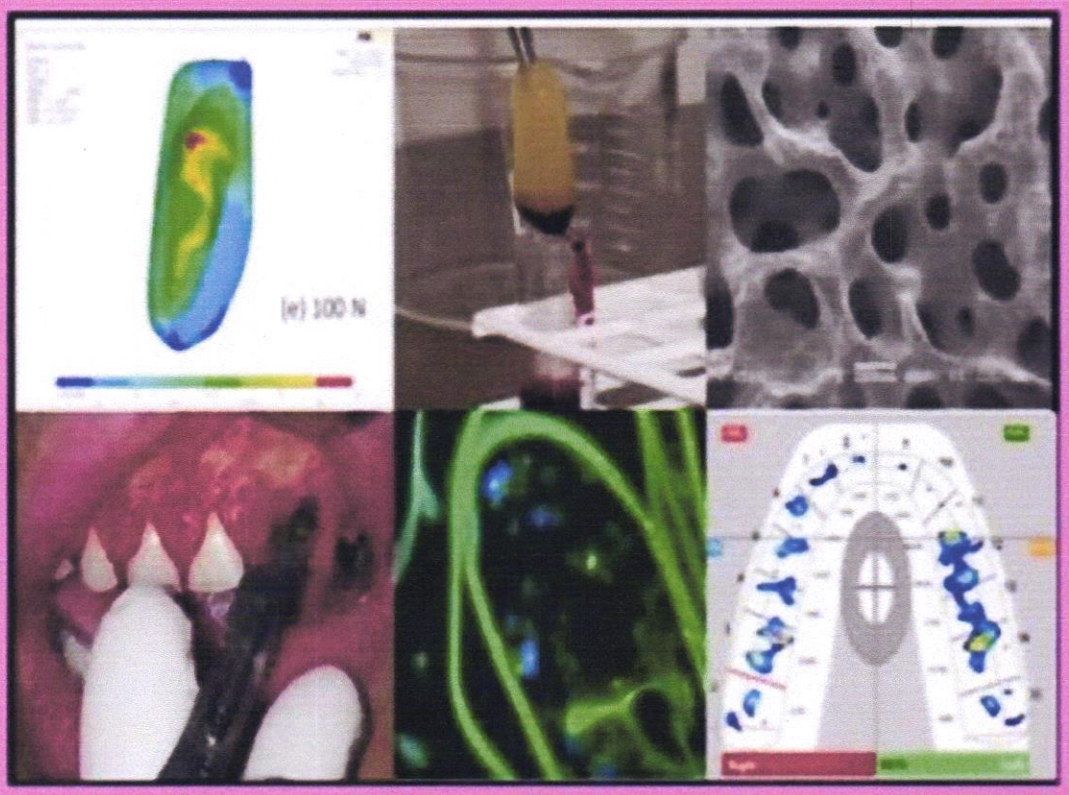
E-ISSN: 1309-100X

Journal of International Dental and Medical Research



2019 - Vol. 12 – No. 2

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Sabiha Zelal L

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Saul Martins F

Sedat AKDEN

Seher GUNDL

Selahattin ATI

Selahattin TEI

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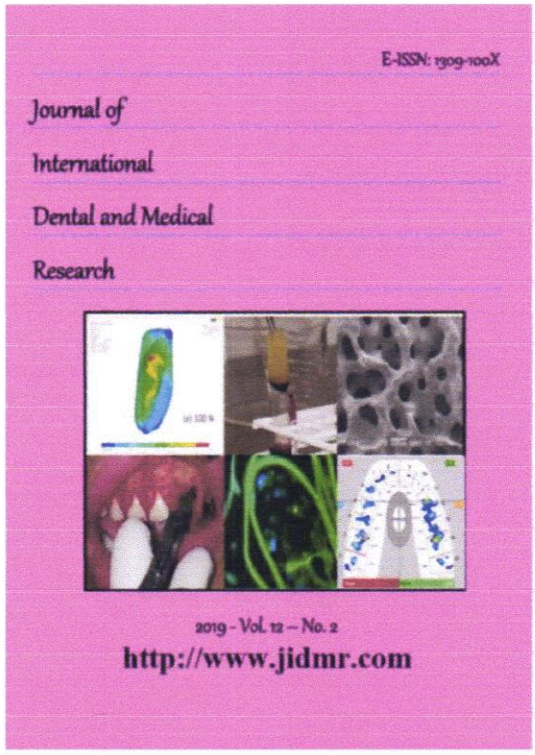
Journal of International Dental and Medical Research

