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Eduarda Blasi Magini Passoni

**Biovidro Mesoporoso revestido com extrato de *Cranberry* para regeneração tecidual
óssea: Análise microbiana e osteogênica *in vitro***

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Orientadora: Prof.^a Dr.^a Ariadne Cristiane Cabral da Cruz
Coorientadora: Prof.^a Dr.^a Helena Cristina de Oliveira Francisco

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Passoni, Eduarda Blasi Magini

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O presente trabalho em nível de Doutorado foi avaliado e aprovado, em 31 de Julho de 2025, pela banca examinadora composta pelos seguintes membros:

Profa. Ariadne Cristiane Cabral da Cruz, Dra.
Universidade Federal de Santa Catarina

Profa. Carolina dos Santos Santinoni, Dra.
Universidade Federal de Santa Catarina

Prof. João Manuel Mendez Caramês, Dr.
Faculdade de Medicina Dentária da Universidade de Lisboa

Prof. Cesar Augusto Magalhães Benfatti, Dr.
Universidade Federal de Santa Catarina

Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Doutor em Odontologia, área de concentração - Implantodontia

Coordenação do Programa de Pós-Graduação

Profa. Ariadne Cristiane Cabral da Cruz, Dra.
Orientadora

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(Leonardo da Vinci)

RESUMO

Este estudo teve como objetivo sintetizar um Biovidro Mesoporoso 58S revestido com extrato seco de *Cranberry* (MBG-58S/CB) e avaliar seus efeitos na formação de biofilme e na diferenciação osteogênica *in vitro*. Os arcabouços de MBG-58S foram sintetizados pelo método sol-gel, seguido por uma solução hidroalcolica de revestimento de extrato de *Cranberry* (MBG-58S/CB). Os arcabouços foram submetidos à caracterização físico-química por microscopia eletrônica de varredura e espectroscopia de energia dispersiva de raios X. A liberação do extrato seco de *Cranberry* a partir dos arcabouços foi avaliada até 35 dias. A influência dos arcabouços MBG-58S, MBG-58S/CB, Nanosynt[®] e Bio-Oss[®] na formação de biofilmes mono e multiespécies foi determinada. A viabilidade celular e a diferenciação osteogênica foram avaliadas nos seguintes grupos: MBG-58S, MBG-58S/CB e Bio-Oss[®] utilizando células mesenquimais derivadas da polpa de dentes decíduos humanos (SHED). Os ensaios osteogênicos envolveram a avaliação da atividade da fosfatase alcalina (ALP) e da mineralização da matriz extracelular por meio da coloração de vermelho de Alizarina. O arcabouço MBG-58S/CB apresentou uma estrutura porosa, permitindo a liberação sustentada do extrato de *Cranberry* por até 35 dias. O arcabouço Bio-Oss[®] promoveu maior quantidade de biofilme de *Streptococcus oralis* ($p < 0,001$) após 24h de cultura, enquanto o MBG-58S/CB promoveu a menor quantidade de bactérias no biofilme multiespécies no dia 8. O arcabouço MBG-58S/CB promoveu a maior viabilidade celular no dia 7 e exibiu maior atividade de ALP em comparação ao Bio-Oss[®]. Por outro lado, o Bio-Oss[®] promoveu a maior mineralização da matriz extracelular no dia 15. Em conclusão, o revestimento com extrato de *Cranberry* em arcabouços de Biovidro Mesoporoso preveniu a formação de biofilme multiespécies, aumentou a biocompatibilidade do Biovidro Mesoporoso 58S e estimulou a expressão da ALP, marcador precoce de osteogênese. No entanto, essa capacidade osteogênica não foi observada para a mineralização da matriz extracelular. Estudos futuros são necessários para explorar o potencial do *Cranberry* na biofuncionalização de biomateriais. Esta nova abordagem destaca o potencial da aplicação do extrato de *Cranberry* para potencializar a regeneração do tecido ósseo, aprimorando assim a bioatividade dos substitutos ósseos.

Palavras-chave: *Cranberry*, Biovidro Mesoporoso, Biofilme, Biomaterial, Regeneração óssea, Engenharia tecidual.

ABSTRACT

This study aimed to synthesize a Cranberry-coated Mesoporous Bioactive Glass 58S (MBG-58S/CB) and evaluate its effects on biofilm formation and osteogenic differentiation *in vitro*. MBG-58S scaffolds were synthesized using the sol-gel method, followed by a hydroalcoholic solution of Cranberry extract coating (MBG-58S/CB). The scaffolds underwent physicochemical characterization using scanning electron microscopy and energy-dispersive X-ray spectroscopy. Cranberry release kinetics were assessed. The interference of MBG-58S, MBG-58S/CB, Nanosynt®, and Bio-Oss® on mono- and multi-species biofilm formation was determined. Cellular viability and osteogenic differentiation were evaluated using human-derived mesenchymal stromal cells (SHED) and the following groups: MBG-58S, MBG-58S/CB, and Bio-Oss®. Osteogenic assays involved the assessment of alkaline phosphatase (ALP) activity and extracellular matrix mineralization. The MBG-58S/CB scaffold exhibited a porous structure, allowing for the sustained release of Cranberry extract over 35 days. Bio-Oss® presented the highest amount of *Streptococcus oralis* biofilm ($p < 0.001$) after 24h of culture, while MBG-58S/CB promoted the lowest number of bacteria in the multi-species biofilm on day 8. The MBG-58S/CB scaffold promoted the highest cellular viability on day 7 and exhibited higher ALP activity compared to Bio-Oss®. Conversely, Bio-Oss® promoted the highest extracellular matrix mineralization on day 15. Cranberry coating on Mesoporous Bioactive Glass scaffolds prevented the multi-species biofilm formation, enhanced the Mesoporous Bioactive Glass biocompatibility, and stimulated the early osteogenic marker (ALP). Nevertheless, this osteogenic capacity was not observed for extracellular matrix mineralization. Future studies are warranted to explore the potential of Cranberry for biomaterial biofunctionalization. This novel approach highlights the potential application of Cranberry extract for improved outcomes in bone tissue regeneration, thereby enhancing the bioactive performance of the bone substitutes.

Keywords: Cranberry, Mesoporous Bioactive Glass, Biofilm, Scaffold, Bone, Tissue engineering.

LISTA DE ABREVIATURAS E SIGLAS

ALP	Atividade de Fosfatase Alcalina
BHI	Infusão Cérebro Coração (Brain Heart Infusion)
Ca	Cálcio
Ca ²⁺	Íon Cálcio
CaO	Óxido de Cálcio
CFU	Unidade Formadora de Colônias
CHAp	Hidroxiapatita Carbonatada
CLSM	Microscopia Confocal de Varredura a Laser
DMEM	Meio de Eagle Modificado por Dulbecco
EDX	Espectroscopia de Raios X por Dispersão em Energia
FBS	Soro Fetal Bovino
HA	Hidroxiapatita
IL-6	Interleucina 6
IL-8	Interleucina 8
kV	Quilovolt
LAE	Etil Lauroil Arginato
MAPKs	Proteínas Cinasas Ativadas por Mitógenos
MBG	Biovidro Mesoporoso
MBG-58S/CB	Biovidro Mesoporoso 58S Revestido com Extrato Seco de Cranberry
mg	Miligrama
mL	Mililitro
mm	Milímetro
MPa	Megapascal
Na ⁺	Íon Sódio
Na ₂ O	Óxido de Sódio
NaOH	Hidróxido de Sódio

NDM	Material não Dialisável
NF- κ B	Fator Nuclear Kappa B
nm	Nanômetro
OD	Densidade Óptica
P	Fósforo
P ₂ O ₅	Óxido de Fósforo
PACs	Proantocianidinas do tipo A
PBS	Solução Salina Tamponada com Fosfato
PLGA	Ácido Poli-Lático-Co-Glicólico
PVP	Polivinilpirrolidona
rpm	Rotações por Minuto
SD	Desvio Padrão
SEM	Microscopia Eletrônica de Varredura
SHED	Células-tronco Derivadas da Polpa de Dentes Decíduos Humanos Esfoliados
Si	Silício
SiO ₂	Dióxido de Silício
β -TCP	β -Fosfato Tricálcico
TEOS	Tetraetoxissilano
TEP	Trietil Fosfato
μ L	Microlitro
μ m	Micrômetro
ρ -NP	p-Nitrofenol
ρ NPP	p-Nitrofenol Fosfato

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1 INTRODUÇÃO

O tecido ósseo é um compósito natural, constituído por matriz orgânica e uma fase mineral (HU et al., 2019), que apresenta capacidade mecânica de autorreparo e tenacidade à fratura (LEE et al., 2019). No entanto, essa capacidade regenerativa é limitada frente a defeitos ósseos críticos (LEE et al., 2019), definidos como aqueles defeitos que não apresentam capacidade de cicatrização espontânea (RODDY et al., 2018; SCHMITZ; HOLLINGER, 1986). Tais defeitos constituem um desafio clínico importante, com impacto significativo na qualidade de vida dos pacientes. Suas causas incluem trauma, infecções, ressecção tumoral, malformações congênitas ou intervenções cirúrgicas (MAJIDINIA; SADEGHPOUR; YOUSEFI, 2018). Na odontologia, a perda dentária é altamente prevalente, e está frequentemente associada a cáries extensas e doença periodontal avançada (ANWANDTER et al., 2016; TAN et al., 2012). As extrações dentárias são procedimentos muito comuns (TAN et al., 2012), que podem resultar em defeitos ósseos residuais. Após a exodontia ocorre uma reabsorção fisiológica do rebordo alveolar, resultando em alterações dimensionais (PARK et al., 2020) e modificações nos tecidos moles subjacentes (TAN et al., 2012). Essas alterações podem comprometer a saúde, estética e gerar dificuldades na instalação de implantes em posições ideais, ocasionando desafios funcionais (LANDSBERG et al., 2021).

