

Antimicrobial TiN-Ag Coatings in Leather Insole for Diabetic Foot

Volume 15 · Issue 6 | March (II) 2022

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Website (<https://www.docenti.unina.it/#!/professor/4749414e5249434f535041474e554f4c4f53504747524337324530374638333956/riferimenti>)

Section Associate Editor

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Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples "Federico II", 80131 Naples, Italy

Interests: oral medicine; dental materials; operative dentistry; oral health

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Prof. Dr. Javier Gil (<https://sciprofiles.com/profile/481998>) *

Website (<https://www.uic.es/ca/teacher/xavier.gil>)

Section Editor-in-Chief

Bioengineering Institute of Technology, Medicine and Health Sciences Faculty, Universitat Internacional de Catalunya, C/ Josep Trueta, s/n, 08195 Sant Cugat del Vallès, Barcelona, Spain

Interests: biomaterials; titanium and its alloys; shape memory alloys; dental materials

* Section EiC of Biomaterials

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Prof. Dr. Daniela Iannazzo (<https://sciprofiles.com/profile/539656>)

Website (<https://www.unime.it/it/persona/daniela-iannazzo>)

Editorial Board Member

Department of Engineering, University of Messina, Contrada Di Dio, I-98166 Messina, Italy

Interests: organic synthesis; advanced synthetic methodologies for the organic functionalization of nanomaterials for applications in drug delivery, biosensors, tissue engineering and in environmental field

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Dr. Isabel Izquierdo-Barba (<https://sciprofiles.com/profile/551493>)

Website (<https://www.ucm.es/cavp1/profra-dra-karen-arriaza-ibarra>)

Editorial Board Member

Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid. Instituto de Investigación Sanitaria Hospital 12 de Octubre i + 12, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain

Interests: silica-based mesoporous materials; bioceramics; functionalization; drug delivery; scaffolds for bone tissue regeneration; bone infection

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Dr. Karen Vickery (<https://sciprofiles.com/profile/555197>)

Website (<https://researchers.mq.edu.au/en/persons/karen-vickery>)

Editorial Board Member

Surgical Infection Research Group, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

Interests: biofilms; infection control; disinfectants; medical implants; cleaning

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Dr. Sergio Adamo (<https://sciprofiles.com/profile/251911>)

Website (<https://gomppublic.uniroma1.it/Docenti/Render.aspx?UID=743587a0-70bb-4278-8730-37beb875b80c>)

Section Board Member

Histology and Medical Embryology, "Sapienza" University of Rome School of Pharmacy and Medicine Section of Histology & Medical Embryology, Department AHFOS, via A. Scarpa 16, 00161 Rome, Italy

Interests: muscle differentiation; muscle homeostasis; hormonal signaling; signal transduction



Prof. Dr. Saverio Affatato (<https://sciprofiles.com/profile/229364>).

Website (<http://www.ior.it/laboratori/lab-di-tecnolog-medica/laboratorio-di-tecnologia-medica>).

Section Board Member

Laboratorio di Tecnologia Medica, IRCCS – Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy ([toggle desktop layout cookie](#)) 🔍 ☰

Interests: biotribology; ceramic; metal; composite; biomaterials; hip; knee; simulator; in silico; prosthesis

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Prof. Dr. Javadi Aliyar

Website (https://tu-dresden.de/ing/maschinenwesen/ifvu/tpg/die-professur/beschaefigte/Aliyar_Javadi)

Section Board Member

1. Institute of Fluid Dynamics, Helmholtz-Zentrum, Dresden-Rossendorf (HZDR), Bautzner Landstrasse 400, 01328 Dresden, Germany;
2. Institute of Process Engineering and Environmental Technology, Faculty of Mechanical Science and Engineering, TU-Dresden, Dresden, Germany

Interests: soft matters; bio-interfaces; self-assembly and nano structures; surfactants; proteins, polymers; bacteria and nanoparticles; foams and emulsions; complex adsorbed layers; interfacial transport phenomena in multiphase systems



Prof. Dr. Marco Annunziata (<https://sciprofiles.com/profile/340676>).

Website (<https://www.dipmdsmco.unicampania.it/dipartimento/docenti?MATRICOLA=059384>)

Section Board Member

Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania “Luigi Vanvitelli”, 80138 Naples, Italy

Interests: periodontal diseases; implant dentistry; peri-implant diseases

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Dr. Roman Perez Antoñanzas (<https://sciprofiles.com/profile/534645>).

Website (<https://www.uic.es/es/teacher/rperezan>).

Section Board Member

1. Bioengineering Institute of Technology, Universitat Internacional de Catalunya, Sant Cugat del Vallès, 08195 Barcelona, Spain
2. Basic Science Department, Universitat Internacional de Catalunya, Sant Cugat del Vallès, 08195 Barcelona, Spain

Interests: biomaterials; tissue engineering; bioactive ceramics; drug delivery; cell delivery; functionalization; bone regeneration

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Prof. Dr. Carla Renata Arciola (<https://sciprofiles.com/profile/1570940>).

Website (<http://www.ior.it/ricerca-e-innovazione/Prof.ssa%20Carla%20Renata%20Arciola>)

Section Board Member

1. Head of the Research Unit on Implant Infections, Rizzoli Orthopaedic Institute, Via di Barbiano 1/10, 40136 Bologna, Italy
2. Professor of General Pathology, Medical School, University of Bologna, Via S. Giacomo 14, 40126 Bologna, Italy

Interests: anti-adhesive surfaces; anti-biofilm agents; anti-biofouling materials; antibiotic-loaded biomaterials; anti-infective materials; anti-infective tissue regeneration membranes; bioactive antibacterial coatings; materials delivering antimicrobials; covalent conjugation of antimicrobial peptides; (GTR/GBR) membrane with anti-infective properties; implant infections; multilayer antibacterial films; periprosthetic infections; photocatalytic coatings for hygienic surfaces; technologies and nano-technologies for infection-resistant surfaces

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Prof. Dr. Dimitris S. Argyropoulos (<https://sciprofiles.com/profile/49991>).