Contents of JIDMR-2019-Vol.12–No.2

07/07/2019 /

Journal of International Dental and Medical Research

ISSN: 1309-100X



Cover page

[http://www.jidmr.com/journal/wp-content/uploads/2019/07/Cover.jpg]

Current issue of JIDMR

Table of Contents 2019 volume Vol.12–No. 2

[http://www.jidmr.com/journal/wp-content/uploads/2019/07/12_2_2019_Table_2.pdf]

DENTISTRY

EXPERIMENTAL ARTICLE

1. Biomechanical Evaluation of Temporomandibular Joint Disc by Using 3-D Finite Element Analysis During Loading

Yi-Hsiang Su, Ming-Lun Hsu

Pages 377-382

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/1_19356-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

2. Secang Heartwood Extract in Serial Dilution as Antibacterial Agent Against Biofilm E. faecalis Clinical Isolate

Sasi Suci Ramadhani, Ratna Meidyawati, Dewa Ayu NPA

Pages 383-388

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/2_16065-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

3. Porphyromonas gingivalis Induces Alveolar Bone Loss and Brain Lesions in Rabbit

Fouad Hussain Al-Bayaty, Omar Emad Ibrahim, Methil Kannan Kutty, Farah Fadzreen Binti Harun,

Siti Amanani Binti Selamat, Mohd Noor Arif Bin Adam and Mazen M. Jamil Al-Obaidi

Pages 389-395

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/3_D18_680_Fouad_AL_Bayaty1_layout-Kh.pdf]*

EXPERIMENTAL ARTICLE

4. Advanced Platelet Rich Fibrin (A-PRF) Supplemented Culture

4. Advanced Platelet Rich Fibrin (A-PRF) Supplemented Culture Medium for Human Dental Pulp Stem Cell Proliferation

Illmilda, Dini Asrianti, Anggraini Margono, Indah Julianto, Mettasari Puspa Wardoyo

Pages 396-400

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/4_16322-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

5. Antibacterial Properties of Clinacanthus nutans Extracts Against Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An In-Vitro Study

Rohazila Mohamad Hanafiah, Khairine Alia Che Kamaruddin, Nurul Amira Ahmad Saikin,

Wan Nur Alwani Binti Wan Abdul Aziz, Muhammad Fahmi Yakop, Vuanghao Lim, Siti Aisyah Abd Ghafar, Nuramirah Azizan, Shahida Mohd Said

Pages 401-404

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/5_D18_691_Rohazila_Mohamad_Hanafiah_I*

EXPERIMENTAL ARTICLE

6. Storage Temperature Effect on Degree of Polymerization and Surface Hardness of Bulk-Fill Composite Resin

Dewi Puspitasari, Adianto Prasetyo, Muhammad Deni Rahman, Sherli Diana,

Muhammad Yanuar Ichrom Nahzi

Pages 405-410

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/6_16895-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

7. In-vitro Inhibitory Effect of Cinnamomum zeylanicum and Eugenia caryophyllata Oils on Multispecies Anaerobic Oral Biofilm

Nuramirah Azizan, Shahida Mohd-Said, Mohd Khairul Firdaus Mazlan, Kurudeven Tamil Chelvan,

Rohazila Mohamad Hanafiah, Zamirah Zainal-Abidin

12. A 12.5% Virgin Coconut Oil Solution as an Alginate Impression Material Disinfectant

Ratna Sari Dewi, Henny Kusumaningati, Nuh Thalib

Pages 443-447

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/12_18088-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

13. The Two-Dimensional Effects of Salvadora persica Mechanical Brushing on the Surface of Polymethyl Methacrylate Denture Base Material

Wan Ali W.N.S., Sapon M.S.1, Rosdi N.M., Halib N.2, Mohamed N.