Para reparar estes defeitos, são frequentemente utilizados materiais de enxertia óssea (GARCÍA-GARETA; CATHUP; BLUNN, 2015). Entretanto, a recuperação da função e estrutura óssea, definido como regeneração, ainda representa um grande desafio clínico (HU et al., 2019). Embora diferentes técnicas e materiais de enxertia óssea sejam amplamente utilizados, as limitações associadas aos enxertos autógenos, alógenos e xenógenos, como a disponibilidade limitada, morbidade na área doadora, risco de transmissão de doenças e custos elevados, estimulam a busca por alternativas biocompatíveis, seguras e eficazes (MCGOVERN; GRIFFIN; HUTMACHER, 2018; OLIVEIRA et al., 2009).

Neste contexto, a engenharia tecidual surge como uma abordagem promissora, com o objetivo de promover a regeneração óssea por meio da combinação de biomateriais, células e fatores de crescimento (MCGOVERN; GRIFFIN; HUTMACHER, 2018). Um biomaterial ideal para enxertia óssea deve apresentar alta

biocompatibilidade, com porosidade e permeabilidade adequadas para favorecer interações celulares e vascularização (FRÖHLICH et al., 2008).

Adicionalmente, infecções associadas a biomateriais tornaram-se complicações pós-operatórias frequentes, limitando severamente a capacidade de regeneração tecidual, podendo levar a necessidade de procedimentos cirúrgicos adicionais (FERNANDES et al., 2017; KARGOZAR et al., 2018; ZHOU; GARCIA; KOTSAKIS, 2022). A formação de biofilmes bacterianos, especialmente relevante em contextos como doenças periodontais e peri-implantares, compromete diretamente a eficácia da regeneração óssea, devido à elevada resistência bacteriana aos agentes antimicrobianos e ao próprio sistema imune do hospedeiro (LLAMA-PALACIOS et al., 2019; SÁNCHEZ et al., 2020). Portanto, há crescente interesse no desenvolvimento de biomateriais inovadores e multifuncionais, que além de promover a osteogênese apresentem também propriedades antimicrobianas, com a finalidade de reduzir a formação de biofilmes e prevenir complicações infecciosas (FERNANDES et al., 2017; ZHOU; GARCIA; KOTSAKIS, 2022).

Entre os biomateriais sintéticos, destaca-se o Biovidro, inicialmente desenvolvido por Larry Hench, na composição 45S5, contendo 45% de SiO_2 , 24,5% de Na_2O , 24,5% de CaO e 6% de P_2O_5 (HENCH et al., 1971). Este biomaterial apresenta a capacidade de formar ligações químicas com o tecido ósseo, estimulando a formação de uma camada de hidroxiapatita carbonatada (CHAp) na sua superfície e liberando íons bioativos como Na^+ e Ca^{2+} , que favorecem a osteogênese (HOPPE; GÜLDAL; BOCCACCINI, 2011).

Entretanto, os Biovidros convencionais, obtidos por fusão a altas temperaturas, apresentam limitações estruturais, como baixa porosidade, área de superfície reduzida e menor capacidade de incorporação de agentes bioativos (WU; CHANG; XIAO, 2011). Com o objetivo de superar essas restrições e o avanço das pesquisas, surgiu o Biovidro Mesoporoso (MBG), desenvolvido por Yan et al. (YAN et al., 2004), utilizando a metodologia sol-gel associada a surfactantes, que se diferencia por apresentar uma arquitetura altamente ordenada de poros mesoscópicos (YAN et al., 2004, 2006). Essa estrutura confere ao MBG uma área superficial significativamente maior, maior volume de poros, melhor eficiência na liberação de íons bioativos (Ca^{2+} , Si^{4+} e P^{5+}) e bioatividade aprimorada (WU; CHANG, 2012). Essas características presentes no Biovidro Mesoporoso permitem a incorporação eficiente e liberação sustentada de agentes terapêuticos, tornando-o um biomaterial extremamente promissor para engenharia

tecidual óssea e utilização em ambientes desafiadores, como a cavidade oral (WU; CHANG, 2012).

Paralelamente ao desenvolvimento de biomateriais, cresce o interesse pela utilização de compostos naturais com propriedades terapêuticas, especialmente aqueles com efeitos antimicrobianos, anti-inflamatórios e antioxidantes (GALARRAGA-VINUEZA et al., 2017; SÁNCHEZ et al., 2020). O *Cranberry* (*Vaccinium macrocarpon*), notoriamente conhecido por sua aplicação na prevenção de infecções do trato urinário, tem despertado interesse crescente na odontologia devido aos seus efeitos contra patógenos cariogênicos e periodontais (PHILIP; WALSH, 2019). Este fruto é rico em compostos fenólicos, incluindo ácidos fenólicos, flavonoides e, especialmente, proantocianidinas do tipo A (PACs), que demonstraram forte capacidade de inibir a adesão bacteriana, modular a resposta inflamatória e reduzir a atividade de enzimas proteolíticas periopatogênicas (FEGHALI et al., 2012; PELLERIN; BAZINET; GRENIER, 2021; PHILIP; WALSH, 2019; SÁNCHEZ et al., 2020; TANABE et al., 2011).

Além dos efeitos antibacterianos, estudos também evidenciam que as PACs presentes no *Cranberry* são capazes de modular as vias de sinalização associadas à diferenciação e atividade dos osteoclastos, contribuindo para a preservação do tecido ósseo (FEGHALI et al., 2012; TANABE et al., 2011). Adicionalmente, nosso grupo de pesquisa demonstrou que o extrato de *Cranberry* apresenta potencial osteogênico moderado *in vitro*, promovendo a expressão de marcadores osteogênicos tanto em fases iniciais quanto tardias da diferenciação celular. Observou-se uma relação dose-dependente entre a concentração do extrato e a expressão dos marcadores: concentrações mais baixas do extrato de *Cranberry* foram eficazes na estimulação da atividade da fosfatase alcalina, enquanto níveis mais elevados foram necessários para induzir uma maior mineralização extracelular (BAUER et al., 2024).

Diante deste cenário, a incorporação de extrato seco de *Cranberry* no Biovidro Mesoporoso 58S configura-se como uma estratégia inovadora para potencializar as propriedades osteogênicas, antibacterianas e anti-inflamatórias deste biomaterial. Assim, este trabalho tem como objetivo desenvolver e caracterizar um biomaterial composto por Biovidro Mesoporoso 58S funcionalizado com extrato seco de *Cranberry*, avaliando *in vitro*, sua biocompatibilidade, capacidade osteoindutora e atividade antibiofilme.

2 DESENVOLVIMENTO

2.1 BIOMATERIAIS PARA REGENERAÇÃO ÓSSEA

A regeneração óssea é vista como um aspecto crítico na implantodontia e na reabilitação oral pois muitos pacientes apresentam volume ósseo reduzido devido à perda dentária, doenças degenerativas ou trauma (SORDI et al., 2021). Desta forma, o reparo de grandes defeitos ainda representa um desafio clínico significativo (MCGOVERN; GRIFFIN; HUTMACHER, 2018). Nesse contexto, várias abordagens surgiram para promover o reparo destes defeitos ósseos. Um substituto ósseo ideal deve apresentar propriedades essenciais além de sua capacidade de promover a formação óssea. Para ser considerado adequado, o material deve apresentar alta biocompatibilidade, porosidade e permeabilidade, fatores que facilitam as interações celulares, o transporte de nutrientes, crescimento tecidual e vascularização (FRÖHLICH et al., 2008).

Embora os enxertos ósseos autógenos sejam considerados o padrão de referência, suas limitações, incluindo morbidade do local doador e disponibilidade restrita, estimularam o desenvolvimento de materiais de enxertia óssea sintéticos, capazes de oferecer biocompatibilidade e osteocondutividade (UZEDA et al., 2017). Entre os biomateriais sintéticos utilizados para regeneração óssea na odontologia, destacam-se as cerâmicas bioativas, como os Biovidros e os fosfatos de cálcio (hidroxiapatita - HA, β -fosfato tricálcico - β -TCP e cerâmicas bifásicas - HA + β -TCP) (GAO et al., 2014).

A hidroxiapatita fornece excelente biocompatibilidade e osteocondutividade, mas apresenta baixa taxa de reabsorção, limitando o osso recém-formado durante o processo de cicatrização (NASCIMENTO et al., 2021). O β -TCP exhibe osteocondutividade e alta porosidade, que promovem a adesão celular e estimulam a vascularização (KAO; SCOTT, 2007). No entanto, apresenta menor resistência à compressão (ZHAO et al., 2021) e taxa de degradação mais rápida (NASCIMENTO et al., 2021; WANG; YEUNG, 2017). Para combinar os benefícios de ambos os materiais, as cerâmicas bifásicas de fosfato de cálcio foram desenvolvidas (HA + β -TCP) afim de atingir uma taxa de reabsorção equilibrada capaz de suportar a migração celular e ativar os processos biológicos envolvidos no reparo ósseo (NASCIMENTO et al., 2021).

