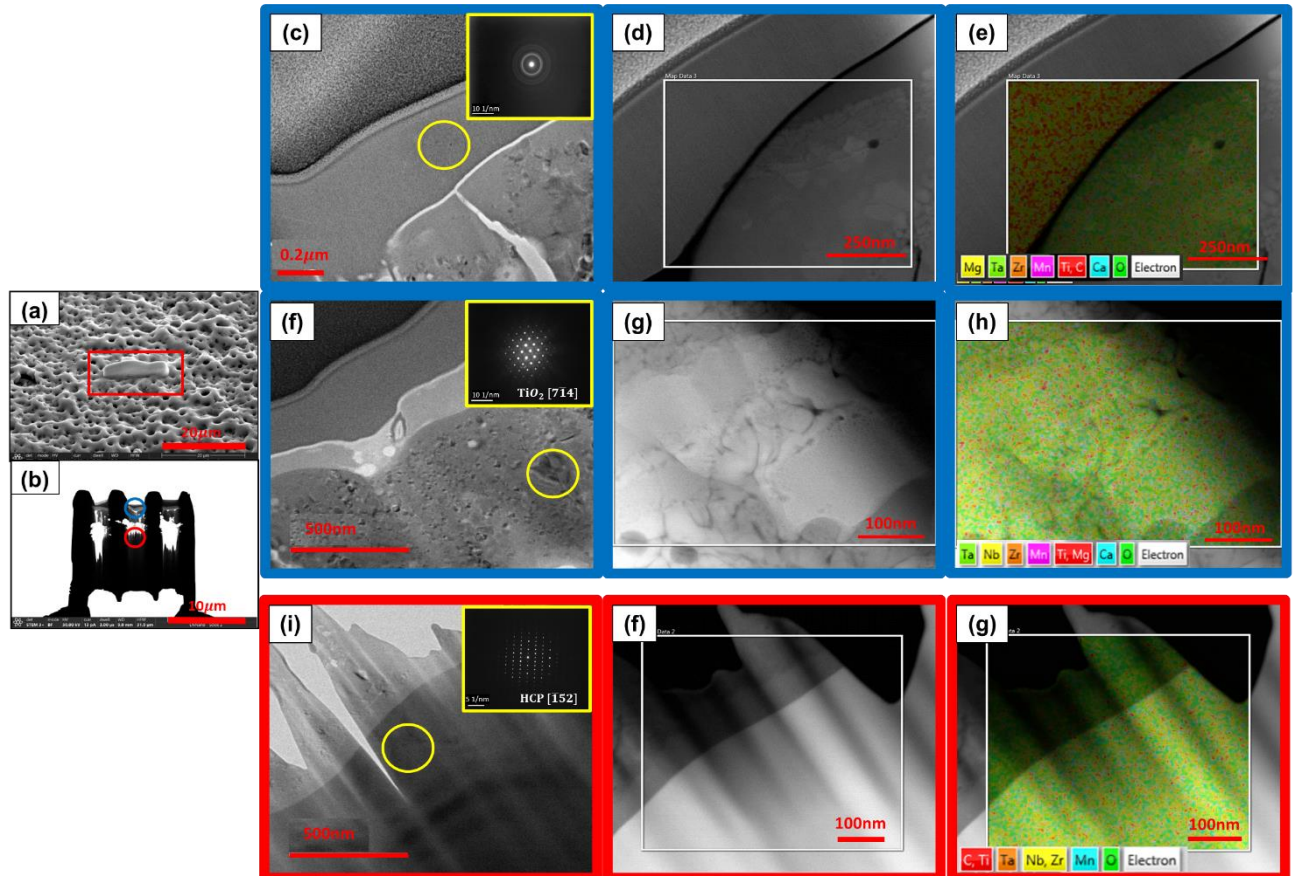


Fig 6: TEM results of the MAO-treated TaMn sample at 300 V: (a) selected area of the surface for FIB milling (b) cross-section view of the FIB lamella with selected analyzed regions (red and blue circles); (c) STEM image of the outer amorphous layer and SAED pattern of the yellow circle; (d) selected area and (e) elemental EDS mapping; (f) STEM image of the intermediary nano-crystalline layer and SAED pattern of the yellow circle; (g) selected area and (h) elemental EDS mapping; (i) STEM image of the bulk and SAED pattern of the yellow circle; (d) selected area and (e) elemental EDS mapping.