Website (<http://www4.ncsu.edu/~dsargyro>)

Section Board Member

Departments of Forest Biomaterials & Chemistry, North Carolina State University, 2820 Faucette Drive, Rm 3104, Raleigh, NC 27695-8005, USA

Interests: materials; chemicals and energy from forest biomass; organic chemistry of wood components; bio-refining of lignin; cellulose and nano-cellulose based smart materials; NMR spectroscopy and polymer chemistry of biopolymers

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Prof. Dr. Elaine Armelin (<https://sciprofiles.com/profile/304679>)

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Website (<https://futur.upc.edu/ElaineArmelinDigroc>)

Section Board Member

Chemical Engineering Department, Universitat Politècnica de Catalunya, Av. Eduard Maristany, 10-14, I2.2, Barcelona, Spain

Interests: Biomaterials; conducting polymers; hybrid materials; multifunctional coatings; plasma-polymers; thin films; corrosion wear

Dr. Iliaria Armentano (<https://sciprofiles.com/profile/479040>)

Website (<http://www.ing1.unipg.it/ricerca/gruppi-di-ricerca/gruppo-di-ricerca-di-scienza-e-tecnologia-dei-materiali/ilaria-armentano>)

Section Board Member

Materials Engineering Centre, UdR INSTM, NIPLAB, University of Perugia, 4 - 05100 Terni, Italy

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Dr. Manuel Arruebo (<https://sciprofiles.com/profile/122655>)

Website (<http://iqtma.unizar.es/>)

Section Board Member

Department of Chemical Engineering, University of Zaragoza, 50018 Zaragoza, Spain

Interests: nanoparticle synthesis; microfluidics; drug delivery; biomaterials



Dr. Emanuel Axente (<https://sciprofiles.com/profile/476679>)

Website (https://cetal.inflpr.ro/newsite/phil_team)

Section Board Member

Center for Advanced Laser Technologies (CETAL), National Institute for Laser, Plasma and Radiation Physics (INFLPR), 077125 Măgurele, Romania

Interests: laser processing of biomaterials; surface nanostructuring; biomimetic materials; tissue engineering; laser synthesis of bioactive coatings; biointerfaces; microfluidic platforms; lab-on-a-chip devices; 3D bioprinting

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Dr. Csaba Balázi (<https://sciprofiles.com/profile/1794202>)

Website (<https://ceramics.org/award-winners/csaba-balazi>)

Section Board Member

Centre for Energy Research, Eötvös Lóránd Research Network, 1121 Budapest, Hungary

Interests: bioceramics; biomaterials; ceramic dispersion strengthened steels; ceramics and nanocomposites for high temperature and tribological applications; open structured functional materials for sensorics; fiber polymers; composites and coatings; layered ceramics

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Dr. Johnny Beaugrand (<https://sciprofiles.com/profile/958008>)

Website (https://www6.angers-nantes.inrae.fr/bia_eng/Home/Staff-members/B/BEAUGRAND-Johnny)

Section Board Member

Biopolymères Interactions Assemblages (BIA), INRA, Nantes, France

Interests: fiber-based composites; biopolymers structures-properties relationships; lignocellulosic cell wall



Dr. Sompop Bencharit (<https://sciprofiles.com/profile/580346>)

Website (<https://www.prosthodontics.org/2021-distinguished-researcher-award-dr-sompop-bencharit/>)

Section Board Member

Office of Oral Health Innovation, Department of Oral Rehabilitation, Medical University of South Carolina, Charleston, SC 29425, USA

Interests: digital dentistry; guided implant surgery; structural biology; protein structure; salivary biomarkers; salivary proteomics

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Prof. Dr. Daniela Berger (<https://sciprofiles.com/profile/789309>),



Section Board Member

Department Inorganic Chemistry, Physical Chemistry and Electrochemistry, Faculty of Chemical Engineering and Applied Technologies, University of Bucharest, 1-7 Gh. Polizu Street, 011061 Bucharest, Romania

Interests: drug-delivery systems; mesoporous materials; inorganic nanoparticles

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Prof. Dr. Gordon Blunn (<https://sciprofiles.com/profile/1094688>),

Website (<https://www.port.ac.uk/about-us/structure-and-governance/our-people/our-staff/gordon-blunn>)

Section Board Member

School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK

Interests: musculoskeletal tissue regeneration; orthopaedic implants; stem cells

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Dr. Antonio Boccaccio (<https://sciprofiles.com/profile/564641>),

Website (<https://www.dmmm.poliba.it/index.php/it/profile/userprofile/boccaccio>)

Section Board Member

Dipartimento di Meccanica, Matematica e Management (DMMM), Campus "Ernesto Quagliariello", Politecnico di Bari, Via Edoardo Orabona, 4, I-70125 Bari, Italy

Interests: bioengineering; morphological optimization of biomaterials; modeling and simulation of biomedical devices and mechanobiological processes; optical techniques for reverse engineering; characterization of biomedical materials

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Prof. Dr. Dariusz Bogdal

Website (<https://home.cyf-kr.edu.pl/~pcbogdal/>)

Section Board Member

Department of Biotechnology and Physical Chemistry, Politechnika Krakowska, ul. Warszawska 24, 31-155 Krakow, Poland

Interests: bio-based polymers; biomedical applications; photo- and electroluminescent polymers and materials; polymer nanocomposites; microwave chemistry



Dr. Assunta Borzacchiello (<https://sciprofiles.com/profile/123863>),

Website (<http://www.ipcb.cnr.it/index.php/it/personale/strutturato/70-assunta-borzacchiello>)

Section Board Member

National Research Council, Rome, Italy

Interests: biomaterials; tissue engineering; drug delivery; rheology

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Prof. Dr. Maria Filomena Botelho (<https://sciprofiles.com/profile/847785>),

Website (<https://www.uc.pt/en/fmuc/icbr/researchlines/oncobiology/MIO/researchteam/MFBotelho>)

Section Board Member

Faculty of Medicine, Biophysics Institute, University of Coimbra, 3004-531 Coimbra, Portugal

Interests: biophysics of ionizing radiation; radiobiology and radiation effects; biomaterials; animal models for human diseases; new cancer therapies

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Dr. Daniele Botticelli (<https://sciprofiles.com/profile/1437140>),

Website (https://www.paro.zmk.unibe.ch/ueber_uns/team/personen/dr_botticelli_daniele/index_ger.html)

Section Board Member



Prof. Dr. Nikolaos Bouropoulos (<https://sciprofiles.com/profile/265000>).

[Website \(http://www.iceht.forth.gr/staff/bouropoulos.html\)](http://www.iceht.forth.gr/staff/bouropoulos.html)

Section Board Member

Department of Materials Science, University of Patras Greece and Institute of Chemical Engineering Sciences (FORTH/ICE-HT), Patras, Greece

Interests: biological mineralization; calcium phosphates; calcium phosphate bone cements; crystal growth; controlled drug delivery systems based on biopolymers; synthesis and characterization of ZnO

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Prof. Dr. Rene Buchet (<https://sciprofiles.com/profile/208230>).

[Website \(https://orcid.org/0000-0002-7966-3856\)](https://orcid.org/0000-0002-7966-3856)

Section Board Member

Institute for Molecular and Supramolecular Chemistry and Biochemistry, Université Lyon 1, French National Centre for Scientific Research, F-69622 Lyon, France

Interests: mineralization in norm and pathology; mineralization competent cells; matrix vesicles

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Prof. Dr. Sebastian Bürklein (<https://sciprofiles.com/profile/900590>).