O Biovidro apresenta capacidade de se ligar ao tecido ósseo e suportar a osteocondutividade, promovendo grande proliferação e diferenciação celular ao liberar

íons e formar uma camada de hidroxiapatita biologicamente ativa em sua superfície (HOPPE; GÜLDAL; BOCCACCINI, 2011). Como sua evolução, foi desenvolvido o Biovidro Mesoporoso (MBG), que apresentam propriedades capazes de aumentar sua eficácia na engenharia de tecido ósseo.

2.2 BIOVIDRO MESOPOROSO

O Biovidro foi desenvolvido em 1969 por Larry Hench na Universidade da Flórida, conhecido pela composição 45S5 Bioglass® (HENCH, 2006; HENCH et al., 1971; JONES, 2015). Este biomaterial é fabricado por um processo de fusão em altas temperaturas (1300–1500 °C) que apresenta a capacidade de se ligar intimamente ao tecido ósseo devido à liberação de íons e à capacidade de formar uma camada de hidroxiapatita carbonatada (CHAp) (WU; CHANG, 2012).

Os vidros bioativos têm desempenhado um papel cada vez mais importante na regeneração óssea em virtude de sua osteocondutividade, osteoestimulação e ótima taxa de degradação (WU; CHANG, 2012). O desenvolvimento do Biovidro revolucionou o campo de materiais bioativos (EL-RASHIDY et al., 2017) e a sua evolução pode ser dividida em três gerações. A primeira geração corresponde ao 45S5 Bioglass®, que demonstrou a eficácia do vidro bioativo na promoção da integração com o tecido. A segunda geração, introduzida em 1991, utilizou a técnica sol-gel, que se destacou pela sua capacidade superior de ligação óssea e por melhores propriedades de reabsorção e degradação em comparação com os vidros preparados por fusão. A busca por um vidro bioativo multifuncional levou ao desenvolvimento da terceira geração de vidros bioativos: os Biovidros Mesoporosos Nanoestruturados (MBGs). Estes materiais foram desenvolvidos em 2004 por meio da combinação de métodos sol-gel e química supramolecular de surfactantes (YAN et al., 2004). O processo de síntese deste biomaterial resulta em um alto volume e distribuição uniforme de poros, formando uma estrutura ordenada de canais mesoporosos e maior área de superfície (KARGOZAR et al., 2018; LALZAWMLIANA et al., 2019; WU; CHANG, 2012).

Os MBGs surgiram como uma resposta às limitações dos vidros bioativos convencionais, que carecem de estruturas mesoporosas bem definidas para aprimorar a regeneração óssea (LALZAWMLIANA et al., 2019). A organização mesoporosa permite que os MBGs apresentem propriedades texturais e bioativas superiores às observadas nos

biovidros convencionais (obtidos por fusão e sol-gel) (IZQUIERDO-BARBA; VALLET-REGI, 2015; LALZAWMLIANA et al., 2019). Esta terceira geração apresenta uma maior bioatividade, excelente biocompatibilidade, área de superfície otimizada e maior volume de poros, favorecendo a indução de mineralização de apatita (WU; CHANG, 2012). Estudos que avaliaram a bioatividade e a capacidade de formação da camada de hidroxiapatita carbonatada (CHAp) demonstraram que os Biovidros obtidos por fusão em altas temperaturas apresentaram bioatividade após 7 dias de imersão em fluido corpóreo simulado (SBF). Em comparação, os Biovidros produzidos pelo método sol-gel mostraram bioatividade em 3 dias, enquanto os Biovidros Mesoporosos destacaram-se por apresentar bioatividade em apenas 4 horas de imersão (IZQUIERDO-BARBA; VALLET-REGI, 2015).

Devido à estas propriedades estruturais e de composição, como a presença de grupos silanol (Si-OH), que favorecem as interações com biomoléculas por meio de ligações de hidrogênio (BAINO; HAMZEHLU; KARGOZAR, 2018; WU; CHANG, 2014), os Biovidros Mesoporosos destacam-se como sistemas eficientes para incorporação e liberação controlada de fármacos, favorecendo a engenharia tecidual (WU; CHANG, 2012).

Estudos comparativos demonstraram que os MBGs são capazes de incorporar o dobro de substâncias bioativas em relação aos arcabouços de biovidro convencional, além de apresentarem uma taxa de liberação significativamente menor, favorecendo um efeito terapêutico prolongado (BAINO; HAMZEHLU; KARGOZAR, 2018). A possibilidade de modular tanto a estrutura de poros quanto a composição química destes materiais permite otimizar a incorporação e ajustar o perfil de liberação de fármacos, atendendo a diferentes demandas terapêuticas (IZQUIERDO-BARBA; VALLET-REGI, 2015; WU; CHANG, 2012). Desta forma, os MBGs se destacam como candidatos promissores para o transporte eficiente e controlado de substâncias bioativas em aplicações regenerativas (WU; CHANG, 2014), representando um avanço relevante para área da regeneração óssea (LALZAWMLIANA et al., 2019).

2.3 CRANBERRY

O *Cranberry* (*Vaccinium macrocarpon*) tem sido amplamente investigado devido aos seus variados benefícios à saúde, especialmente no que se refere ao seu alto

teor de compostos bioativos, como as proantocianidinas (PACs) (TIPTON; BABU; DABBOUS, 2013). Estes compostos atuam como agentes preventivos e terapêuticos em diversas condições humanas, apresentando também propriedades benéficas para a saúde oral. Estudos demonstraram que os extratos de *Cranberry* tem a capacidade de inibir a formação de biofilmes, modular a resposta inflamatória do hospedeiro e inibir a atividade de enzimas proteolíticas derivadas de periodontopatógenos (LA; HOWELL; GRENIER, 2009). Além de apresentar propriedades antibacterianas contra patógenos cariogênicos e periodontais, estes extratos também exibem efeitos anti-inflamatórios, que podem modular as respostas imunológicas (GALARRAGA-VINUEZA et al., 2020; TIPTON; BABU; DABBOUS, 2013). Estas características ressaltam o potencial do *Cranberry* como um agente terapêutico valioso para diversas condições de saúde oral.

Uma das propriedades mais notáveis dos constituintes do *Cranberry* é a fração de alto peso molecular, conhecida como material não dialisável (NDM), que demonstrou a capacidade de inibir a produção de citocinas e quimiocinas pró-inflamatórias de diversos periodontopatógenos (TIPTON; BABU; DABBOUS, 2013), e de inibir a secreção de mediadores inflamatórios de células imunológicas ativadas, como a IL-6 e IL-8 (GALARRAGA-VINUEZA et al., 2020; TIPTON; BABU; DABBOUS, 2013). Estas propriedades posicionam os extratos de *Cranberry* como potenciais agentes no controle de condições inflamatórias que afetam a saúde oral, incluindo as doenças periodontais, que podem resultar na perda óssea alveolar (FEGHALI et al., 2012).

Além das propriedades anti-inflamatórias, as PACs derivadas do *Cranberry* também demonstraram um impacto positivo na inibição da diferenciação e função dos osteoclastos. Estudos indicam que estas proantocianidinas podem inibir a maturação dos osteoclastos por meio de diversas vias de sinalização, como a via NF- κ B e vias de sinalização das proteínas quinases ativadas por mitógeno (MAPKs) (BAUER et al., 2024; FEGHALI et al., 2012). Esta inibição representa uma característica significativa, já que os osteoclastos são responsáveis pela reabsorção óssea, e sua atividade excessiva pode contribuir para condições patológicas (FEGHALI et al., 2012).

Além de seus efeitos na osteoclastogênese, estudos sugerem que os extratos de *Cranberry* podem atuar na osteogênese. Evidências indicam que os componentes do *Cranberry* podem interagir com as vias de sinalização osteogênica, promovendo a diferenciação e proliferação de osteoblastos (BAUER et al., 2024). A modulação destas vias é fundamental para otimizar a cicatrização e a regeneração óssea, sendo um aspecto

importante a ser levado em consideração para a engenharia tecidual e incorporação de agentes terapêuticos em arcabouços.

Com o crescente interesse na utilização de compostos naturais para benefícios à saúde, os compostos derivados do *Cranberry* apresentam uma abordagem inovadora para potencializar os resultados em procedimentos de enxertia óssea. A incorporação ou revestimento de extratos de *Cranberry* em biomateriais de substituição óssea pode aprimorar sua eficácia, proporcionando benefícios osteogênicos, antibacterianos e anti-inflamatórios adicionais.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar *in vitro* a biocompatibilidade, capacidade antibiofilme e osteogênica do Biovidro Mesoporoso 58S revestido por um extrato seco de *Cranberry*.

3.2 OBJETIVOS ESPECÍFICOS

- Desenvolver um Biovidro Mesoporoso 58S revestido com extrato seco de *Cranberry*.
- Determinar a taxa de liberação do extrato seco de *Cranberry* das amostras de Biovidro Mesoporoso 58S.
- Analisar a ação antibiofilme do Biovidro Mesoporoso 58S revestido pelo extrato seco de *Cranberry*.
- Avaliar a biocompatibilidade do Biovidro Mesoporoso 58S com extrato seco de *Cranberry*.
- Avaliar a capacidade osteogênica do Biovidro Mesoporoso revestido pelo extrato seco de *Cranberry*.