Pages 448-453

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/13_D18_745_Wan_Nor_Syariza_bt_Wan_Ali*

EXPERIMENTAL ARTICLE

14. Comparative In Vitro Evaluation of The Novel Remineralizing Agents' Effects on Enamel Surface Hardness

Pooja M Rai, Darshana Devadiga, Jithesh Jain, Rohit.A.Nair

Pages 454-459

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/14_19100-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

15. Javanese Turmeric (Curcuma Xanthorrhixza Roxb.) Ethanol Extract Has Inhibitory Effect on The Development of Intermediate Phase of Candida Albicans Biofilm

Sofwan Ardiansyah, Fithrotul Hashiinah, Ratna Farida, Ria Puspitawati

Pages 460-464

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/15_19280-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

16. Morphological Analysis of Goniopora Species Coral Powder and Composite Scaffold Using Micro-Computed Tomography

Vera Julia, Fourier Dzar Eljabbar Latief, Rachmat Mauludin, Rahmana Emran Kartasasmita,

Benny Sjahersjan Lauer

Pages 465-471

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/16_19297-ED-2-OK_layout.pdf]*

EXPERIMENTAL ARTICLE

17. Effect of Alkaline Environment on Micro-Hardness of Mineral Trioxide Aggregate with Different Setting Times

Diatri Nari Ratih, Sylvia Widhihapsari

Pages 472-475

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/17_D18_696_Diatri_Nari_Ratih_layout.pdf]*

EXPERIMENTAL ARTICLE

18. Cytotoxicity of Sodium Hypochlorite, Chlorhexidine and Propolis on Human Periodontal Ligament Fibroblast Cell

Latief Mooduto, Clarrisa Fredline, Galih Sampoerno, Setyabudi Goenharto, Fikarini Hadi Puteri,

Dian Agustin Wahjuningrum

Pages 476-480

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/18_D18_698_Dian_Agustin_Wahjuningrum_I*

EXPERIMENTAL ARTICLE

19. Effectiveness Photodynamic Inactivation with Wide Spectrum Range of Diode Laser to Staphylococcus aureus Bacteria with Endogenous Photosensitizer: An in vitro Study

Suryani Dyah Astuti, Indira Wastu Widya, Deny Arifianto, Retna Apsari

Pages 481-486

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/19_D18_707_Suryani_Dyah_Astuti_layout.pd*

EXPERIMENTAL ARTICLE

20. The Influence of Pandalus Borealis Shell Nano Chitosan on Permanent Teeth Enamel Integrity against Caries

Fidya, Mohammad Chair Effendi, Mutiara Fauzia Nurmawlidina

Pages 558-562

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/32_D18_669_Piolina_Wiwin_Nurgalih_layout*

CLINICAL ARTICLE

33. The Psychosocial Impact of Dental Aesthetics on Orthodontics Patients

Azrul Hafiz, Diana Zahid, Ainuddin Yushar, Zurairah Ibrahim, Syiral Mastura, Fitri Octavianti, A. Noor Sam

Pages 563-568

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/33_D18_686_Azrul_Hafiz_b_Abdul_Aziz_lay*

CLINICAL ARTICLE

34. Cognitive Comprehension of Dental Health Education Using a Busy Book "Ayo Sikat Gigi" in Down Syndrome Children

Astrinia Ristia Putri, Margaretha Suharsini, Mochammad Fahlevi Rizal, Sarworini B. Budiardjo, Heriandi Sutadi, Ike Siti Indiarti, Eva Fauziah

Pages 569-572

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/34_16983-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

35. Competency of Dental Students to Detect Lesions Suspicious for Oral Cancer and Oral Potentially Malignant Disorders

Kamis Gaballah, Fatma Sabry Mahmoud, Eynas Albalkhi, Omar Kujan

Pages 573-578

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/35_D18_712_Kamis_Gaballah_layout.pdf]*

CLINICAL ARTICLE

36. Angle's Malocclusion Classification and Soft Tissue Facial Profile in People with Down Syndrome in Jakarta – Angle's Malocclusion Classification and Soft Tissue Facial Profile in Down Syndrome

Aditya Eka Nurcahya, Margaretha Suharsini, Sarworini Budiardjo, Heriandi Sutadi, Ike Siti Indiarti, Mochammad Fahlevi Rizal, Eva Fauziah

Pages 579-583

Pages 579-583

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/36_17299-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

37. The Audit of Comprehensiveness of Dental Records at Dental Hospital, Faculty of Dentistry, Naresuan University, Thailand

Tipruthai Prayoonwong, Thanaphat Chirattanasak, Duangruethai Boonyo, Narathip Yuthim,

Natthapon Kerdphol

Pages 584-590

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/37_D18_715_Tipruthai_Prayoonwong_layout*

CLINICAL ARTICLE

38. Evaluation of Impacted Mandibular Third Molar Position in Relation to Mandibular Canal on Panoramic Radiography compared to Cone-Beam Computed Tomography