[Website \(https://bzaev.de/index.php/component/k2/itemlist/user/644-burkleinprofdrsebastianuniversitatmunster\)](https://bzaev.de/index.php/component/k2/itemlist/user/644-burkleinprofdrsebastianuniversitatmunster)

Section Board Member

Central Interdisciplinary Ambulance in the School of Dentistry, University of Münster, Münster, Germany

Interests: cbct, cyclic fatigue; endodontics; endodontic surgery; endodontic diagnosis; endodontic education (3D-printed teaching teeth/models); nickel-titanium root canal instruments; metallurgy; root canal filling materials; root canal irrigation; root canal sealers, treatment outcome

Dr. Davide Campoccia (<https://sciprofiles.com/profile/407842>).

[Website \(http://www.ior.it/ricerca-e-innovazione/Dr.%20Davide%20Campoccia\)](http://www.ior.it/ricerca-e-innovazione/Dr.%20Davide%20Campoccia)

Section Board Member

Research Unit on Implant Infections, Rizzoli Orthopaedic Institute, 40136 Bologna, Italy

Interests: bacterial interactions with material surfaces; implant-related infections; bioactive biomaterials; bacteria-repelling surfaces; anti-infective biomaterials; antibacterial coatings; intrinsically antimicrobial materials; material-based antibacterial strategies; nanostructured materials with enhanced bactericidal activity; antibacterial materials with immunomodulatory activity

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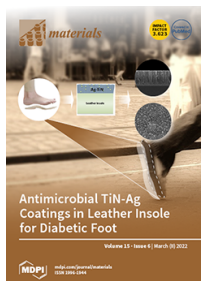
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Cover Story ([view full-size image](#) (/files/uploaded/covers/materials/big_cover-materials-v15-i6.png)): This work reports on TiN-Ag antimicrobial coatings deposited by DC magnetron sputtering on leather for insoles on the footwear industry and studies involving the antimicrobial properties of these coatings. The XRD results suggested the presence of a crystalline fcc-TiN phase and a fcc-Ag phase in the samples containing silver. SEM analysis shows that coatings were homogeneous, and dispersed Ag clusters were found on samples with silver content above 8 at.%. ICP-OES spectrometry analysis showed that the ionization of silver over time depends on the morphology of coatings. The samples did not present cytotoxicity and only samples with silver presented antibacterial and antifungal activity, highlighting the potential of the TiN-Ag insole coatings for diseases such as diabetic foot. [View this paper](#) (<https://www.mdpi.com/1996-1944/15/6/2009>)

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

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[Design, Fabrication, and Mechanical Properties of T-700TM Multiaxial-Warp-Knitting-Needled-C/SiC Composite and Pin](#) (/1996-1944/15/6/2338)



Materials **2022**, *15*(6), 2338; <https://doi.org/10.3390/ma15062338> (<https://doi.org/10.3390/ma15062338>) - 21 Mar 2022

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  [/1996-1944/15/6/2337/pdf?version=1647997820](https://www.mdpi.com/1996-1944/15/6/2337/pdf?version=1647997820)


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Fabrication and Characterization of Submicron-Scale Bovine Hydroxyapatite: A Top-Down Approach for a Natural Biomaterial [\(/1996-1944/15/6/2324\)](https://doi.org/10.3390/ma15062324)






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Suitability of Constitutive Models of the Structural Concrete Codes When Applied to Polyolefin Fibre Reinforced Concrete (1996-1944/15/6/2323)

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Optimization of Digital Light Processing Three-Dimensional Printing of the Removable Partial Denture Frameworks; The Role of Build Angle and Support Structure Diameter (1996-1944/15/6/2316)

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Correction: Chen et al. The Effect of High-Quality RDX on the Safety and Mechanical Properties of Pressed PBX. *Materials* 2022, 15, 1185 (1996-1944/15/6/2315)

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Materials **2022**, 15(6), 2311; <https://doi.org/10.3390/ma15062311> (https://doi.org/10.3390/ma15062311) - 21 Mar 2022



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MDPI (U)

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Multilayer Nonwoven Inserts with Aerogel/PCMs for the Improvement of Thermophysiological Comfort in Protective Clothing against the Cold [\(/1996-1944/15/6/2307\)](https://doi.org/10.3390/ma15062307)

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Characterization of Hydroxyapatite Film Obtained by Er:YAG Pulsed Laser Deposition on Sandblasted Titanium: An In Vitro Study [\(/1996-1944/15/6/2306\)](https://doi.org/10.3390/ma15062306)

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Experimental Study on Subgrade Material of Calcium Silicate Slag [\(/1996-1944/15/6/2304\)](https://doi.org/10.3390/ma15062304)

Materials **2022**, *15*(6), 2304; <https://doi.org/10.3390/ma15062304> (<https://doi.org/10.3390/ma15062304>) - 20 Mar 2022



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Microstructural Parameters for Modelling of Superconducting Foams [\(/1996-1944/15/6/2303\)](https://doi.org/10.3390/ma15062303)

Materials **2022**, *15*(6), 2303; <https://doi.org/10.3390/ma15062303> (<https://doi.org/10.3390/ma15062303>) - 20 Mar 2022



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Effect of Ultraviolet Light C (UV-C) Radiation Generated by Semiconductor Light Sources on Human Beta-Coronaviruses' Inactivation [\(/1996-1944/15/6/2302\)](https://doi.org/10.3390/ma15062302)

Materials **2022**, *15*(6), 2302; <https://doi.org/10.3390/ma15062302> (<https://doi.org/10.3390/ma15062302>) - 20 Mar 2022



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Materials **2022**, *15*(6), 2301; <https://doi.org/10.3390/ma15062301> (<https://doi.org/10.3390/ma15062301>) - 20 Mar 2022



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Application of the Sinter-HIP Method to Manufacture Cr–Mo–W–V–Co High-Speed Steel via Powder Metallurgy [\(/1996-1944/15/6/2300\)](https://doi.org/10.3390/ma15062300)

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Experimental and Numerical Thickness Analysis of TRIP Steel under Various Degrees of Deformation in Bulge Test [\(/1996-1944/15/6/2299\)](https://doi.org/10.3390/ma15062299)

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Medium-Entropy SrV_{1/3}Fe_{1/3}Mo_{1/3}O₃ with High Conductivity and Strong Stability as SOFCs High-Performance Anode [\(/1996-1944/15/6/2298\)](https://doi.org/10.3390/ma15062298)