4 ARTIGO

Cranberry-coated mesoporous bioactive glass for bone tissue regeneration: Biofilm and osteogenic analysis *in vitro*

Eduarda Blasi Magini (<https://orcid.org/0000-0002-1762-7059>, eduardablasimagini@gmail.com)^{1,2,3}, Yasmim Guterres Bauer (<https://orcid.org/0000-0003-4112-2314>, yasinha@gmail.com)^{1,2}, Joana Marques (<https://orcid.org/0000-0002-8740-642X>, jmarques2@edu.ulisboa.pt)³, Helena Francisco (<https://orcid.org/0000-0002-6783-7740>, helenafrancisco@campus.ul.pt)⁴, Bernardo Passoni (<https://orcid.org/0000-0001-6292-4311>, dr.bernardopassoni@gmail.com)³, Neusa Silva (<https://orcid.org/0000-0002-3454-065X>, s.neusa.marina@gmail.com)³, João Caramês (<https://orcid.org/0000-0002-5544-3744>, carames@campus.ul.pt)⁴, Ariadne Cristiane Cabral Cruz (<http://orcid.org/0000-0001-7306-4708>, ariadne.cruz@ufsc.br)^{1,2*}

¹ Post-Graduation Program of Dentistry, Center for Education and Research on Dental Implants, Federal University of Santa Catarina, Florianópolis, 88053-701, Brazil

² Laboratory of Applied Virology, Federal University of Santa Catarina, Florianópolis, 88053-701, Brazil.

³ Oral Biology and Biochemistry Research Group—Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Faculty of Dental Medicine, University of Lisbon, 1600-277 Lisbon, Portugal

⁴ Department of Oral Surgery and Implant Dentistry, Faculty of Dental Medicine, University of Lisbon, 1600-277 Lisbon, Portugal

* Correspondence to: ariadne.cruz@ufsc.br, +55 48 3721-3407 / Fax: +55 48 3721-9523

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Abstract

Objectives: This study aimed to synthesize a Cranberry-coated Mesoporous Bioactive Glass 58S (MBG-58S/CB) and evaluate its effects on biofilm formation and osteogenic differentiation *in vitro*.

Materials and Methods: MBG-58S scaffolds were synthesized using the sol-gel method, followed by a hydroalcoholic solution of Cranberry extract coating (MBG-58S/CB). The scaffolds underwent physicochemical characterization using scanning electron microscopy and energy-dispersive X-ray spectroscopy. Cranberry release kinetics were assessed. The interference of MBG-58S, MBG-58S/CB, Nanosynt[®], and Bio-Oss[®] on mono- and multi-species biofilm formation were determined. Cellular viability and osteogenic differentiation were evaluated using human-derived mesenchymal stromal cells (SHED) and the following groups: MBG-58S, MBG-58S/CB, and Bio-Oss[®]. Osteogenic assays involved the assessment of alkaline phosphatase (ALP) activity and extracellular matrix mineralization.

Results: The MBG-58S/CB scaffold exhibited a porous structure, allowing for the sustained release of Cranberry extract over 35 days. MBG-58S/CB promoted the lowest amount of bacteria in the multi-species biofilm on day 8, the highest cellular viability on day 7, and higher ALP activity compared to Bio-Oss[®] on day 3. Conversely, Bio-Oss[®] promoted the highest extracellular matrix mineralization on day 15.

Conclusions: Cranberry coating on Mesoporous Bioactive Glass scaffolds prevented the multi-species biofilm formation, enhanced the Mesoporous Bioactive Glass biocompatibility, and stimulated the early osteogenic marker (ALP). Nevertheless, this osteogenic capacity was not observed for extracellular matrix mineralization. Future studies are warranted to explore the potential of Cranberry for biomaterial biofunctionalization.

Clinical Relevance: This novel approach highlights the potential application of Cranberry extract for improved outcomes in bone tissue regeneration, thereby enhancing the bioactive performance of the bone substitutes.

Keywords: Cranberry, Mesoporous Bioactive Glass, Biofilm, Scaffold, Bone, Tissue engineering.

Introduction

As the global demand for bone regeneration surgeries continues to rise, research on biomaterials has primarily focused on their regenerative capabilities. However, bacterial contamination remains a critical challenge in bone grafting procedures, potentially compromising bone repair, leading to severe complications, and increasing the necessity for additional surgical interventions [1]. Indeed, biomaterial-associated infections are among the most common postoperative complications, significantly impairing tissue healing and regeneration [2]. Consequently, there is a growing interest in the development of multifunctional biomaterials that not only promote osteogenesis but also exhibit antibacterial properties to mitigate these risks[3].

Among the synthetic biomaterials used as scaffolds, Bioactive Glass is particularly notable for its high osteoconductivity, osteogenesis, controlled degradation rate, and inherent antimicrobial properties [2, 4]. First introduced by Professor Larry Hench in 1969 [5], 45S5 Bioactive Glass (composed of 45% SiO₂, 24.5% Na₂O, 24.5% CaO, and 6% P₂O₅) has demonstrated a unique ability to chemically bond with bone tissue. Through the release of Na⁺ and Ca²⁺ ions and the subsequent formation of a carbonated hydroxyapatite layer, bioactive glass actively supports new bone formation and integration with host tissue [6].

Building on the success of 45S5 Bioactive Glass, Mesoporous Bioactive Glass was later developed by Yan et al. [7], incorporating a highly ordered mesoporous architecture (2–50 nm). This structure modification significantly increases surface area and porosity, leading to enhanced ion exchange kinetics, faster hydroxyapatite formation, and improved bioactivity. Furthermore, Mesoporous Bioactive Glass offers a tunable degradation rate, allowing controlled material resorption while maintaining mechanical integrity. Its mesoporous structure also facilitates the efficient incorporation and sustained release of bioactive molecules, making it particularly advantageous for multifunctional scaffolds in challenging environments such as the oral cavity [4].

Cranberry (*Vaccinium macrocarpum*) bioactive compounds have gained considerable attention due to their antibacterial efficacy against cariogenic and periodontal pathogens [8]. Rich in polyphenolic constituents - including phenolic acids, flavonoids, and tannins - Cranberry exhibits notable anti-inflammatory properties [8–10]. Additionally, Cranberry-derived polyphenols have been shown to inhibit osteoclastic differentiation and activity [11, 12], partly by modulating the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways [12]. These mechanisms suggest a potential role for Cranberry in bone preservation [13]. Our research group has further demonstrated that Cranberry extract exhibits moderate osteogenic potential *in vitro*, promoting both early and late osteogenic markers [14].

In light of Cranberry extract's antimicrobial, anti-inflammatory, and osteogenic properties, this study aims to synthesize a Cranberry-coated Mesoporous Bioactive Glass scaffold and assess its impact on biofilm prevention and osteogenesis *in vitro*.

Material and Methods

Scaffolds preparation

The 58S Mesoporous Bioactive Glass (MBG-58S), composed of 58% SiO₂, 33 % CaO, and 9 % P₂O₅ (wt%), was synthesized using the sol-gel technique [15, 16]. The pluronic triblock copolymer P123 (EO20PO70EO20, 5800, Sigma-Aldrich, USA) was dissolved in ethanol (1:8, m:v) and stirred magnetically at 40°C for 1 hour. Tetraethyl orthosilicate (TEOS, 98%, Sigma-Aldrich, USA), triethyl phosphate (TEP, 99.8% Sigma-Aldrich, USA), and calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O, Vetec, Brazil) served as the silicate, phosphate, and calcium sources, respectively. For this, TEOS was initially dissolved in ethanol (Synth, Brazil) under magnetic stirring at 25°C, followed by the addition of TEP. A solution of Ca(NO₃)₂·4H₂O was prepared in 2 M HNO₃ (Vetec, Brazil) and combined with water at a TEOS:H₂O molar ratio of 1:4, and stirred for 1 hour. The mixture was dried at 70 °C for 24 hours, followed by heat treatment at 600 °C. The resultant material was pulverized using a planetary ball mill (PM100, Retsch, Germany) at 400 rpm for 1 hour to achieve a fine bioactive glass powder. This powder was then deagglomerated in an agate mortar using acetone and sieved to a particle size of 106 µm. Fine powders were then compressed at 80 MPa to form scaffolds with a diameter of 6 mm and a thickness of 3 mm.

For the Cranberry extract coating, a 500 mg/mL Cranberry dry extract (Shaanxi Honghao, Xi'an, China) solution was prepared [14] using a 50% (v/v) hydroalcoholic solution. Subsequently, 150 µL of this solution was applied onto each scaffold, protected from light, and dried at 37°C for 24 hours. This group was named as MBG-58S/CB. Scaffolds without Cranberry extract were named as MBG-58S. The scaffolds were sterilized using gamma irradiation (Radiation Technology Center, CTR-IPEN, São Paulo, Brazil), as shown in Fig. 1.