Chemical details of the outer surface in the MAO-treated samples are shown in the survey spectrums (**Fig. 7**) and the corresponding semi-quantitative results (**Fig. 8**). The survey spectrums exhibited typical peaks from the alloying elements as well as the bioactive species (Ca, P, and Mg), and incorporated C and O. The corresponding semi-quantitative results of the MAO-treated samples depicted some critical differences. For the TaMo sample, the surface had a composition almost entirely by C and O (over 80%), probably indicating the prominent presence of oxides and

carbonate-based compounds. Increasing the applied voltage, the outer surface was enriched by bioactive species (Ca, P, and Mg) compared to the alloying elements. For the alloying elements, Ti was the most predominant one, probably due to its greater affinity with oxygen. The other elements could be present as oxides or melted metals, which were removed from the substrate during the dielectric breakdown and plasma discharges (CLYNE; TROUGHTON, 2018; NIE; CAI; ZHAO; SUN *et al.*, 2022). The TaMn and FeMo results were quite similar but had a non-linear relationship with the applied voltage, which indicated distinct growth mechanisms. Several studies have highlighted the positive biological effects of these kinds of multicomponent oxides enriched with bioactive species after MAO treatment (XUE; ATTARILAR; LIU; LIU *et al.*, 2020). Thus, these MAO-treated HEAs could also exhibit similar behavior when in contact with the human body.

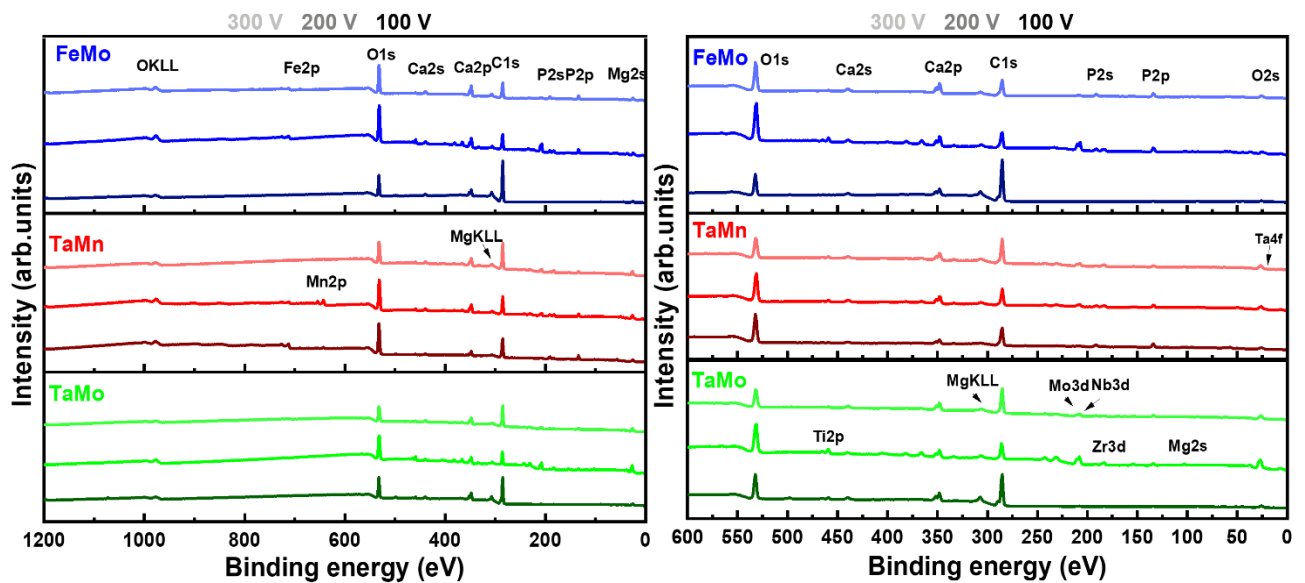


Fig 7: Survey spectrums of the MAO-treated samples: (left) full spectrum and (right) zoomed view