Saptadi A, Lilies DS, Menik P, Benny S. Latief

Pages 591-596

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/38_18082-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

39. Dental Procedures During Fasting: Perceptions Among Muslims In Malaysia

Farah Natashah Mohd, Abdul Hadi Said

Pages 597-601

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/39_D18_714_ABDUL_HADI_BIN_SAID_layo*

CLINICAL ARTICLE

40. The Effect of Low Bone Mineral Density in Stomatognatic System

Irene Edith Rieuwpassa, Nurul Fitri, Rafikah Hasyim, Dwi Putri Wulansari

Pages 602-606

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/40_D18_720_Dwi_Putri_Wulansari.pdf]*

CLINICAL ARTICLE

41. Prosthetic Rehabilitation with Use of Palatal Augmentation Prosthesis in Patients Affected by Functional Limitations of the Tongue

Di Carlo Stefano, De Angelis Francesca, Armida Matteo, Sara Jamshir, Fabretti Michele, Edoardo Brauner

Pages 607-611

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/41_D19_856_Matteo_armida-last-manuscript-9.pdf]*

CLINICAL ARTICLE

42. The Effects of Scaling and Root Planing on Mrna Expression of Matrix Metalloproteinase-1 and Clinical Parameters

Melissa Patricia, Robert Lessang, Fatimah Maria Tadjoedin, Boy M. Bachtiar

Pages 612-616

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/42_18183-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

43. Effects of Social Networking Education on HIV/AIDS Knowledge, Attitude and Practice in Dentistry Students

Sharare Karimi, Khaled Rahmani, Vahid Veisi, Abdorrahim Afkhamzadeh

Pages 617-621

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/43_D18_736_Khaled_Rahmani-Layout.pdf]*

CLINICAL ARTICLE

44. Association of Propolis Fluoride with Arrested Dentinal Caries and Dental Plaque Levels

Risqa Rina Darwita, Febriana Setiawati, Salsabila Ghina Andiani

Pages 622-627

Pages 622-627

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/44_18527-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

45. Occlusal Force Distributions in Various Angle'sMalocclusions an Evaluation by T–Scan III System

Titirat Chutchalermpan, Jittima Pumklin, Ratchawan Tansalarak, Saengdao Sirijaroenpun,

Aphiwat Sedtasuppana, Thosapol Piyapattamin

Pages 628-632

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/45_D18_754_Thosapol_Piyapattamin_Layou*

CLINICAL ARTICLE

46. Validity and Reliability of the Indonesian Version of Oral Hygiene Behavior Index Questionnaire: A Cross Sectional Study among Young Adolescents in Junior High School in Bandung, Indonesia

Netty Suryanti, Armasastra Bahar, Anton Rahardjo, Ali Nina Liche Seniati, Diah Ayu Maharani

Pages 633-639

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/46_18575-ED-2-B4-OK_layout.pdf]*

CLINICAL ARTICLE

47. Comparison of Visual Oral Health Literacy Level Pre and Post Oral Health Education among Adolescents

Haslina Rani, Michelle Su Yean Hua, Alicia Ding Sue Ann, Nurul Asyikin Yahya, Azlan Jaafar

Pages 640-644

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/47_D18_728_Haslina_Rani_Layout.pdf]*

CLINICAL ARTICLE

48. Xerostomia and Salivary Flow Rates in Methamphetamine Abusers in Jakarta, Indonesia

Cut Mytha Fitriana, Gus Permana Subita, Masita Mandasari

Pages 645-649

Pages 645-649

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/48_19328-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

49. The Impact of Fixed Appliances (Braces) on Quality of Life

Azrul Hafiz, Atiqah Jamal, Nurazfalina Azura, Rahimah Sahudi, Murshida Marizan Nor

Pages 650-654

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/49_D18_690_Azrul_Hafiz_bin_Abdul_Aziz_la*

CLINICAL ARTICLE

50. Development of the Indonesian version of the Psychosocial Impact of Dental Aesthetic Questionnaire (PIDAQ) in Prosthodontics

Saraventi Mursid, Lindawati S. Kusdhany, Diah Ayu Maharani, R. Irawati Ismail

Pages 655-662

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/50_19362-ED-2-B4-OK_layout.pdf]*