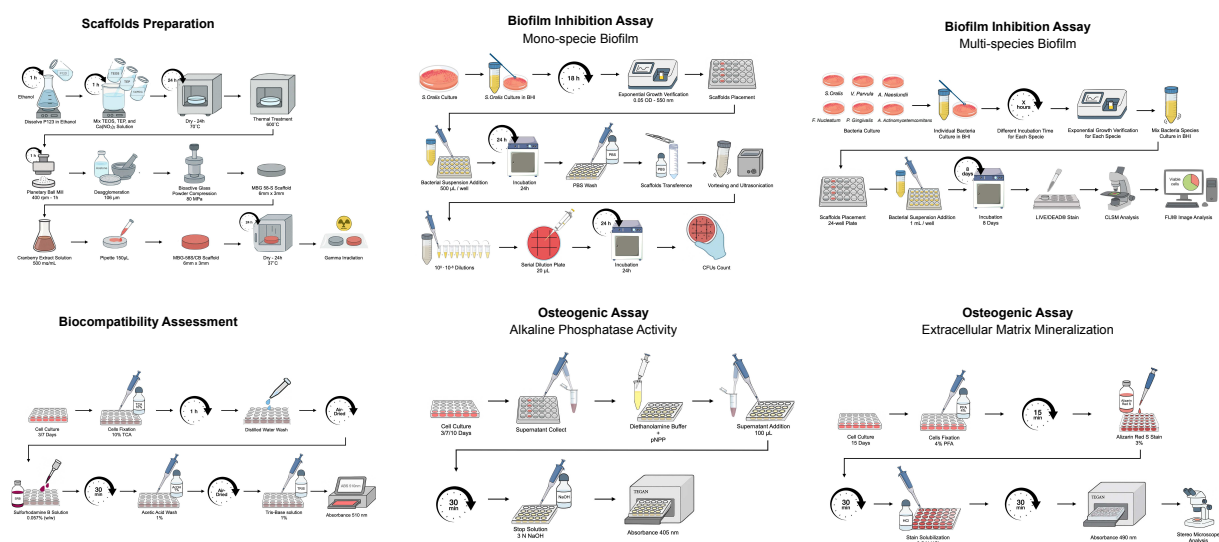


Fig. 1 Schematic representation of the study methodology. Legend. *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*; *A. naeslundii*: *Actinomyces naeslundii*; BHI: Brain–heart infusion medium; CFUs: Colony-forming units; CLSM: confocal laser scanning microscopy; *F. nucleatum*:

Fusobacterium nucleatum; MBG-58S: 58S Mesoporous Bioactive Glass; MBG-58S/CB: Cranberry-coated MBG-58S; PFA: paraformaldehyde; PBS: Phosphate buffer saline; *P. gingivalis*: *Porphyromonas gingivalis*; *S. oralis*: *Streptococcus oralis*; TEOS: Tetraethyl orthosilicate; TEP: Triethyl phosphate; *V. parvula*: *Veillonella parvula*.

Physic and Chemical Characterization

Microstructural analysis of MBG-58S and MBG-58S/CB scaffolds (n=3 for each group) was performed using a scanning electron microscope (SEM, JEOL JSM-6390LV, Tokyo, Japan) at 10 kV. Scaffolds were coated with a 300 Å gold layer prior to imaging at 500x and 1000x magnifications. Energy-dispersive X-ray spectroscopy (EDX, Hitachi Swift 2000, Japan) was used to analyze the surface chemical composition [17].

Cranberry Extract Release

The Cranberry extract release from MBG-58S/CB scaffolds (n=6) was assessed in phosphate-buffered saline (PBS) at 37°C and pH 7.4, over 35 days (sampling on days 3, 7, 10, 14, 21, 28, and 35). Absorbance at 550 nm was measured using a spectrophotometer (Infinite M200, TECAN, Austria). A standard curve was generated using concentrations of Cranberry extract ranging from 0.0195 to 5 mg.

Bacterial strains

The reference bacterial strains employed in this study included: *Streptococcus oralis* (*S. oralis*, CECT 907), *Veillonella parvula* (*V. parvula*, CCUG 5123), *Actinomyces naeslundii* (*A. naeslundii*, ATCC 19039), *Fusobacterium nucleatum* (*F. nucleatum*, DSM 20482), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*, DSM 8324), and *Porphyromonas gingivalis* (*P. gingivalis*, ATCC 33277). Bacteria cultures were maintained on blood agar plates (Blood agar Base No.2, Oxoid, Basingstoke, UK) supplemented with 5 mg/mL Hemin (Sigma, St. Louis, USA), 1 mg/mL menadione (Merk, Darmstadt, DE), and 5% (v/v) sterile horse blood (TCS, Buckingham, UK), under anaerobic conditions (10% H₂, 10% CO₂, and 80% N₂) at 37°C. Liquid cultures were prepared in modified brain–heart infusion medium (BHI, BD, New Jersey, USA), supplemented with 2.5 g/L mucin (Sigma), 1 g/L yeast extract (Sigma), 0.1 g/L cysteine (VWR, Ohio, USA), 2 g/L sodium bicarbonate (Sigma), 5 mg/mL hemin (Sigma), 1 mg/mL menadione (Merck), and 0.25% (v/v) glutamic acid (Sigma).

Biofilm Inhibition Assays

The following groups were established to develop the biofilm inhibition assays: MBG-58S, MBG-58S/CB, Nanosynt® (FGM, Brazil), and Bio-Oss® (Geistlich Biomaterials, Switzerland) (n=9 for each group).

Mono-specie Biofilm

S.oralis was cultured anaerobically at 37°C for 18h in a modified BHI medium. After incubation, the bacterial inoculum was adjusted with fresh medium to reach an optical density (OD) of 0.05 at 550 nm, ensuring exponential growth. Scaffolds were placed in a 48-well plate (Corning®, Corning, NY, USA), and

500 μL of the bacterial suspension, at a final concentration of 10^8 colony-forming units (CFUs) mL^{-1} , was added to each well. The plates were incubated under anaerobic conditions at 37°C for 24 hours. To assess the capacity of the scaffolds to prevent biofilm formation, bacterial adhesion was quantified by CFU counting. Following incubation, the scaffolds were washed once with phosphate buffer saline (PBS, VWR®, Radnor, PA, USA). Each scaffold was then transferred to a tube containing 3 mL of PBS and subjected to vortexing for 1 minute at 1600 rpm, followed by ultrasonication for 4 minutes and an additional 2 minutes of vortexing under the same conditions. Serial ten-fold dilutions were prepared up to 10^{-8} , and 20 μL of each dilution were plated in duplicate on supplemented blood agar plates. The plates were incubated under anaerobic conditions at 37°C for 24 hours before determining total CFUs per millimeter [18].

Multi-species Biofilm

The *in vitro* multi-species biofilm model was established according to the protocol described by Sánchez et al [19]. Individual bacterial strains were cultured anaerobically at 37°C in a modified BHI medium. Upon reaching the exponential phase (verified by spectrophotometry), bacterial suspensions were mixed in fresh modified BHI medium to achieve a final concentration of 10^3 CFUs mL^{-1} for *S. oralis*, 10^5 CFUs mL^{-1} for *V. parvula* and *A. naeslundii*, and 10^6 CFUs mL^{-1} for *F. nucleatum*, *A. actinomycetemcomitans*, and *P. gingivalis*. Scaffolds were then placed in a 24-well plate (Corning®, Corning, NY, USA), and 1 mL of the mixed bacterial suspension was added to each well. Plates were incubated anaerobically at 37°C for up to 8 days, with media changes occurring every 48 hours, starting at 72 hours post-inoculation. The capacity of the scaffolds to prevent biofilm formation was assessed by confocal laser scanning microscopy on day 8. Biofilms formed on the scaffolds were stained using the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Thermo Fisher Scientific, Milan, Italy) and analyzed by confocal laser scanning microscopy. Fluorescence signals were optimized for the detection of SYTO9® (515–530 nm) and propidium iodide (>600 nm). Three representative regions per scaffold were selected for imaging. Image processing and analysis were performed using FIJI® software (ImageJ, National Institute of Health, Bethesda, Maryland, USA). A z-stack series (XYZ) was obtained, and images were processed by adjusting brightness and contrast, converting them to 8-bit format, and applying a threshold to eliminate background noise. Particle analysis was performed to quantify live and dead cells, and the percentage of viable cells was calculated.

Cell Culture Conditions

Mesenchymal stromal cells from human exfoliated deciduous teeth (SHED, Curityba Biotech™ Cell Processing Center, Brazil) were used to assess the biocompatibility and osteogenic potential of the scaffold. SHED were cultured in Dulbecco's modified Essential culture medium (DMEM, Gibco, Brazil) supplemented with 10% fetal bovine serum (FBS, Gibco, Brazil). Culture media were refreshed every 2-3 days, and cells were used up to passage #9, incubated at 37°C in a 5% CO_2 atmosphere condition. Since the Bio-Oss® demonstrated reduced multi-species biofilm formation among the control group, it was decided to follow with these experimental groups: MBG-58S, MBG-58S/CB, and Bio-Oss®. To determine the sample size (n) for independent groups, data from a pilot study were utilized [20]. All assays were

performed in biological triplicate and experimental duplicate (n=9 for each group). Cells were cultured in 48-well plates with 2×10^4 cells per well.

Biocompatibility Assessment

Scaffold biocompatibility was evaluated using the Sulforhodamine B assay on days 3 and 7. Therefore, SHED (2×10^4 cells/scaffold) were seeded onto the scaffolds' surface, in 150 μ L of DMEM supplemented with 10% FBS, and maintained 4 hours to allow cells to adhere. Subsequently, 400 μ L of culture medium was added to each well. At the specific experimental times, SHED were fixed with 10% trichloroacetic acid for 1 hour at 4°C, washed with distilled water, and air-dried. Following this, 0.057% (w/w) Sulforhodamine B solution was added for 30 minutes, followed by washing with 1% acetic acid. Wells were air-dried, and 10 mM Tris-Base solution (pH 10.5) was added to dissolve bound dye, followed by agitation for 5 minutes to dissolve the dye bound to cellular proteins. Absorbance was measured at 510 nm (Infinite M200, TECAN, Austria) (VICHAI; KIRTIKARA, 2006). Cell viability percentages were calculated relative to the control group (Bio-Oss®).