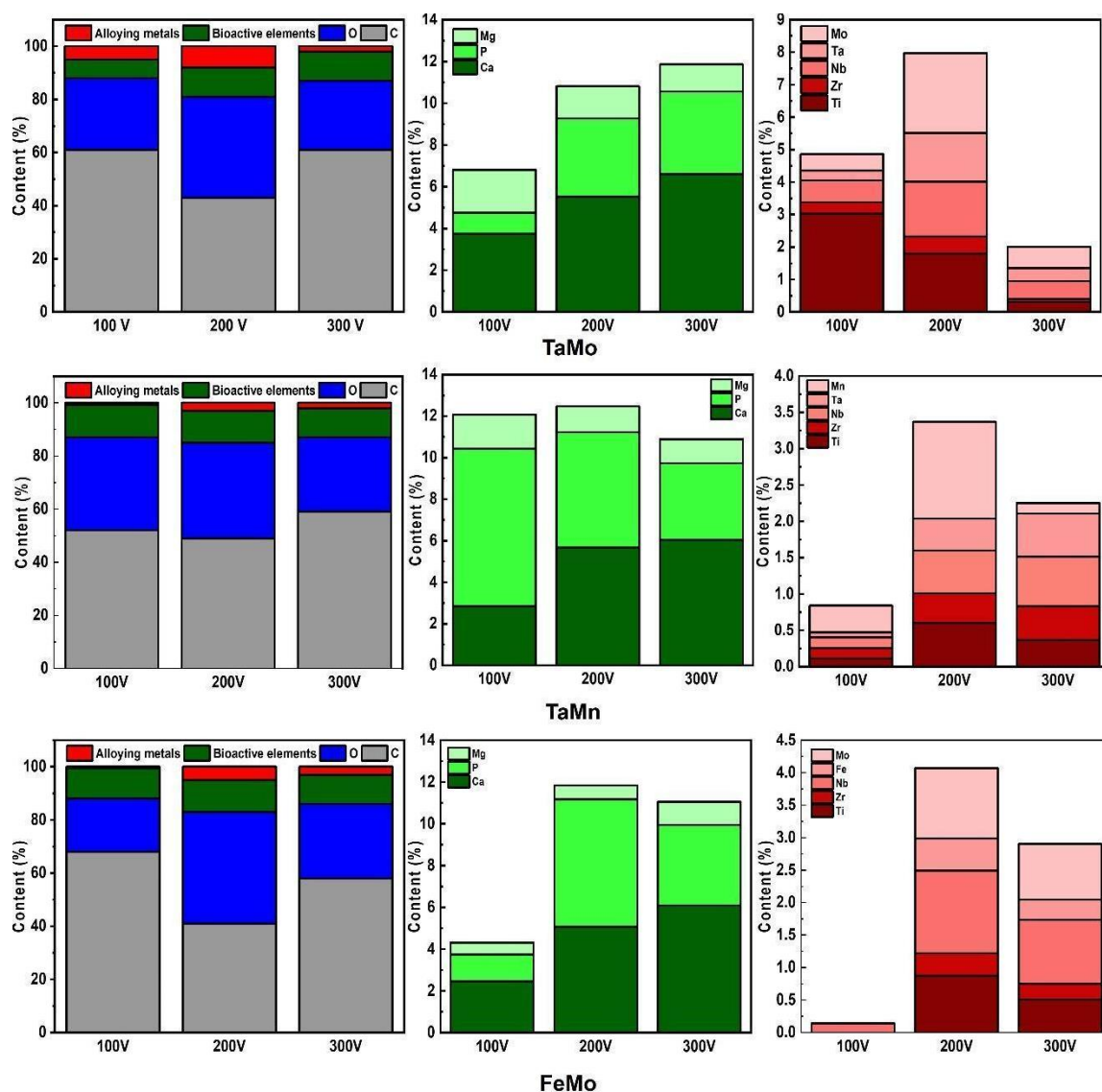


Fig 8: Semi-quantitative results of the XPS survey spectrum for the MAO-treated TaMo/TaMn/FeMo sample: (left) overall content, (center) bioactive elements, and (right) alloying metals.

Fig. 9, 10, and 11 depict the chemical state of some elements acquired in the high-resolution (HR) spectrums and the corresponding proportion. The HR spectrums of the alloying elements exhibited typical duplets related predominantly to the most stable oxides, which were formed during the anodic oxidation (e.g., TiO_2 , ZrO_2 , Nb_2O_5 , Ta_2O_5 , MoO_3 , MoO_2 , MnO_2 , and Fe_2O_3). In addition, the HR spectrums of the bioactive species indicated the presence of carbonates and phosphates, following the XRD results. Du *et al.* (DU; WEI; WANG; CHENG *et al.*, 2019) pointed out that these MAO-treated surfaces decorated with Ca- and P-rich compounds can significantly impact bioactivity, nucleating calcium phosphate precipitates and facilitating cell mineralization.