CLINICAL ARTICLE

51. Awareness of Fake Braces Usage Among Y- Generations

Rohaya Megat Abdul Wahab, Siti Khadijah Hasan, Nor Ermamarsila Mohd Yamin, Zurairah Ibrahim

Pages 663-666

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/51_D18_694_Rohaya_Megat_Abdul_Wahab_*

CLINICAL ARTICLE

52. Biological Factors in 2 – 3 years old Children in Determining Risk Factors of Early Childhood Caries: Pilot Study

Asty Samiaty Setiawan, Risqa Rina Darwita, Sri Susilawati, Diah Ayu Maharani, Ariadna Adisattya Djais

Pages 655-671

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/52_19377-ED-2-B4-OK_layout.pdf]*

CLINICAL ARTICLE

CLINICAL ARTICLE

53. Clinical Evaluation of Osseointegration on Dental Implants with Resonance Frequency Analysis

Ratna Sari Dewi, Roselani W Odang, Fariza Gita

Pages 672-676

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/53_19431-ED-2-OK_layout.pdf]*

CLINICAL ARTICLE

54. Demography of Oral Health Status for Students and Teachers in Islamic Boarding School

Muhammad Ruslin, Rasmidar Samad, Burhanuddin DP, A. Tajrin, Abul Fauzi, M. Irfan Rasul,

Nursyamsi Djamaluddin, A. St. Hajrah Yusuf, A. Sri Permatasari, Adam Malik H.

Pages 677-681

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/54_D18_796_Muhammad-Ruslin-5-M.-Ruslin_Layout.pdf]*

CLINICAL ARTICLE

55. Influence of Waiting Times in Dental Offices Towards Patient Satisfaction and Evaluations of Patient/Provider Relationships in Bantaeng District, South Sulawesi, Indonesia, 2018

Fuad Husain Akbar, Rasmidar Samad, Burhanuddin Daeng Pasiga, Rini Pratiwi, Ayub Irmadani Anwar, Nursyamsi Djamaluddin, Andi Nursakina Tri Meilana

Pages 682-687

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/55_D18_796_Muhammad-Ruslin-6-Fuad_edit_new_Layout.pdf]*

CLINICAL ARTICLE

56. Description of the Level of Knowledge, Attitude, Preparedness and Willingness of the Faculty of Dentistry Faculty of Hasanuddin University in Caring for People with Hiv/Aids

Rini Pratiwi, Fuad Husain Akbar, Burhanuddin Pasiga, Rasmidar Samad, Ayub Irmadani Anwar,

Nursyamsi Djamaluddin, Ratu Syamsiah Nila Kencana

Pages 688-694

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/56_D18_796_Muhammad-Ruslin-7-Rini-Pratiwi_edit_Layout.pdf]*

CLINICAL ARTICLE

57. The Prevalence of Caries and Gingivitis in Elementary School Children in Grade IV, V and VI in East Sinjai District

Adam M. Hamudeng, Farah Fadhillah

Pages 695-699

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/57_D18_796_Muhammad-Ruslin-12-ADAM-M.-HAMUDENG_Layout.pdf]*

CASE REPORT

58. Oral Candidiasis in Grave's Disease after Dental Surgery

Fanny Margaretha Laihad, Lita Agustia, Eddy Hermanto, Kristanti Parisihni, Syamsulina Revianti,

Widyasri Prananingrum, Linda Rochyani, Meinar Nur Ashrin, Noengki Prameswari

Pages 700-704

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/58_D18_716_Noengki_Prameswari.pdf]*

CASE REPORT

59. Management of Gingival Enlargement Caused by Phenytoin Used in Epilepsy Patients: A Case Report

Arni Irawaty Djais, A. Mardiana Adam, Hasanuddin Thahir, Sri Oktawati, Asdar Gani, Supiaty, Muliaty Yunus, Dwi Putri Wulansari, Fuad Husain Akbar, Vidya Yuniati Tope

Pages 705-708

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/59_D18_796_Muhammad-Ruslin-2-Arny-Irawati-Djais_edit_Layout.pdf]*

CASE REPORT

60. Efficacy of corticosteroids in oral lesion treatment associated with Steven-Johnson syndrome and toxic epidermal necrolysis in HIV patient (A Case Report)

Mega Rafika,Irna Sufiawati

Pages 709-715

Pages 733-737

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/64_D18_533_Benso_Sulijaya_Suyono_layou*