Osteogenic Differentiation

Alkaline Phosphatase Activity

The alkaline phosphatase (ALP) activity was assessed in the cell supernatant by measuring ρ -nitrophenol (ρ -NP) release from ρ -nitrophenol phosphate (ρ NPP) on days 3, 7, and 10. The reaction was conducted in diethanolamine buffer (1.02 M, pH 9.8, containing 0.6 mM magnesium chloride) with 60.8 mM ρ NPP for 2 minutes at 37°C. After, 0.1 mL of the supernatant was added. The reaction was stopped with 3 N NaOH after 30 minutes. Absorbances were measured at 405 nm (Infinite M200, TECAN, Austria), and ALP activity was calculated based on a ρ NP standard curve [21].

Extracellular Matrix Mineralization

Alizarin Red S staining was used to quantify the extracellular matrix mineralization on day 15. Briefly, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, USA) for 15 minutes and stained with 3% Alizarin Red S (Sigma-Aldrich, St. Louis, USA) for 30 minutes. A stereo microscope (SZX16® Olympus Corporation, Tokyo, Japan) was employed for analysis, and images were captured using the Olympus DP73® camera and the acquisition software (CellSens Dimension 1.12®, Olympus Corporation, Tokyo, Japan). Absorbances were measured at 490 nm using a spectrophotometer (Infinite M200, TECAN, Salzburg, Austria) after solubilizing the stain in 0.5 N hydrochloric acid (Sigma-Aldrich, St. Louis, USA) for 30 minutes [20].

Statistical Analysis

Data were analyzed using GraphPad Prism 8 Software (GraphPad Software Inc., San Diego, CA, USA). Group comparisons were conducted at each experimental time, using one-way ANOVA followed by Tukey's post hoc test. Values of $p < 0.05$ were considered statistically significant.

Results

Physic and chemical characterization

As depicted in Fig. 2, SEM micrographs revealed a high porous volume in Mesoporous Bioactive Glass scaffolds with a non-smooth porous structure. In contrast, the Cranberry-coated 58S Mesoporous Bioactive Glass scaffolds showed a more homogeneous layer due to the application of the Cranberry extract solution, with some cracks present in this layer. EDX analysis confirmed the presence of Silicon (Si) and Calcium (Ca) elements in both MBG-58S and MBG-58S/CB scaffolds.

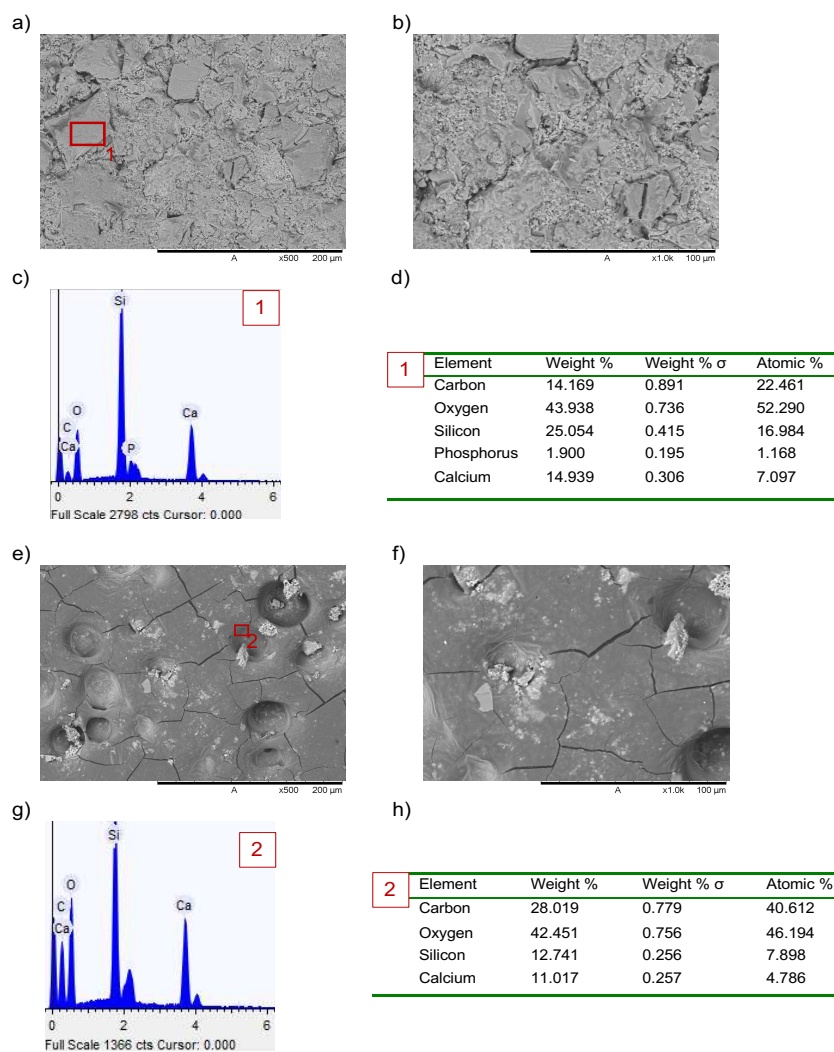


Fig.2 Physic and chemical characterization of MBG-58S and MBG-58S/CB (n=3 for each group). Representative image obtained by SEM of MBG-58S at magnifications of a) x500 and b) x1,000, with scale

bars of 200 μm and 100 μm , respectively. c) EDX graph of MBG-58S scaffold and d) weight percentages of chemical composition. Representative SEM images of MBG-58S/CB at e) x500 and f) x1.000 magnifications, with scale bars of 200 μm and 100 μm , respectively. g) EDX graph of MBG-58S/CB scaffold and h) weight percentages of chemical composition. Legend: EDX: Energy dispersive X-ray spectroscopy; MBG-58S: 58S Mesoporous Bioactive Glass; MBG-58S/CB: Cranberry-coated 58S Mesoporous Bioactive Glass; SEM: Scanning electron microscopy.

Cranberry Extract Release

As demonstrated in Fig. 3, the Cranberry extract was gradually and steadily released from the MBG-58S/CB scaffolds. By day 3, 1.53% (1.15mg) of the Cranberry was released. This sustained-release profile continued consistently over the 35 days, culminating in a total release of 3.43%, corresponding to 2.57 mg. The Cranberry extract release profile exhibited a linear sustained-release rate, indicating prolonged availability of the bioactive compound.

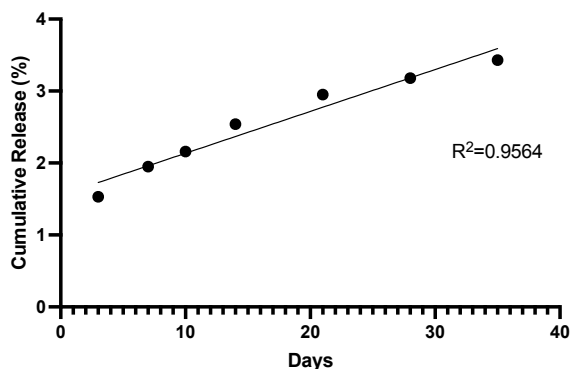


Fig.3 Cranberry extract released from MBG-58S/CB scaffolds in phosphate-buffered saline solution for up to 35 days. The analysis periods were 1, 3, 7, 10, 14, 28, and 35 days. Values reported are an average of $n=6 \pm$ standard deviation. Legend: MBG-58S/CB: Cranberry-coated 58S Mesoporous Bioactive Glass.

Capacity to prevent the mono-specie biofilm formation

As depicted in Fig. 4a, according to the CFU assays, Bio-Oss[®] presented the highest amount of *S. oralis* biofilm ($p < 0.001$) at 24 h of culture. There was no difference among MBG-58S, MBG-58S/CB, and Nanosynt[®] results ($p > 0.05$).

Capacity to prevent the multi-species biofilm formation

Concerning the capacity to prevent the multi-species biofilm formation, Cranberry-coated 58S Mesoporous Bioactive Glass scaffolds promoted the lowest amount of bacteria in the multi-species biofilm on day 8, according to Fig. 4b-c. Conversely, Nanosynt[®] demonstrated a higher amount of bacteria. Additionally, Cranberry-coated 58S mesoporous bioactive glass scaffolds presented less viable bacteria compared to MBG-58S.