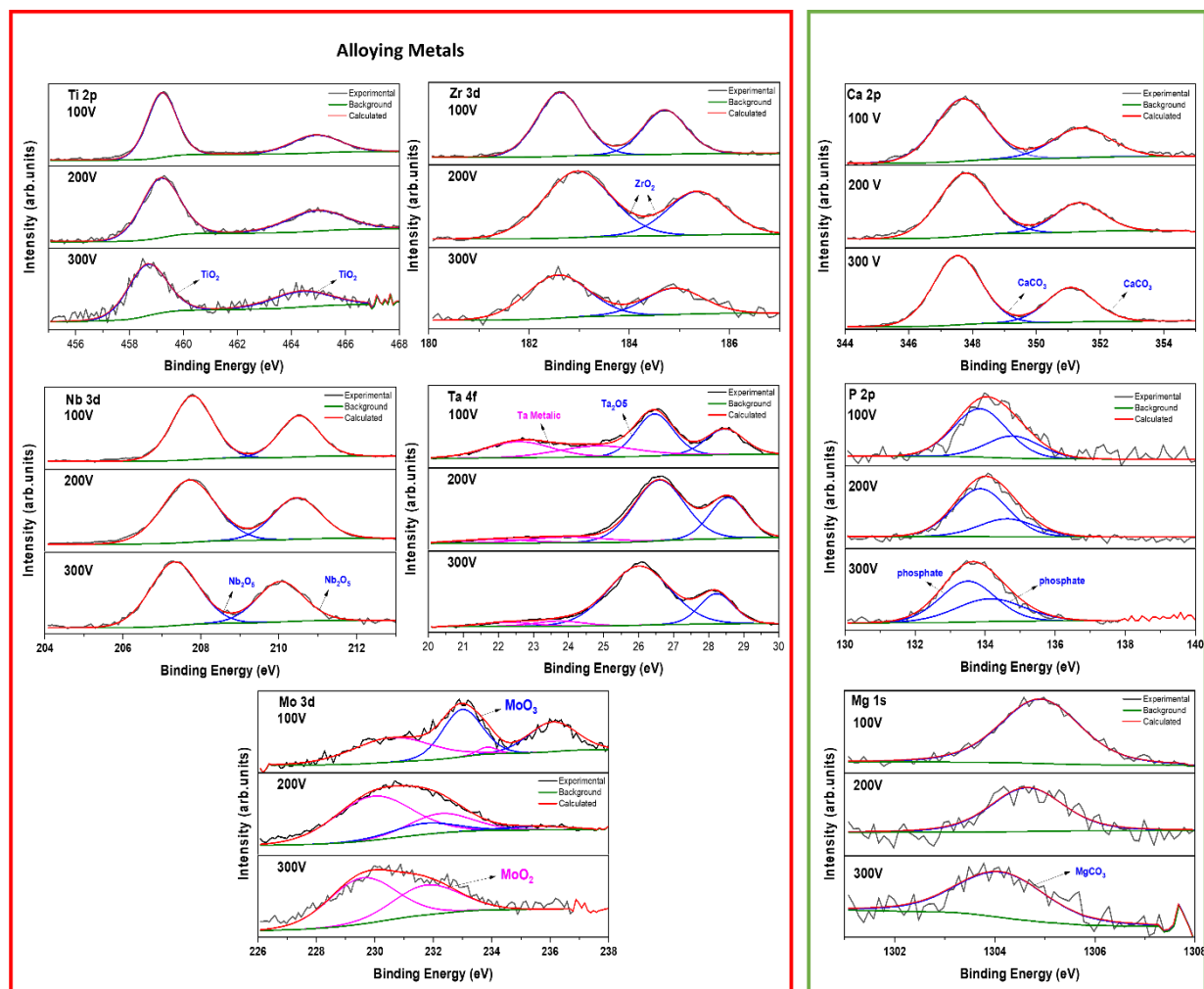


Fig 9: HR spectrums of the MAO-treated TaMo samples.