REVIEW

65. A Review of MicroRNA Associated with Oral Cancer

Khor Goot Heah, Nur Rawaidah Bt Mohd Shobri, Syairah Nabila Bt Suhaimi, Nurhanani Harun,

Nur Aliana Hidayah Mohamed, Gabriele Ruth Anisah Froemming

Pages 738-743

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/65_D18_685_Khor_Goot_Heah_layout.pdf]*

REVIEW

66. Oral Microbiome in Forensic Odontology to Identify Bioterrorism Attack

Bambang Tri Hartomo, Elza Ibrahim Auerkari

Pages 744- 747

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/66_D18_796_Muhammad-Ruslin-10-Bambang-Tri-Hartomo_edit_Layout.pdf]*

REVIEW

67. Graphene Applications in Dentistry

Di Carlo Stefano, Brauner Edoardo, Di Carlo Fabio, Visca Annalisa, Piccoli Luca, De Angelis Francesca

Pages 748-754

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/67_D19_855_FRANCESCA_DE_ANGELIS_I*

REVIEW

68. Combination of Periodontic and Orthodontic in Treating Pathological Migration: Systematic Review

Sri Oktawati, Fatmawati M

Pages 755-759

Full Text PDF *[http://www.jidmr.com/journal/wp-content/uploads/2019/07/68_D18_796_Muhammad-Ruslin-11-Sri-Oktawati-Fatmawati-M_edit_Layout.pdf]*

MEDICINE

EXPERIMENTAL ARTICLE

69. Experimental Partial Transection of Spinal Cord and Its Bioengineering Reconstruction

Bolshakov I Nikolaevich, Svetlakov A Vasilevich, Eremeev A Valerievich, Sheina Y Igorevna

Pages 760-779

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70. Biolarvicidal Effects of Papaya Leaves Juice Against Aedes Aegypti Linn Larvae

Widya Hary Cahyati, Widya Asmara, Sitti Rahmah Umniyati, Budi Mulyaningsih

Pages 780-785

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EXPERIMENTAL ARTICLE

71. LIF and LIF-R Protein Expression in Macaca nemestrina Midluteal Endometrial Tissues After Controlled Ovarium Stimulation

Nurhuda, Rosalina T, R Muharam, Mujihartini N, Dwi Ari P

Pages 786-790

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EXPERIMENTAL ARTICLE

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Sri Yusnita Irda Sari, Azyyati Ridha Alfian, Titik Respati, Dwi Agustian, Ardini Saptaningsih Raksanagara

Pages 791-796

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Injectable Bone Substitute of Hydroxyapatite-Gelatin Composite with Alendronate for Bone Defect Due to Osteoporosis

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Abstract

World Health Organization mentioned that there were 200 million people in the world suffered from osteoporosis in 2013 and half of them has bone fractures. Hydroxyapatite as a bioactive material was explored as a substitute for bone defect or osteoporosis. The conventional bone filler was lack of the ability to let the filler to fill the irregular defect due to osteoporosis. Thus, a new method was introduced called an injectable bone substitute. While the bone filler performs its function to stabilize the mechanical property of the defected bone, the addition of a drug, such as an alendronate, would be beneficial. The injectable bone substitute (IBS) based on hydroxyapatite-gelatin was synthesized with the addition of alendronate. The Fourier Transform Infrared (FTIR) result showed a bond formation of hydroxyapatite and gelatin by shifting of carboxyl group wavenumber from 1332,72 cm^{-1} to 1559-1543 cm^{-1} from gelatin with Ca^{2+} from hydroxyapatite. The IBS viscosity was (38.7±0.53) dPa.s and was able to be extruded from the syringe. The IBS could become suspension again after sedimentation and did not change the pH of SBF solution. The IBS was precipitated with a suitable substrate. The cytotoxicity test showed that the samples were non-toxic. The results of IBS characterizations demonstrated that it has potential to be used as a bone filler as well as drug delivery system to the bone defect due to osteoporosis.

Experimental article (J Int Dent Med Res 2019; 12(2): 813-818)

Keywords: Injectable bone substitute, Hydroxyapatite-gelatin composite, Alendronate, Osteoporosis, Bone defect.