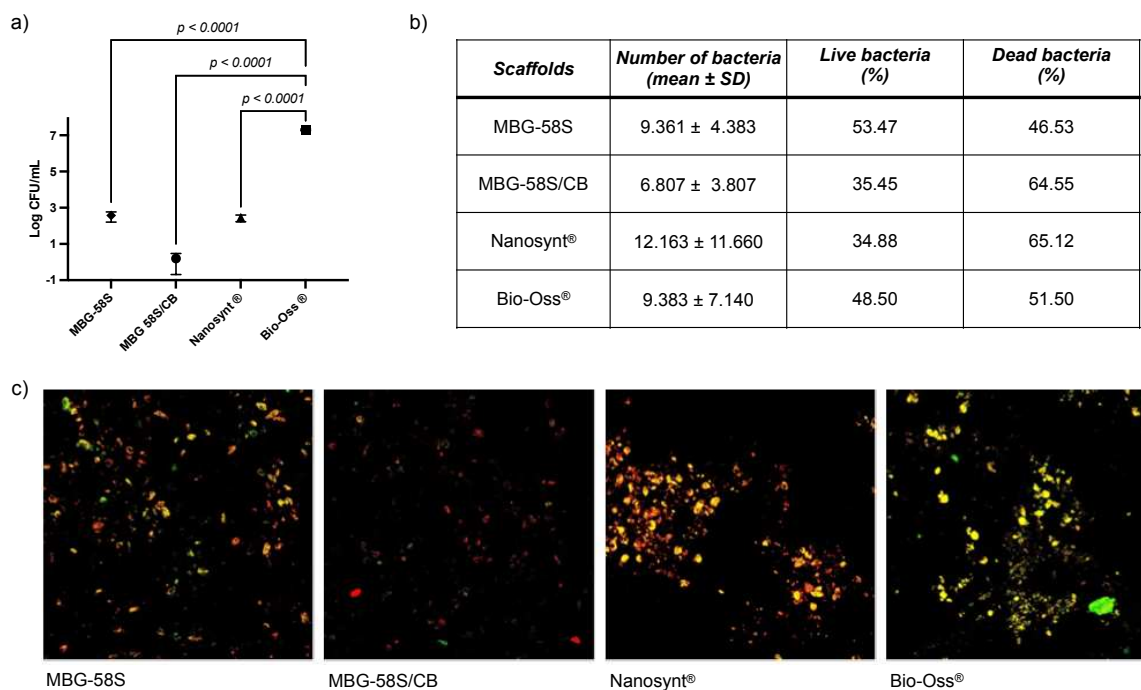


Fig.4 a) Capacity of the scaffolds ($n=9$ for each group) to prevent the *Streptococcus oralis* biofilm formation by CFU at 24 h of culture. Statistical analysis employed a one-way ANOVA test ($p < 0.0001$), followed by Tukey's multiple comparisons test. The post-test p values are presented in the graph. b) Capacity of the scaffolds to prevent the multi-species biofilm formation. Biofilm was composed of *Aggregatibacter actinomycetemcomitans*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus oralis*, and *Veillonella parvula*. The b) quantification of bacteria and c) their viability were assessed using LIVE/DEAD® BacLight™ Bacterial Viability Kit and confocal laser scanning microscopy on day 8 ($n=9$ for each group). The number of bacteria (mean ± SD) is reported per sample. Statistical analysis employed a one-way ANOVA test ($p = 0.6237$), followed by Tukey's multiple comparisons test. Legend: CFU: colony forming unit; MBG-58S: 58S Mesoporous Bioactive Glass; MBG-58S/CB: Cranberry-coated 58S Mesoporous Bioactive Glass. SD: standard deviation.

Biocompatibility Assessment

Fig. 5 presents cellular viability data, showing that on day 7, Cranberry-coated 58S Mesoporous Bioactive Glass scaffolds promoted the highest cellular viability, compared to MBG-58S scaffolds and Bio-Oss® ($p = 0.0481$ and $p = 0.0253$, respectively), suggesting a positive effect of Cranberry extract on cellular survival. It is worth mentioning that all scaffolds maintained cellular viability above 70%, indicating biocompatibility with SHED [22].

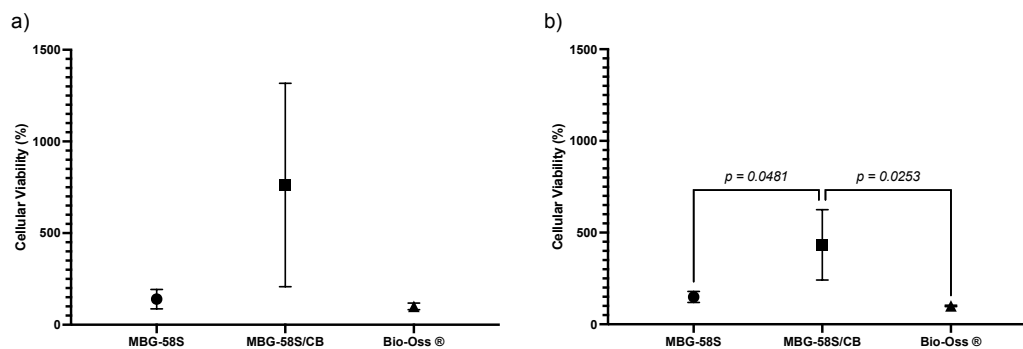


Fig.5 Biocompatibility of MBG-58S and MBG-58S/CB in SHED on days (a) 3 and (b) 7 using the Sulforhodamine B assay. Cell viability percentages were determined in reference to the positive control (Bio-Oss®). Values reported are an average of $n=9 \pm$ standard deviation. No significant difference was found among groups on day 3. Statistical analysis employed a one-way ANOVA test for day 3 data ($p = 0.0789$) and day 7 data ($p = 0.0220$), followed by Tukey's multiple comparisons test. The post-test p values are presented in the graph. Legend: MBG-58S: 58S Mesoporous Bioactive Glass; MBG-58S/CB: Cranberry-coated 58S Mesoporous Bioactive Glass; SHED: Stromal cells from human exfoliated deciduous teeth.

Alkaline Phosphatase Activity

As depicted in Fig. 6a-c, the Cranberry-coated 58S Mesoporous Bioactive Glass scaffolds induced more ALP activity compared to the positive control group (Bio-Oss®) on days 3, 7, and 10 ($p = 0.0384$, $p = 0.0030$, and $p = 0.0009$, respectively). Additionally, the Cranberry coating promoted more ALP activity compared to Bioactive Glass without the coating on day 3, which was the peak day for ALP activity, indicating that the Cranberry coating induced this positive effect.

Extracellular Matrix Mineralization

According to Fig. 6d-e, Bio-Oss® scaffold induced the highest extracellular matrix mineralization compared to MBG-58S ($p = 0.0007$) and MBG-58S/CB ($p = 0.0040$) on day 15 through Alizarin Red S quantification.

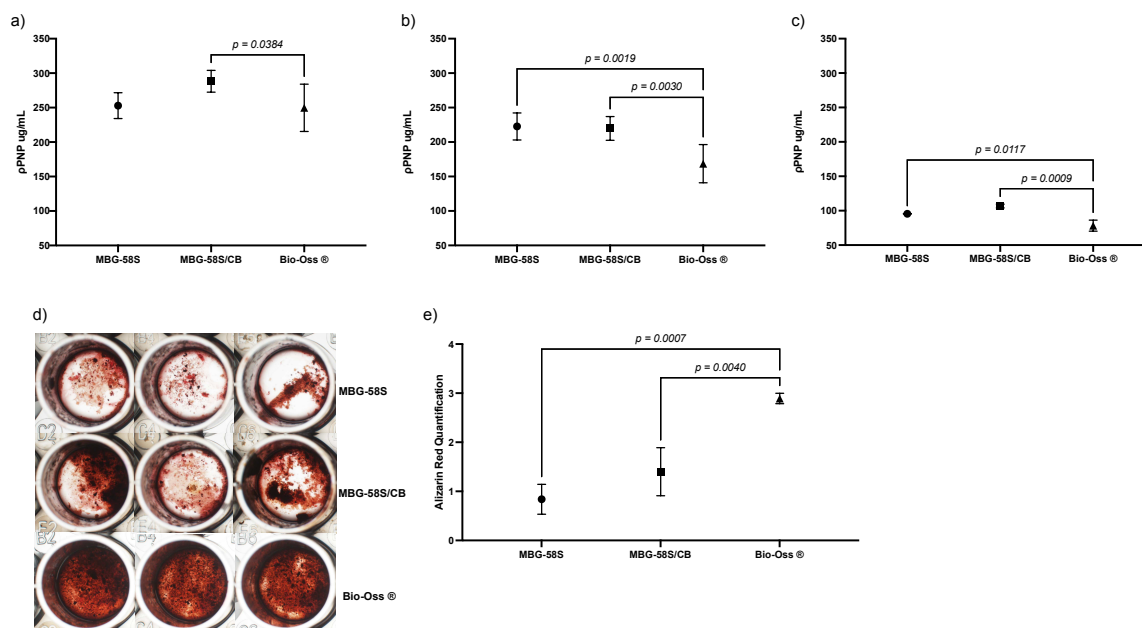


Fig. 6 ALP activity assessed by pPNP produced from pPNPP measured on days a) 3, b) 7, and c) 10 in SHED. d) Representative images of Alizarin Red S staining and e) Alizarin Red S quantification on day 15. Values reported are an average of $n=9 \pm$ standard deviation. Statistical analysis was based on the one-way ANOVA test, followed by Tukey's multiple comparisons test. The post-test p values are presented in the graph. Legend- ALP: Alkaline phosphatase; MBG-58S: 58S Mesoporous Bioactive Glass; MBG-58S/CB: Cranberry-coated 58S Mesoporous Bioactive Glass; pPNP: p -nitrophenyl; pPNPP: p -nitrophenyl phosphate; SHED: Stromal cells from human exfoliated deciduous teeth.

Discussion

The development of innovative biomaterials has notably advanced the field of bone tissue engineering, offering novel solutions to overcome challenges in bone regeneration. Mesoporous Bioactive Glass (MBG), with its highly porous structure, bioactivity, and capacity for controlled delivery of therapeutic agents, is a particularly promising candidate. In this study, we investigated the loading of Cranberry extract as a surface coating on MBG-58S scaffold to assess its effect on biofilm inhibition and osteogenic differentiation. Our findings indicate that Cranberry-coated MBG scaffolds exhibited improved capacity to prevent multi-species biofilm formation, biocompatibility for human undifferentiated cells, and significantly elevated alkaline phosphatase activity - a recognized early marker of osteogenic differentiation - compared to commercial gold standard Bio-Oss®. This novel approach highlights the potential application of Cranberry extract for improved outcomes in bone tissue regeneration, thereby enhancing the bioactive performance of bone substitutes.