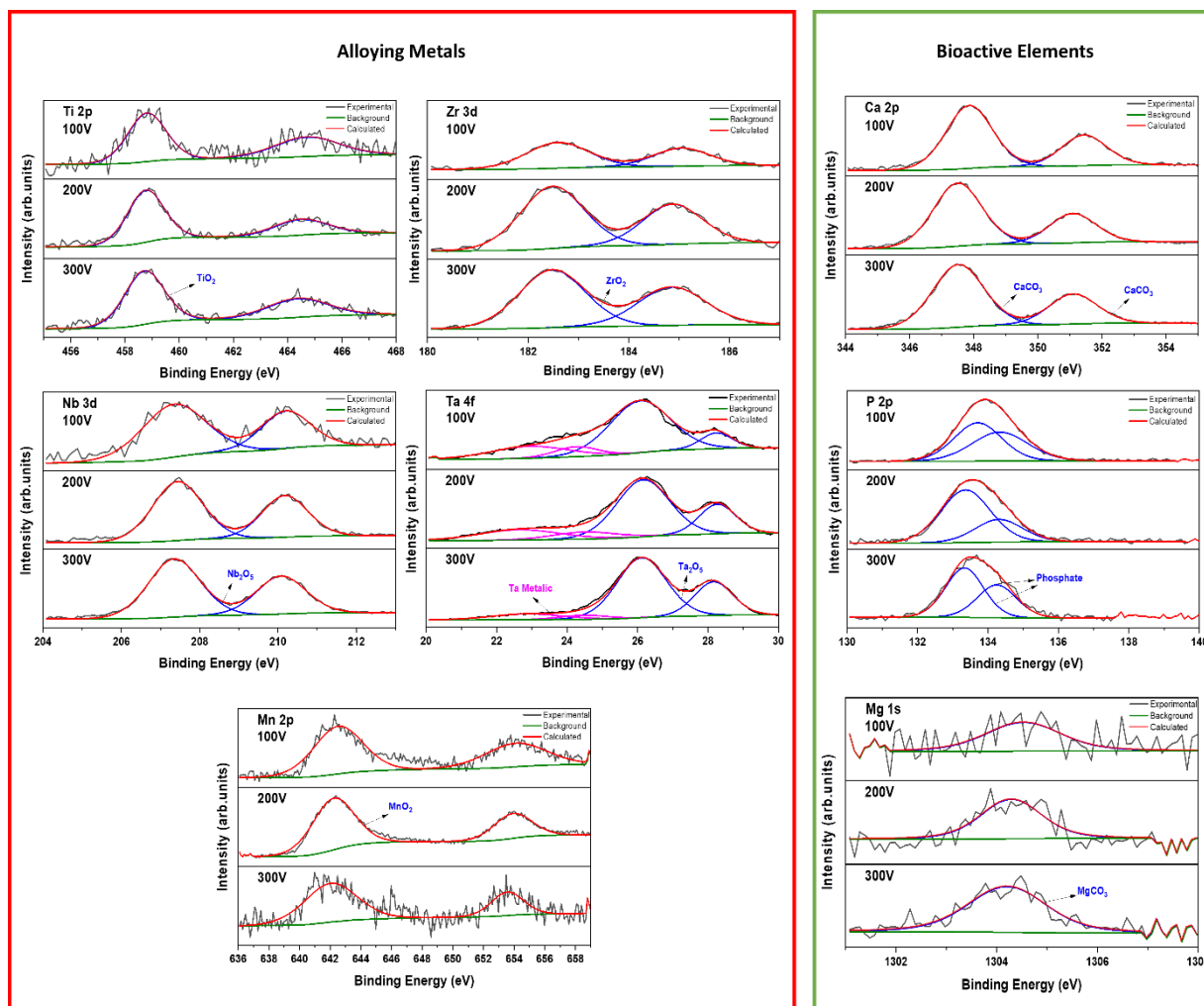


Fig 10: HR spectrums of the MAO-treated TaMn samples.

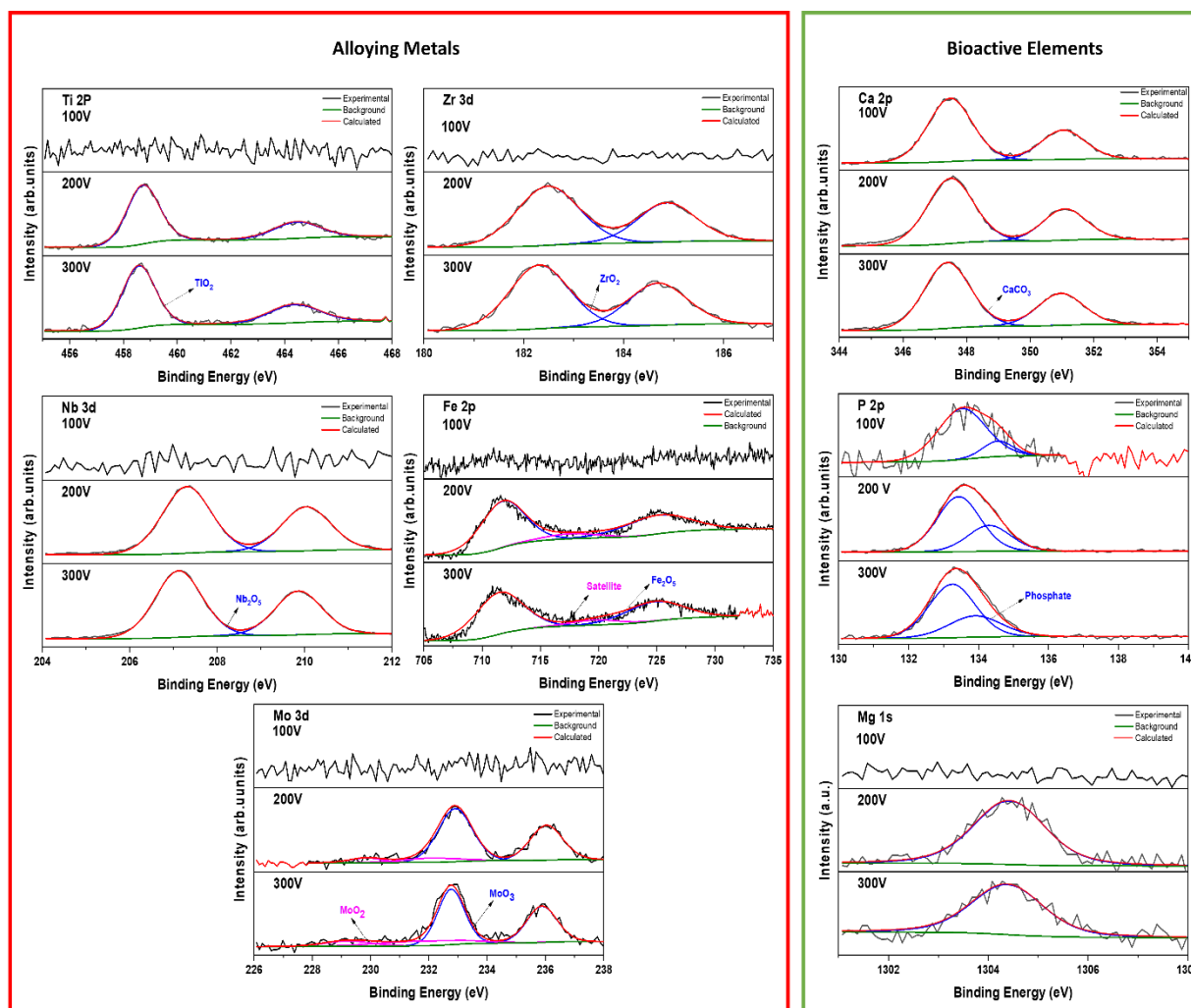


Fig 11: HR spectrums of the MAO-treated FeMo samples.