Received date: 28 June 2018 **Accept date:** 07 October 2018

Introduction

Bone defects caused by traumatic accident, tumor and total joint reconstruction often occurred and needed bone substitute materials. There were some implanted synthetic materials in the bone defect and still encapsulated by fibrous tissue and did not attach to bone.[1] Osteoporosis was bone deformation marked by bone strength reduction and influenced by increasing of bone fracture risk.[2] World Health Organization (WHO) in 2013 reported that around 200 million people in the world suffer from osteoporosis, and 50% of them had a bone fracture, especially in the upper leg[3]. Bone defect was caused by external factor and osteoporosis was caused by an internal factor, such as reduction of bone ability to do bone remodeling process because of an unbalanced process of osteoblast and osteoclast. This case could be handled by increasing the bone density or filling the bone defect with a suitable material. Hydroxyapatite as a bioactive material was explored as a substitute for bone defect or osteoporosis.[4] Hydroxyapatite is brittle. So, in its application, it needs another material from polymer group, like gelatin to support it. The addition of gelatin was aimed to increase osteoblast adhesion, migration, and mineralization. The hydroxyapatite-gelatin composite was already studied and proved that this composite was suitable to use as a bone substitute material with high biocompatibility and non-toxicity.[5]

The application of bone filler nowadays was not effective for the defect caused by the osteoporosis since the defect was in an irregular shape. The bone filler should fill the irregular defect while maintaining its mechanical function. Because of that, a new method called injectable bone substitute was introduced. Injectable bone

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substitute (IBS) was bone substitute material in an injectable form. There were two kinds of IBS, IBS in ready-to-use suspension form and IBS with ionic hydraulic cement that could harden *in vivo* after injection.[6] Weiss et al. (2007) already conducted research about calcium phosphate-based IBS paste that could harden after injection.[6] The results showed that the paste could form suspension or paste by using hydroxypropyl methylcellulose (HPMC) polymer 2% w/v as a suspending agent. Another study from Warastuti and Abbas (2012) already succeeded to synthesize IBS from hydroxyapatite and chitosan with HPMC 2% w/v as suspending agent. That research showed the effect of irradiation in physics characterization of IBS. The results showed that irradiation 25 kGy did not affect the IBS.[7] The function of IBS could be added as drug delivery to help bone defect healing by using a bisphosphonate drug, like alendronate. Alendronate has high-affinity electron to Ca^{2+} ion that could improve the interaction with bone calcium and inhibit the osteoclast in the bone remodeling process.[8] Thus, there is a need to investigate the potential of hydroxyapatite-gelatin-based injectable bone substitute with the addition of alendronate for application in bone defect due to osteoporosis.

Materials and methods

Materials. Materials used in this research were hydroxyapatite powder from cow bone (Tissue Bank dr. Soetomo General Hospital, Surabaya, Indonesia), Gelatin (150 bloom Rousselot, Guangdong, China) from cow skin, Alendronate (Arshine Technology Co., Limited, Wanchai, China), *Hydroxypropylmethylcellulose* (HPMC) (Sigma Aldrich H7509, Singapore), distilled water, and *Simulated Body Fluid* (SBF) solution.

Methods. The samples were synthesized by dissolving 2% w/v HPMC in distilled water at 90-100°C and dissolving 5% w/v gelatin in distilled water at 40°C. The hydroxyapatite (HA) powder was added to the gelatin solution with a variation of 45:55, 50:50 and 55:45. The alendronate was added to that solution with a ratio of 1 to 10 compared to the mass of hydroxyapatite.[9] The final step was added HPMC solution to hydroxyapatite-gelatin-alendronate solution gradually until that solution became a white suspension.

Fourier Transform Infrared (FTIR) Analysis. The samples were characterized by Fourier Transform Infrared (FTIR) in Faculty of Mathematics and Natural Science, Institute of Sepuluh November, Surabaya, Indonesia. The sample was freeze-dried first at -20°C for 4 hours. KBr was added to the sample and compacted into a pellet. The FTIR test was performed in the wavenumber range of 4000 - 400 cm^{-1}.

Viscosity test. This test was performed in the Material Physics Laboratory Faculty of Science and Technology, Universitas Airlangga, Indonesia by using Viscotester VT-04F RION Co. Ltd. The composite suspension of hydroxyapatite-gelatin poured to the 300 ml Becker glass. The test used Rotor Number 1. The motor would stir the solution and the reader would read the viscosity of the solution corresponding to the second scale due to the use of rotor no. 1. The test was replicated 5 times.

Resuspension test. This test aimed to observe the ability of the solution to form suspension again after sedimentation in term of time to reform a suspension again and the pH of the solution. The test was performed by adding PBS to the suspension and shacked them. The time and pH after shaking until forming suspension again were recorded. The pH was measured by using pH-indicator strips (Merck©).