Materials **2022**, *15*(6), 2298; <https://doi.org/10.3390/ma15062298> (<https://doi.org/10.3390/ma15062298>) - 20 Mar 2022


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Effect of Water–Solid Mixing Sequence and Crystallization Water of Calcium Sulphate on the Hydration of C₃A [\(/1996-1944/15/6/2297\)](https://doi.org/10.3390/ma15062297)

Materials **2022**, *15*(6), 2297; <https://doi.org/10.3390/ma15062297> (<https://doi.org/10.3390/ma15062297>) - 20 Mar 2022



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  [./\(1996-1944/15/6/2296/pdf?version=1647768619\)](https://doi.org/10.3390/ma15062296)


A Simple Structure for an Independently Tunable Infrared Absorber Based on a Non-Concentric Graphene Nanodisk [\(/1996-1944/15/6/2296\)](https://doi.org/10.3390/ma15062296)

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  [./\(1996-1944/15/6/2295/pdf?version=1647768787\)](https://doi.org/10.3390/ma15062296/pdf?version=1647768787)

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Materials 2022, 15(6), 2295; <https://doi.org/10.3390/ma15062295> (https://doi.org/10.3390/ma15062295) - 20 Mar 2022



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Analysis on Seismic Performance of Steel-Reinforced Concrete-Filled Circular Steel Tubular (SRCFST) Members Subjected to Post-Fire (1996-1944/15/6/2294)

Materials 2022, 15(6), 2294; <https://doi.org/10.3390/ma15062294> (https://doi.org/10.3390/ma15062294) - 20 Mar 2022

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Influence of Monocalcium Phosphate on the Properties of Bioactive Magnesium Phosphate Bone Cement for Bone Regeneration (1996-1944/15/6/2293)

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Magnetic Instabilities in the Quasi-One-Dimensional $K_2Cr_3As_3$ Material with Twisted Triangular Tubes (1996-1944/15/6/2292)

Materials 2022, 15(6), 2292; <https://doi.org/10.3390/ma15062292> (https://doi.org/10.3390/ma15062292) - 20 Mar 2022


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Acacia nilotica Pods' Extract Assisted-Hydrothermal Synthesis and Characterization of ZnO-CuO Nanocomposites (1996-1944/15/6/2291)

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Foundational Investigation on the Characterization of Porosity and Fiber Orientation Using XCT in Large-Scale Extrusion Additive Manufacturing (1996-1944/15/6/2290)

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


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
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
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
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
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
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


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Fabrication and Characterization of Submicron-Scale Bovine Hydroxyapatite: A Top-Down Approach for a Natural Biomaterial

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Abstract: Submicron hydroxyapatite has been reported to have beneficial effects in bone tissue engineering. This study aimed to fabricate submicron-scale bovine hydroxyapatite (BHA) using the high-energy dry ball milling method. Bovine cortical bone was pretreated and calcined to produce BHA powder scaled in microns. BHA was used to fabricate submicron BHA with milling treatment for 3, 6, and 9 h and was characterized by using dynamic light scattering, scanning electron microscope connected with energy dispersive X-Ray spectroscopy, Fourier-transform infrared spectroscopy, and X-ray diffractometry to obtain its particle size, calcium-to-phosphorus (Ca/P) ratio, functional chemical group, and XRD peaks and crystallinity. Results showed that the particle size of BHA had a wide distribution range, with peaks from ~5 to ~10 μm . Milling treatment for 3, 6, and 9 h successfully gradually reduced the particle size of BHA to a submicron scale. The milled BHA's hydrodynamic size was significantly smaller compared to unmilled BHA. Milling treatment reduced the crystallinity of BHA. However, the treatment did not affect other characteristics; unmilled and milled BHA was shaped hexagonally, had carbonate and phosphate substitution groups, and the Ca/P ratio ranged from 1.48 to 1.68. In conclusion, the fabrication of submicron-scale BHA was successfully conducted using a high-energy dry ball milling method. The milling treatment did not affect the natural characteristics of BHA. Thus, the submicron-scale BHA may be potentially useful as a biomaterial for bone grafts.

Keywords: submicron material; nanomaterial; bone scaffold; bone graft; calcium phosphate; neglected disease



Citation: Gani, M.A.; Budiati, A.S.; Lestari, M.L.A.D.; Rantam, F.A.; Ardianto, C.; Khotib, J. Fabrication and Characterization of Submicron-Scale Bovine Hydroxyapatite: A Top-Down Approach for a Natural Biomaterial. *Materials* **2022**, *15*, 2324. <https://doi.org/10.3390/ma15062324>

Academic Editors: Swarup Roy and Antonio Scarano

Received: 8 February 2022

Accepted: 19 March 2022

Published: 21 March 2022

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1. Introduction

Bone defects represent a neglected form of disease that may severely interfere with the physiological function of bones [1–3]. This form of disease may also affect an individual's quality of life and the patient's economic burden [1]. Every year, more than 2.2 million people globally undergo bone grafting procedures to treat bone defects [4]. Therefore, the development of osteoconductive biomaterial should focus on bone tissue engineering to accelerate bone defect repairs.

The development of nanoscale materials has been rapidly increasing in recent decades. Nanoscale biomaterials have been reported to have beneficial effects in bone tissue engineering, such as increasing the adhesion, differentiation, and proliferation of osteoblasts and providing vascularization and bone formation in vivo [5,6]. However, the effects of

nanomaterials, particularly in osteoclasts, were not as expected in osteoblasts. Chen et al. [7] reported that HA in nanoscale grain sizes (~100 nm) impaired osteoclastic formation and function. This was evidenced by inhibited cell fusion, reduced osteoclast size, increased osteoclast apoptosis, suppressed expression of osteoclast specific genes and proteins, and hampered resorption activity compared to submicron HA (~500 nm). Other than that, nanoparticles smaller than 10 nm were indicated to act similar to gas and may cross various biological barriers, reaching sensitive organs and disturbing normal cell behavior [8,9].

Furthermore, the study begins to report the beneficial effect of submicron +- scale material for bone reconstruction. The submicron +-scale material was also found to be the most influential surface dimension in promoting osteoblast differentiation, as reported by Khang et al. [10]. In addition, submicron-scale ceramic (grain size: ~800 nm) was observed to enhance bone regeneration after 12 weeks of implantation [11]. Particularly in regard to osteoclasts, it has been reported that hydroxyapatite with a particle size of ~500 nm beneficially influences osteoclast formation and function compared to smaller-sized materials [7]. Thus, considering the controversy of material sizes, it is suggested that the submicron-scale materials potentially have superior action on bone cells, particularly osteoclasts, and may be combined with other ceramics to produce desired characteristics for bone tissue regeneration [12–15].