The average roughness values (**Fig. 12**) presented a non-linear relationship with the applied voltage, reinforcing the assumption that different chemical reactions took place during the dielectric breakdown and plasma generation. Overall, the MAO samples depicted a roughness value in the order of microscale, which could be feasible for promoting cell viability. A comparison of the contact angle values from the MAO-treated samples using distilled water and diiodomethane droplets is shown in **Fig. 13**. It is possible to note that the MAO-treated samples at 100 V depicted a hydrophilic behavior, probably indicating a strong effect of the bulk in the droplet, considering the small thickness of the oxide layer. Contrarily, the MAO treatment above 200 V significantly augmented the contact angles of the samples, remaining hydrophobic ($> 90^\circ$), probably because of the porous surface. Chu *et al.* (CHU; LIU; CHEN; LI, 2020) produced TiO_2 -rich anodic surfaces on the Ti-6Al-4V alloy and analyzed the influence of porous morphology on the wettability results. According to the authors, larger pores

result in hydrophilic surfaces. In comparison, smaller pores trap atmospheric air and make it difficult for the liquid to contact the surface, creating a hydrophobic surface. It is probably related to the electrolyte's Ca, P, and Mg enrichment, which can promote a more hydrophobic effect than the hydrophilic character of the porous structure when added to oxides or carbonates (POPA; ANASTASESCU; GIFU; CALDERON MORENO, 2022). Thus, these results show that it is possible to handle the wettability of the surface of these HEAs by using MAO treatment, which could be helpful in the development of novel biomaterials. For example, hydrophobic and hydrophilic surfaces can result in distinct interaction with cells, promoting different adhesion, proliferation, and differentiation rates, which could be useful for some long-term implants and external bone fixation devices (KIM; KIM; KIM, 2010). The corresponding surface energy of the MAO-treated samples with the polar and dispersive components are shown in **Fig. 14**. Considering that the polar and dispersive components of the surface energy influence the adhesion and wetting of proteins and cells into the surface (HAMIEH), it is possible to note that the MAO treatment can affect the surface wettability of the HEA samples. In addition to it, the dispersive component of the surface energy is related mainly with hydrocarbon chains over the surface (HAMIEH), its majority in the MAO-treated samples agree with the TEM results. Further studies measuring the contact angle and surface energy in a simulated body fluid is welcome for a fully assessment of the surface physical and chemical characteristics of the samples for the targeted biomedical application.