Incorporating natural compounds, such as Cranberry extract, is a novel strategy for improving biomaterial properties. Cranberry extract, rich in polyphenols, such as proanthocyanidins, is known for its antibacterial and anti-inflammatory properties, which make Cranberry extract a promising additive for dental applications [8]. Studies demonstrate that Cranberry polyphenols can disrupt the physiology of

osteoclastic cells, inhibiting their function while promoting osteoblastic differentiation [11, 12]. Polyphenols can influence bone regeneration by inhibiting osteoclast differentiation and protecting against inflammatory bone loss [23], while also regulating inflammatory cytokines involved in bone resorption and enhancing anti-inflammatory cytokines, targeting macrophagic cells and osteoblasts [13]. Additionally, the direct osteopromotive effects of polyphenols on osteoblast differentiation and proliferation through various biological pathways are also well-documented [13, 24, 25], with evidence of increased alkaline phosphatase activity and enhanced mineralization capacity [24]. Furthermore, previous studies have reported that Cranberry-derived proanthocyanins possess multifunctional anti-inflammatory, anti-biofilm, and macrophage-modulating properties that may be beneficial in targeting periodontal and peri-implant disease sites [26].

In our study, the MBG-58S/CB scaffolds showed promising antibacterial behavior by reducing multi-species biofilm formation. The use of a multi-species biofilm model is particularly as it better reflects the complex microbial interactions found in the oral cavity. Unlike mono-species models, multi-species biofilms exhibit enhanced resilience and virulence, making them a more stringent and clinically relevant test for evaluating the antibacterial performance of biomaterials. Therefore, demonstrating efficacy against multi-species biofilms strengthens the translational potential of the tested scaffold for bone tissue regeneration in contaminated environments.

Alkaline phosphatase is recognized as an early marker of osteoblast differentiation [27, 28]. It plays a central role in mineralization by generating an alkaline microenvironment and increasing phosphate availability, thereby facilitating the formation of hydroxyapatite [29]. Therefore, an increased level of alkaline phosphatase indicates ongoing bone formation [27, 28]. The Cranberry-loaded Mesoporous Bioactive Glass promoted significantly higher alkaline phosphatase activity at all time points, especially on day 3, the peak of activity, suggesting that Cranberry effectively stimulated osteogenic differentiation during the initial phases of differentiation. Nevertheless, this stimulatory effect was not consistently observed during the later stages of osteogenesis. Although all groups exhibited extracellular matrix mineralization by day 15, Bio-Oss® demonstrated significantly higher mineral deposition than both MBG-58S and MBG-58S/CB. These results suggest that while Cranberry extract enhances early osteogenic markers, it may be insufficient to drive mineralization to the same extent as traditional biomaterials. The limited release concentration of Cranberry observed in the sustained delivery profile may account for this discrepancy. Indeed, the Cranberry coating was uniformly distributed and demonstrated a sustained release profile over 35 days, a feature beneficial for maintaining prolonged biological activity at the implantation site. While this prolonged release supports cellular viability [30], it may not deliver a sufficient therapeutic concentration required for effective late-stage osteogenesis. Recent evidence by Bauer [14] highlights a dose-dependent relationship between Cranberry extract concentration and osteogenic marker expression, low concentrations were adequate to stimulate alkaline phosphatase activity, whereas higher concentrations were required for robust mineralization. Conversely, Galarraga-Vinueza et al. [16] reported that high concentrations of Cranberry proanthocyanidins may inhibit

hydroxyapatite formation, suggesting a delicate balance is needed to maximize therapeutic benefit without compromising bioactivity.

Controlled delivery strategies have been explored to address challenges related to polyphenol bioavailability, stability, and dose-dependence, aiming to enhance their local therapeutic effects while minimizing systemic toxicity [25]. Approaches such as polymeric matrices, nanoparticulate systems, microemulsions, and biodegradable coatings enable the controlled release of polyphenols, resulting in increased effectiveness [25]. Previous studies have shown that poly(lactic-co-glycolic) acid (PLGA)-based microencapsulation of Cranberry extract, embedded with stearic acid with polyvinylpyrrolidone (PVP) or Ethyl lauroyl arginate (LAE), led to higher loading of the Cranberry active and inactive compounds, reduced burst effects, and demonstrated effective biofilm inhibition even at lower concentrations, highlighting the critical role of dosing in achieving therapeutic efficacy [31]. Additionally, previous studies have shown that polyphenols can readily bind to the surface of bioactive glass and glass-ceramics due to their high surface area and abundance of silanol groups, forming hydrogen bonds with polyphenolic hydroxyl groups [32–34]. This interaction not only reduces the initial burst release but also prolongs sustained delivery, allowing the controlled dose release that promotes antioxidant and anti-inflammatory properties over time [32, 33, 35]. Moreover, according to Dziadek et al. [32], the presence of bioactive glass particles as modifiers in biodegradable polymer matrices has reduced the polyphenol release rate. The adsorption of polyphenols on the glass surface may have influenced the Cranberry concentration released in our study. Furthermore, the bioactive glass properties, surface area, and the use of different polyphenol concentrations, can be modulated to achieve a superior polyphenol binding capacity and release kinetics, influencing key properties such as hydrophilicity, apatite-forming ability, and osteogenic potential [32–35].

The integration of natural compounds such as Cranberry extract into Mesoporous Bioactive Glass scaffolds offers an attractive strategy for enhancing scaffold performance in bone tissue engineering. While this study provides the first *in vitro* evidence of osteogenic stimulation by Cranberry-coated MBG, future *in vivo* studies and clinical investigations are essential to validate its translational potential and to fully harness its multifunctional properties for regenerative applications. Additionally, future studies should investigate alternative loading or embedding strategies to optimize the release kinetics and enhance the bioavailability of Cranberry compounds at the cellular level. The sustained release kinetics of the Cranberry extract may not have delivered a sufficient concentration to promote significant mineralization. Applying the Cranberry extract as a coating could also have restricted its effective delivery to the cells. Embedding Cranberry extract directly into the MBG-58S during synthesis, rather than simply coating the surface, may improve the delivery and efficacy of the Cranberry extract. Further research is needed to clarify the mechanism by which Cranberry extract acts on bone regeneration, as well as to explore how its benefits and properties can be integrated into novel biomaterial combinations to validate the efficacy of this natural agent in improving bone regeneration in clinical settings.

Conclusions

The Cranberry coating on Mesoporous Bioactive Glass scaffolds prevented multi-species biofilm formation, enhanced the Mesoporous Bioactive glass biocompatibility, and stimulated the early osteogenic marker (alkaline phosphatase). Nevertheless, this osteogenic capacity was not observed for extracellular matrix mineralization. This novel approach highlights the potential application of Cranberry extract for improved outcomes in bone tissue regeneration, thereby enhancing the bioactive performance of bone substitutes. Future studies are warranted to explore the potential of Cranberry for biomaterial biofunctionalization.

Compliance with Ethical Standards

- Conflict of Interests: The authors declare no conflicts of interest.
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- Ethical Approval: Not applicable.
- Informed Consent: Not applicable.

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5 CONSIDERAÇÕES FINAIS

O presente trabalho avaliou o potencial do Biovidro Mesoporoso 58S (MBG-58S/CB) revestido com extrato seco de *Cranberry* para aplicações em engenharia tecidual óssea. Os resultados obtidos demonstraram que a estratégia de biofuncionalização com extrato de *Cranberry* foi eficaz. O revestimento de *Cranberry* proporcionou um aumento na biocompatibilidade do Biovidro Mesoporoso 58S, resultado em uma alta viabilidade de células mesenquimais derivadas de dentes decíduos humanos (SHED) cultivadas sobre o biomaterial. O MBG-58S/CB foi eficaz em reduzir a formação de biofilme multiespécies, o que evidencia a aplicação promissora de compostos naturais atuarem como agentes antimicrobianos em substitutos ósseos. Além disso, foi observado um estímulo significativo na atividade da fosfatase alcalina (ALP), um marcador precoce da diferenciação osteogênica, sugerindo que o recobrimento com *Cranberry* contribuiu para induzir as fases iniciais do processo osteogênico.

Por outro lado, o estudo indicou limitações quanto à mineralização da matriz extracelular. Apesar do aumento na ALP, os níveis de mineralização induzidos pelos arcabouços MBG-58S/CB foram inferiores aos observados para o arcabouço Bio-Oss® (controle positivo). Esta diferença pode estar associada ao perfil de liberação do *Cranberry*, que possivelmente não forneceu doses adequadas para sustentar as fases tardias da diferenciação osteogênica e mineralização. Isso indica a necessidade de otimizar estratégias de incorporação e liberação de agentes terapêuticos para potencializar os seus efeitos.

De forma geral, os achados do presente estudo contribuem para o avanço no desenvolvimento de biomateriais multifuncionais e a abordagem destaca a aplicação potencial do extrato de *Cranberry* para aprimorar a regeneração do tecido ósseo. Estudos futuros são necessários para validar sua aplicação clínica na regeneração óssea.

PRODUÇÃO CIENTÍFICA DURANTE O DOUTORADO

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