Hydroxyapatite (HA) is a derivate of calcium phosphate that is widely used for medical purposes, including orthopedic and dental [16]. HA as a biomaterial can be synthesized from chemical precursors known as synthetic HA or extracted from natural sources such as mammalian bones [16]. One advantage in using natural HA compared to synthetic HA is the characteristics of natural HA. Natural HA, such as bovine hydroxyapatite (BHA), has a carbonate substitution group on its apatite. The natural HA is similar to human bone and was not found in synthetic HA [17–19]. Carbonated HA was found to increase osteoblast proliferation in vitro [20]. Other characteristics of BHA, such as its high compressive strength, prevent premature degradation and support bone formation in vivo [16,21].

Considering the beneficial effects of BHA and submicron material in bone tissue engineering, the development of submicron BHA potentially increases the osteoinductive and osteoconductive properties of BHA. As it was established that submicron material had a superior effect on osteoclasts [7], submicron BHA will also benefit bone diseases related to osteoclast dysfunction.

Studies have reported that critical parameters must be considered when fabricating hydroxyapatite [13,14,22,23]. Due to the high compressive strength property of BHA, an appropriate method should be chosen to reduce the particle size of the BHA. A previous study by Ruksudjarit et al. [24] synthesized a nano-hydroxyapatite using the wet ball milling method with ethanol as the milling media. Ethanol is widely used as a process control agent (PCA) in the milling process of biomaterials [25]. However, the use of ethanol and other organic solvents is known to be the most common source of contamination of obtained materials. These solvents reduced crystallinity, and also changed the morphology and distribution of elements of the starting materials [26,27]. Because of this, it is suggested that the use of organic compounds should be avoided in the milling treatment of biomaterials.

This study aimed to fabricate submicron-scale HA by using high-energy dry ball milling. In this study, bovine bones were given pretreatment (boiled, dried, and calcined) in order to obtain BHA. BHA naturally sized in microns was milled for several hours by using the high-energy dry ball milling method. Milled and unmilled BHA then were characterized by using dynamic light scattering (DLS, Beckman Coulter, Indianapolis, IN, USA), a scanning electron microscope connected with energy dispersive X-Ray spectroscopy (SEM-EDX, Carl Zeiss, Oberkochen, Germany), Fourier-transform infrared spectroscopy (FTIR, Bruker, Leipzig, Germany), and X-ray diffractometry (XRD, PANalytical Corporation, Almelo, The Netherlands). Submicron-scale BHA with innate characteristics of human bone has the potential to be used for bone grafts or drug delivery systems to bone tissues.

2. Materials and Methods

2.1. Extraction of Bovine Hydroxyapatite

BHA was extracted based on the methods used by Budiadin et al. [18], with modifications. The raw material was fresh cortical bone from mature bovines. Bone was cut and cleaned with water, and spongy parts and bone marrow were removed. Furthermore, the bone was boiled for 5 h in distilled water (distilled water was changed every hour). Bone was boiled in the pressurized tank for 3 h (water was changed every one hour). The boiled bone was then dried at 60 °C for three hours. The dried bone was soaked in absolute ethanol (Brataco Chemika, Surabaya, Indonesia) for 24 h while being shaken (ethanol was changed every 12 h). Calcination was conducted for two hours at 1000 °C. Finally, the bone was ground and sieved through an 80-mesh sieve.

This study also examined the BHA-based and HA-based scaffold's compressive strength. The scaffolds were made from extracted BHA and HA (CASs number 1306-06-5, molecular weight 1004.6 g mol⁻¹; further characteristics are present in Figure S1). Briefly, BHA or HA (10 g) was added to 5 mL of a prepared 20% gelatin solution (Cartino, Samut Prakan, Thailand). The mixture was stirred and sieved using a mesh (size: 1.0 mm) and dried at 37 °C. Next, 25 mg of granules were molded into an implant (diameter: 2 mm) using a hydraulic press (2 ton; Graseby-Specac Ltd., Orpington, Kent, London, UK).

2.2. Fabrication of Submicron-Scale BHA Using High-Energy Dry Milling

The fabrication of submicron-scale BHA was conducted based on the methods of Aminatun et al. (2019) [27]. BHA extracted from the previous method was used as the starting material. A milling ball made from alumina was used; the ratio of BHA and the milling ball was 1:20. The milling treatment was conducted for three, six, and nine hours in the milling vial.

2.3. Material Characterization

The compressive strength of the BHA-based and HA-based scaffolds was characterized by using a mini autograph (Autograph Microcomputer Control Universal Testing, LoadCell, YXC-1B (Original Equipment Manufacture, Surabaya, Indonesia), speed 5 mm/min).

The Ca/P ratio of materials was examined using SEM-EDX (EVO MA 10; Carl Zeiss, Oberkochen, Germany). The structural evaluation of particle size was examined using the same instrument and analyzed by measuring the mean particle size in at least two different axes using the ImageJ 1.52a software (National Institutes of Health, Bethesda, MD, USA).

The crystallinity of the materials was examined by using XRD (PANalytical X'Pert³ powder; PANalytical Corporation, Almelo, The Netherland). The XRD pattern was corrected, and the XRD peaks were detected using Origin software (OriginLab Corporation, Northampton, MA, USA). The percent of crystallinity of each material was calculated based on area under peaks by using Origin software.

$$\text{Percent of crystallinity} : \frac{\text{Acrytalline}}{\text{Acrytalline} + \text{Aamorphous}} \times 100\% \quad (1)$$

The hydrodynamic particle size of materials was measured by using DLS (DelsaTM Nano C; Beckman Coulter, Indianapolis, IN, USA), while the functional chemical group of each material was examined with FTIR (Alpha II; Bruker, Leipzig, Germany).

3. Results

Prior to the fabrication and characterization of the material, a compressive strength test was conducted to compare the strength of BHA as natural form of HA with a synthetic HA (characteristics of HA are presented in Figure S1). Figure 1 shows that BHA had a higher compressive strength than the synthetic HA ($p = 0.0254$; unpaired t -test). This indicated that BHA is a compact material compared to HA.

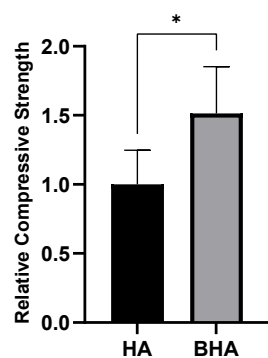


Figure 1. Relative compressive strength of BHA and HA. Each bar shows the mean \pm SD ratio. * $p < 0.05$ based on the unpaired t -test.