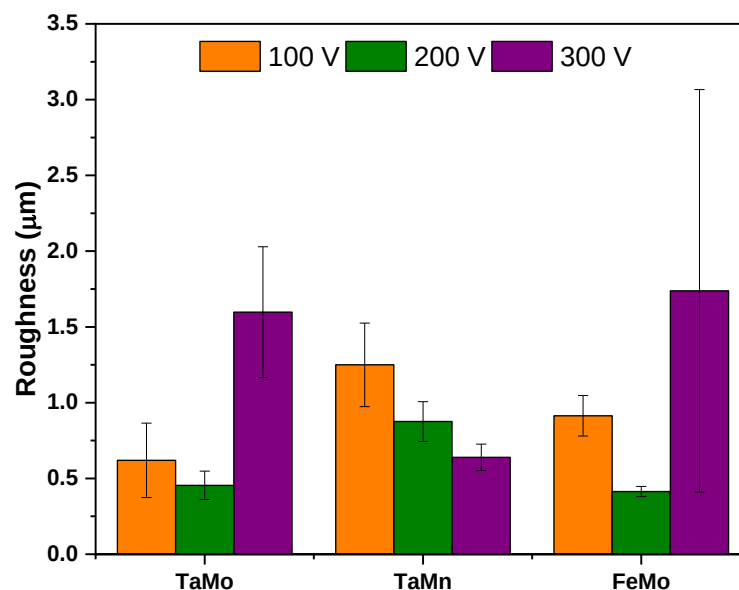


Fig 12: Roughness values of the MAO-treated samples

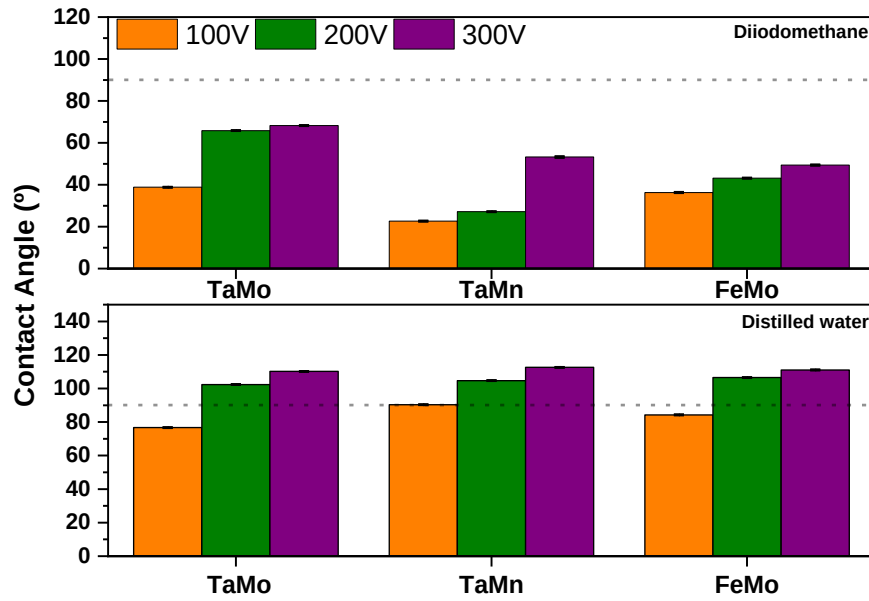


Fig 13: Contact angle values of the MAO-treated samples.

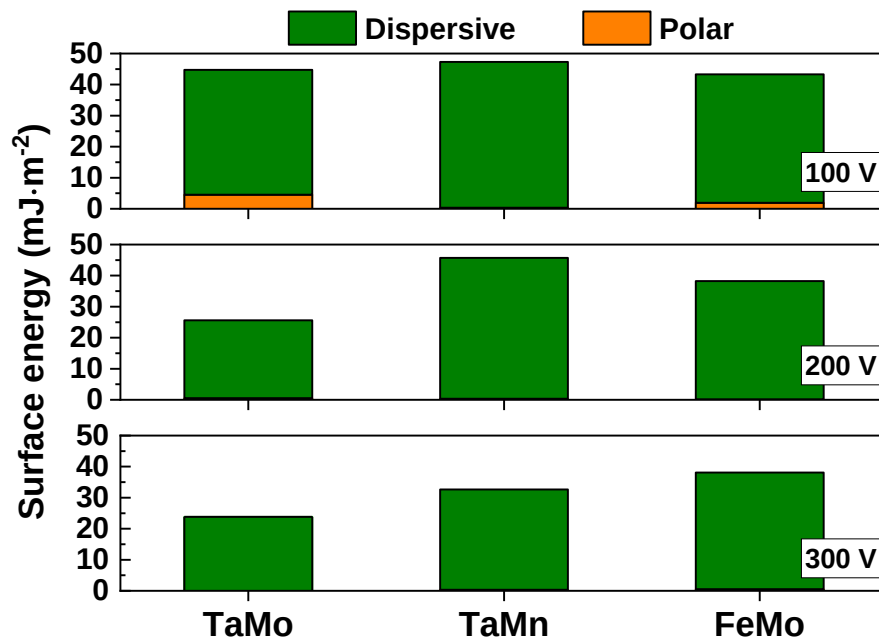


Fig 14: Surface energy of the MAO-treated samples.

4.2.4. Conclusions

From the results reported in this study, it is possible to summarize:

- The HEA samples exhibited distinct proportions of HCP and BCC phases, represented by dendritic and granular structures, respectively. This conformity was confirmed through XRD and SEM analysis;

- The augment of the applied voltage produced distinct growth mechanisms of the MAO coating until the achievement of the typical porous topography enriched with the bioactive ions (Ca, P, and Mg);
- Analysis by TEM evidenced the presence of distinct layers in the MAO-treated coating, such as an amorphous outer layer enriched with C and O, and a nano-crystalline inner layer enriched with O and some alloying elements;
- The results of film composition indicated a large amount of C and O in the outer layer, with a significant presence of bioactive elements (Ca, P, and Mg) in detriment of the alloying elements;
- The HR spectrums detected the presence of stable oxides of the alloying metals together with Ca- and P-based oxides and carbonates, which can be advantageous to input bioactive ability in the surfaces;
- Roughness values depicted a non-linear relationship with the applied voltage, suggesting strong dependence of the oxidative reactions that took place during the dielectric breakdown and plasma generation;
- The contact angle values changed with the applied voltage, presenting a hydrophobic effect in distilled water and hydrophilic in diiodomethane, which was related to the the enrichment of chemical species from the electrolyte;
- The surface energy remained with values below 50 mJm^{-2} , almost entirely composed by the dispersive component, and being dependent of the bulk's chemical composition and applied voltage;
- Considering that HEAs are emerging materials for biomedical applications, the findings of this study shed light on the potential use of MAO treatment to produce functionalized HEAs surfaces with bioactive ability, which can induce different cell interactions in the human body.

4.2.5. Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

4.2.6. Acknowledgments

The authors thank the São Paulo Research Foundation (FAPESP), grant #2021/13921-3, and National Council for Scientific and Technological Development (CNPq), grants #404020/2023-2 and #407251/2018-9, for financial support. Also, the Brazilian National Laboratory (LNNano) from the Brazilian Center for Research in Energy and Materials (CNPEM), research proposals #20232420 and #20230756, for the XPS and TEM analysis, respectively.

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5. Considerações finais

Ao longo deste doutorado, o desenvolvimento de superfícies biomultifuncionais em ligas de alta entropia (HEAs) por oxidação por micro-arco (MAO) proporcionou resultados promissores, conforme evidenciado pelas pesquisas realizadas. Esses resultados foram consolidados em um artigo já publicado e outro em fase final de revisão para submissão, além de outros dois artigos ainda a serem escritos. Também, ao longo do doutorado, foram publicados três artigos em parceria, demonstrando a relevância do trabalho para a comunidade científica.

As conclusões dos estudos reforçam o potencial das ligas TiZrNbTaMo, TiZrNbTaMn e TiZrNbFeMo para aplicação em implantes biomédicos. Os resultados indicaram que as ligas apresentaram microdureza superior à liga Ti-6Al-4V, com módulo elástico próximo, fatores essenciais para minimizar o efeito do *stress shielding*. Além disso, as fases cristalinas formadas (BCC, HCP/Laves C14 e cBCC) foram consistentes com as previsões teóricas, revelando a influência dos elementos de liga na estrutura e nas propriedades mecânicas. As análises *in vitro* demonstraram que as ligas não apresentaram efeitos citotóxicos após 24 horas de cultivo com células osteoblásticas, destacando a biocompatibilidade dessas ligas.

Além disso, o tratamento MAO nas HEAs produziu superfícies enriquecidas com íons bioativos (Ca, P, Mg), promovendo a formação de camadas amorfas e nanocristalinas com características desejáveis para aplicações biomédicas. A presença de óxidos estáveis dos metais da liga e óxidos/carbonatos de cálcio e fósforo sugere um potencial bioativo nas superfícies tratadas. A análise da energia superficial e dos ângulos de contato indicou uma dependência direta da composição química e do processo de oxidação, o que pode influenciar a interação celular nas superfícies tratadas.

Em síntese, o trabalho realizado oferece uma contribuição significativa para o campo das ligas de alta entropia com potencial para implantes biomédicos, destacando tanto a importância dos elementos de liga quanto do tratamento de superfície por MAO. Os achados sugerem novas oportunidades de pesquisa em funcionalização de superfícies de HEAs para otimização de sua bioatividade e desempenho clínico.

Além disso, uma nova linha de pesquisa envolvendo o armazenamento de hidrogênio foi aberta com resultados promissores utilizando as ligas de alta entropia

desenvolvidas. A capacidade dessas ligas de armazenar hidrogênio de forma eficiente, combinada com sua estabilidade estrutural, oferece grande potencial para aplicações em sistemas de energia limpa, como células de combustível e armazenamento de hidrogênio. Essa direção de pesquisa pode trazer avanços significativos na área de materiais para energia sustentável, contribuindo para soluções tecnológicas em transição energética e sustentabilidade ambiental.

6. Produção Científica: Artigos Publicados

APL Materials	ARTICLE	scitation.org/journal/apm
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Development of non-equiatomic Bio-HEAs based on TiZrNbTa-(Mo and Mn)

Cite as: APL Mater. 10, 081113 (2022); doi: 10.1063/5.0100465
 Submitted: 24 May 2022 • Accepted: 8 August 2022 •
 Published Online: 30 August 2022

View Online Export Citation CrossMark

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Materials Characterization 208 (2024) 113678

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Aging temperature effect on the phase composition, microstructure, and selected mechanical properties of non-equiatomic TiZrNbTaMo/Mn HEAs targeted for biomedical implants

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Promising composites for wear resistant load-bearing implant applications: Low elastic moduli of β Ti–Nb alloy reinforced with TiC particles and/or TiB whiskers

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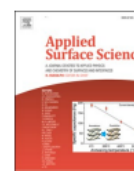


Applied Surface Science 664 (2024) 160227



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Full Length Article

Surface aspects of novel Bio-HEAs MAO-treated in a Ca-, P-, and Mg-rich electrolyte

T.S.P. Sousa^{a,*}, J.E.M. Torrento^a, P.A.B. Kuroda^b, V.R.M. Gonçalves^a, K.S. Coan^a, D.R.N. Correa^a, C.R. Grandini^a

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