The strength of BHA indicated its brittleness, which may affect the submicron-BHA fabrication process. Thus, an appropriate method should be considered in the top-down fabrication process for BHA. In the current study, we chose the high-energy dry ball milling method to fabricate submicron BHA. Figure 2C shows that the particle size of unmilled BHA had a wide distribution range, from submicrons to $\sim 30 \mu\text{m}$, with a peak of ~ 5 to $\sim 10 \mu\text{m}$. Moreover, SEM images presented in Figure 2 also show that milling treatment reduced the particle size of BHA, both qualitatively and semiquantitatively. BHA samples milled for three, six, and nine hours were measured and found to have particle sizes of ~ 3 , $\sim 1 \mu\text{m}$, and in the submicron scale (Figure 2F,I,L). Moreover, all material was shaped hexagonally (Figure 2); this shape of BHA also differentiates BHA from synthetic HA (Figure S1A,B).

Milling treatment did not affect the characteristics of BHA based on its functional chemical groups. FTIR spectra showed that all materials had carbonate and phosphate groups present at a wavenumber of 1455 cm^{-1} and $1000\text{--}1100 \text{ cm}^{-1}$, respectively (Figure 3). Moreover, the carbonate group detected in all BHA samples was not present in HA (Figure S1C). The XRD pattern and peaks of unmilled and milled BHA are presented in Figure 4. BHA had 84.02% crystallinity, while BHA milled for 3, 6, and 9 h had 65.81%, 60.98%, and 60.70% crystallinity, respectively. In addition, the calcium-to-phosphorus (Ca/P) of unmilled and milled BHA ranged from 1.48 to 1.68 (Table 1). The Ca/P of all BHA samples (unmilled and milled) is higher than that found in synthetic HA (Figure S1E).

The current study also conducted a hydrodynamic particle measurement of the unmilled and milled BHA (Figure 5). This measurement was essential to predict the in vivo performance of BHA as submicron materials. In line with previous results by SEM (Figure 2), the milling treatment reduced the particle size of BHA over time. However, the hydrodynamic size of milled BHA was more extensive; the particle size was scaled in micron size with BHA milled for nine hours being the smallest.

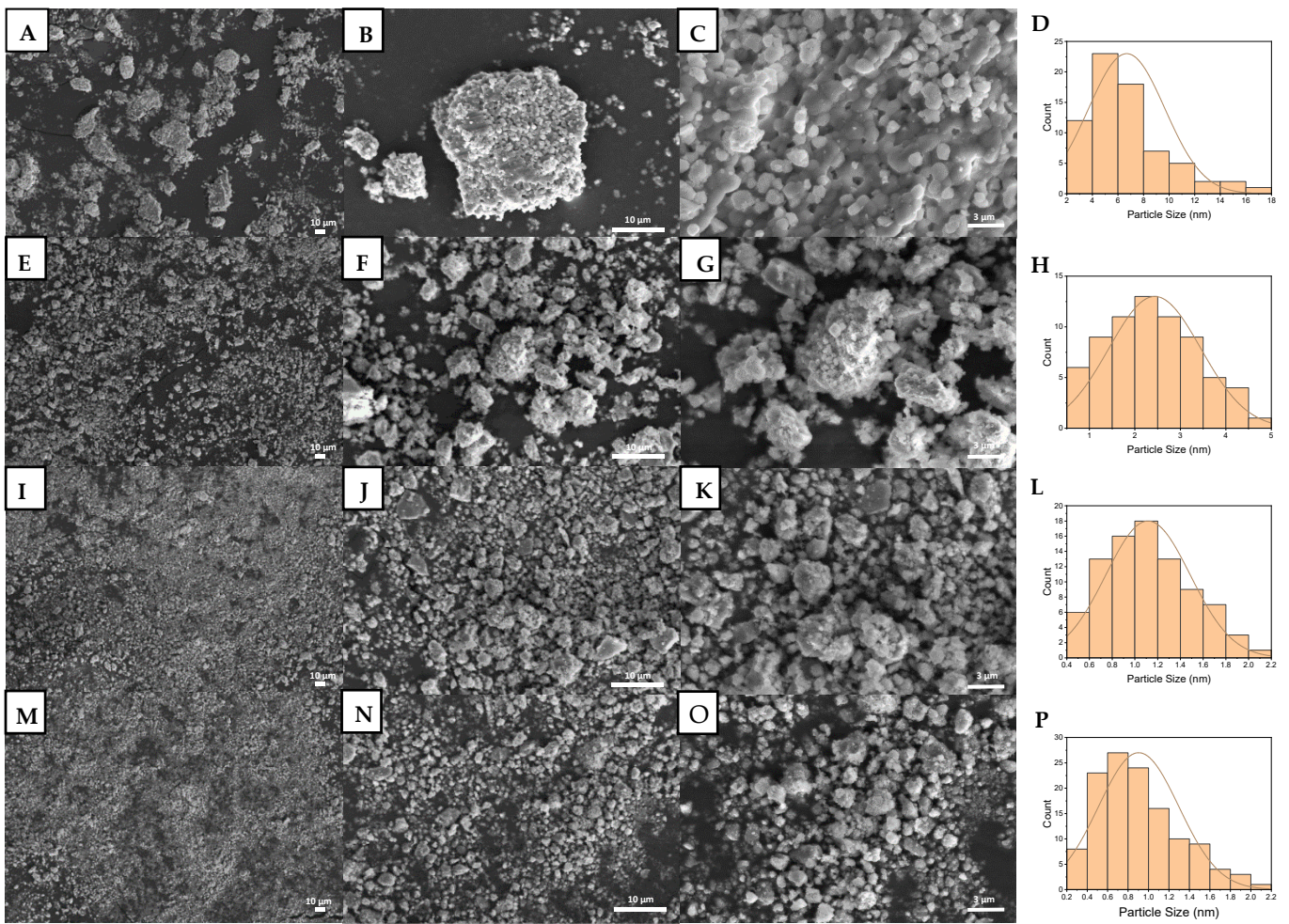


Figure 2. SEM images and particle size distribution of unground BHA (A–D), BHA milled for 3 h (E–H), BHA milled for 6 h (I–L), and BHA milled for 9 h (M–P). (A,E,I,M) Images show total magnification of 1000 \times . (B,F,J,N) Images show total magnification of 5000 \times . (C,G,K,O) Images show total magnification of 15,000 \times . (D,H,L,P) Graphs show the corresponding particle size distributions.

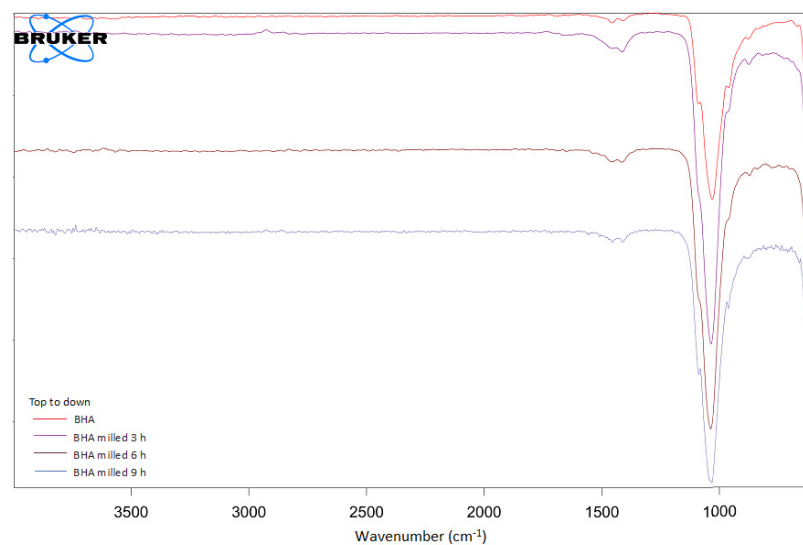


Figure 3. FTIR spectra of unground and milled BHA.

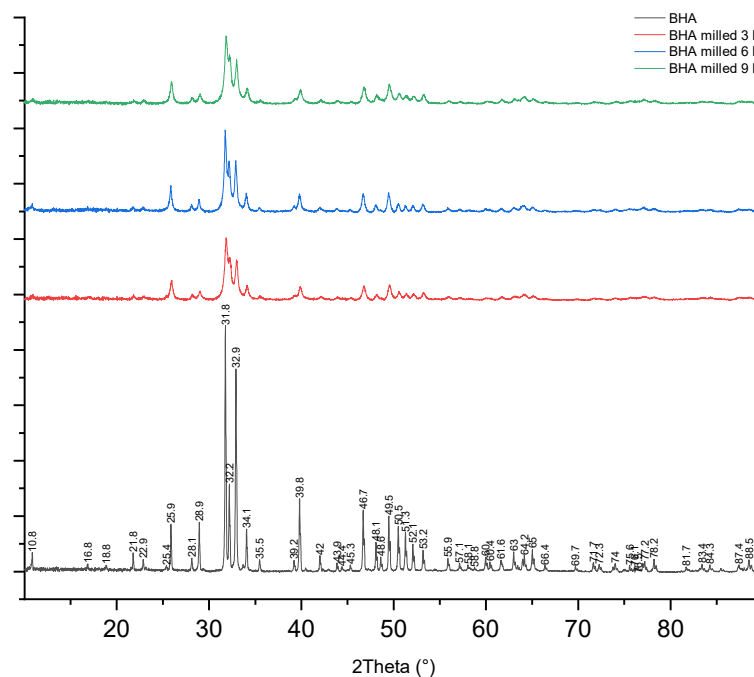


Figure 4. XRD spectra of unground and milled BHA.

Table 1. Ca/P ratio of unground and milled BHA.

Material	Calcium (Ca)		Phosphorus (P)		Ca/P Ratio
	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)	
BHA	68.01	62.16	31.99	37.84	1.64
BHA milled 3 h	68.43	62.62	31.57	37.38	1.68
BHA milled 6 h	65.70	59.69	34.30	40.21	1.48
BHA milled 9 h	65.95	59.95	34.05	40.05	1.50

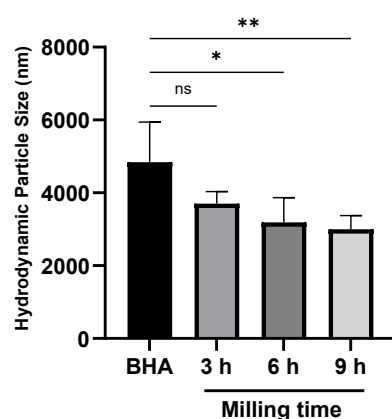


Figure 5. The hydrodynamic particle size of milled and unground BHA. Each bar shows the mean \pm SD value. * $p < 0.05$, ** $p < 0.01$ based on a one-way ANOVA test.

4. Discussion

The current study was conducted to fabricate submicron-scale BHA using the dry ball milling method with various milling times. This milling method uses no media in the milling treatment of materials. Milling media such as ethanol and other organic solvent are widely used as PCA in the milling method [28–30]. PCA functions to avoid cold welding and bonding between the powder particles. However, PCA is also known to change material characteristics [25]. In the case of the milling treatment of BHA, the use of PCA

may change its natural characteristics, which is not desirable. In the current study, we did not vary the milling speed because it was previously reported that milling speed did not influence the particle size of materials [31,32]. Although BHA is a material with high compressive strength, our study proved that a 9-h milling treatment successfully reduced the particle size of BHA to a submicron scale without changing the natural morphology of BHA. The hydrodynamic size of these materials was higher; however, the particle size of milled BHA was significantly smaller than unmilled BHA.

Our currently fabricated submicron BHA has been indicated to have a beneficial effect in bone tissue engineering. A previous study reported that submicron-scale materials (~500 nm) had a beneficial effect on osteoclast formation and function compared to nanomaterials [7]. This event is suggested to occur through the integrin–ligand protein interactions by protein adsorption [10]. In addition, submicron-sized ceramics (grain size: ~800 nm) have also been proven to enhance bone regeneration compared to microscale ceramics (grain size: ~2.5 µm) after 12 weeks of implantation in vivo [11].

Furthermore, our current study also proved that the milling treatment did not change the morphology of BHA. The morphology of these materials was similar to bovine-derived HA in our previous study [18] and the hydrogel composite HA in the study of Slota et al. [33]. This hexagonal-like morphology of biomaterials was reported to promote osteoblast adhesion after five hours of seeding [33]. This morphology is also present in the biomineralization of human bone mesenchymal stem cells [34], suggesting similar properties and better performance in vivo.

Moreover, our current study proved that the top-down treatment did not affect the characteristics of BHA based on its chemical functional groups. Both unmilled and milled BHA showed carbonate and phosphate chemical groups. This was similar to our previous study [18] and another study conducted by Michelot et al. [35]. The carbonate group in HA was reported to accelerate the differentiation and proliferation of osteoprogenitor cells to bone cells compared to uncarbonated HA [20]. Moreover, the carbonated HA was also reported to increase the gene expression of the collagen matrix [36]. In vivo studies also showed that carbonated HA, such as BHA, exhibited higher presentation of new bone formation and higher bone-to-material contact when compared to other types of HA [37,38].

Our current study also found that milling treatment reduced the crystallinity of BHA. Reductions in material crystallinity generally occurs because of milling treatment, such as reported by Ma et al. [39]. The study reported that a high-energy milling treatment reduced the crystallinity of MgCu. This incident is related to grain refinement and the impingement of milling balls with material, which decreased XRD peaks [39].

The Ca/P of human bone is generally considered to have a theoretical Ca/P ratio of 1.67 [40]. In the current study, we also examined the Ca/P of materials. This study showed that the Ca/P ratio of all materials was close to human HA [40]. Furthermore, the Ca/P ratio of 1.67 is also widely used as a reference value for bone grafts, rendering it suitable for orthopedic, dental, and maxillofacial implants [41,42]. Thus, the Ca/Pa value of fabricated submicron BHA supported the potential of this material to be used for bone implants. Moreover, calcium and phosphate play essential roles in bone tissue formation. Calcium ions stimulate the osteoblastic bone synthesis pathway, affects osteoblasts' life span, and regulate the formation and the resorptive functions of osteoclasts. On the other hand, phosphate regulates the differentiation and growth of osteoblastic lineage, increases the expression of bone morphogenetic proteins, and plays a role in the maturation of osteocytes [43,44].

To the best of our knowledge, our current study is the first study that considers the milling treatment of hard and dense natural HA without PCA. The non-hazardous method of milling treatment used in this study reduced the natural particle size of the material without affecting its characteristics. Considering the beneficial effects of submicron material and the characteristics of natural HA, submicron-sized natural HA may have beneficial effects in bone tissue engineering.

5. Conclusions

Submicron-scale natural HA fabrication was successfully conducted using a high-energy dry ball milling method. A milling time of 9 h decreased the particle size of BHA from micron to submicron scale. The milling treatment did not affect the natural characteristics of BHA marked by the morphology, chemical group substitution, crystallinity, and Ca/P ratio of the material. Thus, the submicron-scale BHA with innate characteristics of human bone may be potentially used as a biomaterial that could possibly have better in vitro and in vivo performance. However, further research regarding this should be proven in further studies.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ma15062324/s1>, Figure S1: Characteristics of synthetic HA.

Author Contributions: Conceptualization: M.A.G. and J.K.; methodology: A.S.B. and M.L.A.D.L.; software: M.A.G. and C.A.; validation: M.A.G. and J.K.; formal analysis: M.A.G.; investigation: M.A.G.; resources: A.S.B., M.L.A.D.L. and J.K.; data curation: M.A.G.; writing—original draft preparation: M.A.G.; writing—review and editing: M.A.G. and J.K.; visualization: M.A.G.; supervision: F.A.R., C.A. and J.K.; project administration: M.A.G. and A.S.B.; funding acquisition: J.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Education and Culture of the Republic of Indonesia, through PMDSU research scheme (grant number: 578/UN3.15/PT/2021).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the Faculty of Pharmacy, Universitas Airlangga, for all of the support during the research.

Conflicts of Interest: The authors declare no conflict of interest.

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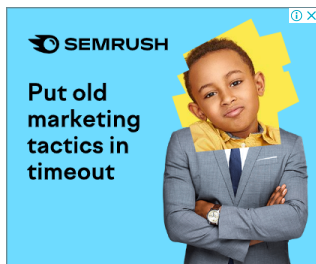
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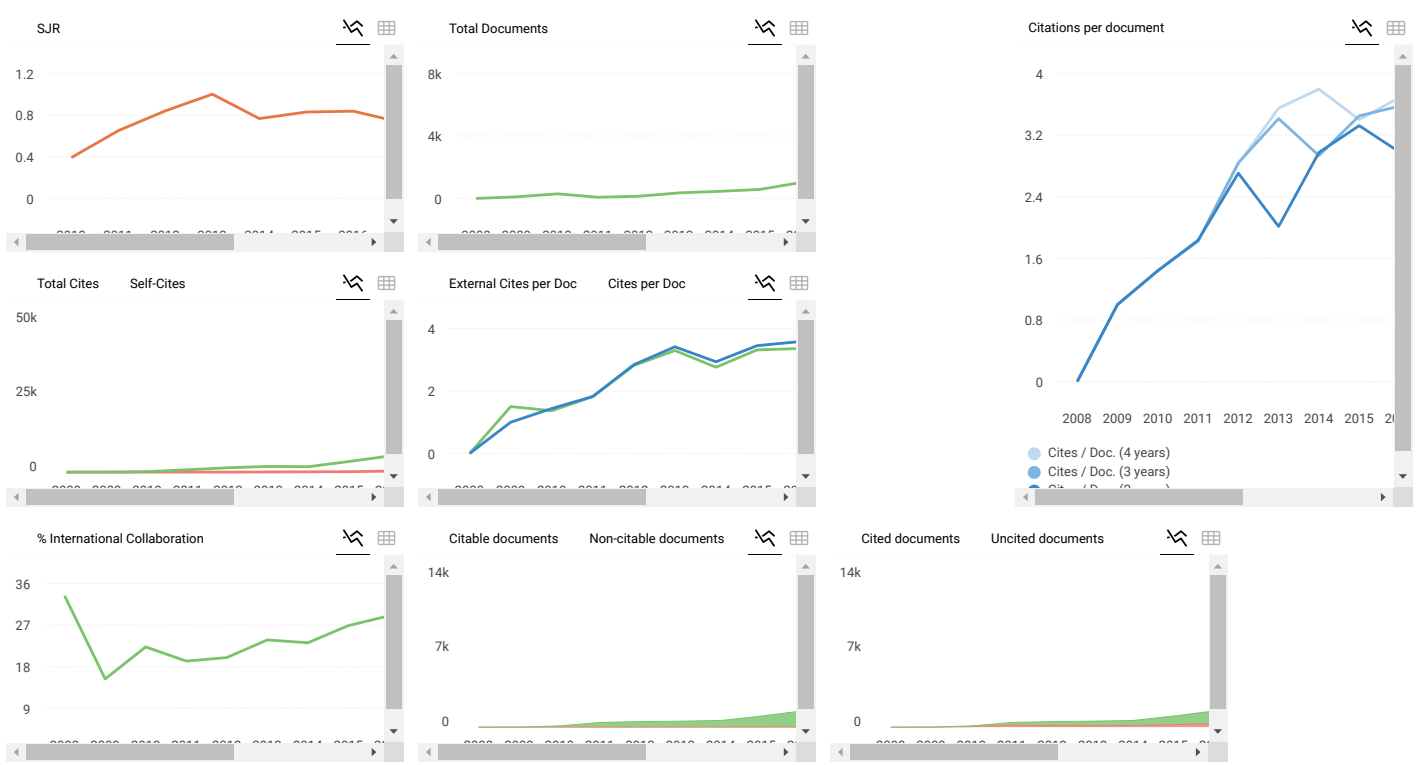
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