Cytotoxicity Test. This test used MTT assay method to evaluate the cytotoxicity properties of materials. The test was performed at PUSVETMA, Surabaya by using fibroblast cell from Baby Hamster Kidney (BHK)-21. The living cell would react by changing the tetrazolium salt to formazan. The fibroblast cell of BHK-21 in Eagle's media was incubated for 48 hours and cleaned by using Phosphate Buffer Saline (PBS). 86% Eagle's media, 1% Penicillin streptomycin and 100 units of Fungizone/ml was prepared for 100 µl and the cultured cell was inserted to it. The cell culture was moved to 96-microwell plate. The suspension sample was diluted four times to be neutral. 1 ml of that suspension was dissolved in 10 ml aquabidest. 1 µl of that sample was placed in each well and was incubated for 24 hours at 37°C. The micro well plate was rinsed with PBS and was added 10 µl of 5mg/ml MTT solution in each well. Every well was added with 50 µl of DMSO and then centrifuged for 5

minutes with 30 rpm. The measurement process was performed by Elisa reader showing the violet level as Optical Density (OD) which represents the cell viability of the material based on Eq. 2.[10] The test was replicated four times. % cell viability = (OD treatment + OD media control)/(OD cell control + OD media control) (2)

Setting time and morphology test. The setting time and morphology test was performed aimed to obtain the time which the suspension needed to set in the application. Two types of substrate were used, glass and hydroxyapatite-collagen scaffold. After that, the substrate that was treated with the suspension was observed in its physical appearance and furthermore, characterized by Scanning Electron Microscope (SEM) Inspect S20 with magnification of 1000.

The statistical test. The data was analyzed by using statistical test which was one-way ANOVA test with p-value of 0.05 and presented as its average and standard deviation.

Results

FTIR test. The sample was freeze-dried and characterized by Fourier Transform Infrared (FTIR) first. The result showed the spectra of the functional bond in the sample as shown in Figure 1.

Based on spectra in Figure 1, there were some absorbance peaks that represented specific functional groups of the material, such as 3262.65 cm^{-1} which represented the hydroxyl O-H group (intermolecular hydrogen bond) that came from HPMC, gelatin, hydroxyapatite, and alendronate.[11] The absorbance at a wave number of 1332.72 cm^{-1} represented the carboxyl (COO^-) bond from proline of the gelatin which was the specific characteristic of type I collagen.[11] Besides that, the absorbance at a wave number of 1627.82 cm^{-1} represented the amine (NH_2) group from gelatin and alendronate. The most important part was the presence of a new bond shifting from 1332.72 cm^{-1} to 1558.65 cm^{-1} which was an absorbance peak of the interaction of Ca^{2+} from hydroxyapatite and carboxyl group from gelatin.[11,12] The P-O-C and phosphate group from hydroxyapatite and alendronate was also present at 1042.24 cm^{-1} and 547.13 cm^{-1}, respectively.[10]

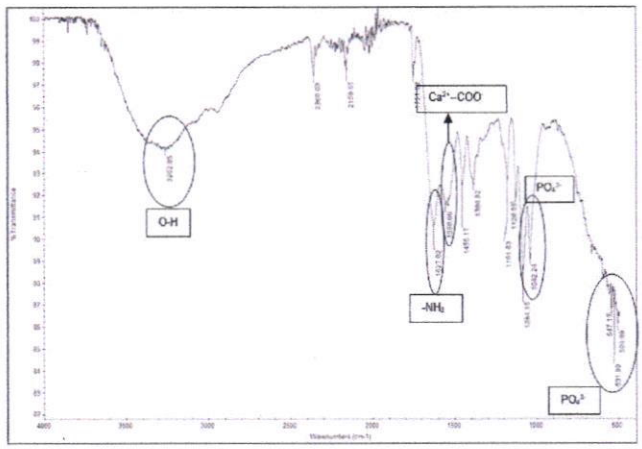


Figure 1. The Hydroxyapatite-gelatin based IBS suspension with addition of alendronate FTIR spectra.

Viscosity test. The viscosity of IBS is essential as the injectability property is defined by the viscosity. The viscosity IBS suspension was characterized by Viscotester VT-04F Rion Co. Ltd. The results of viscosity test showed in Figure 2.

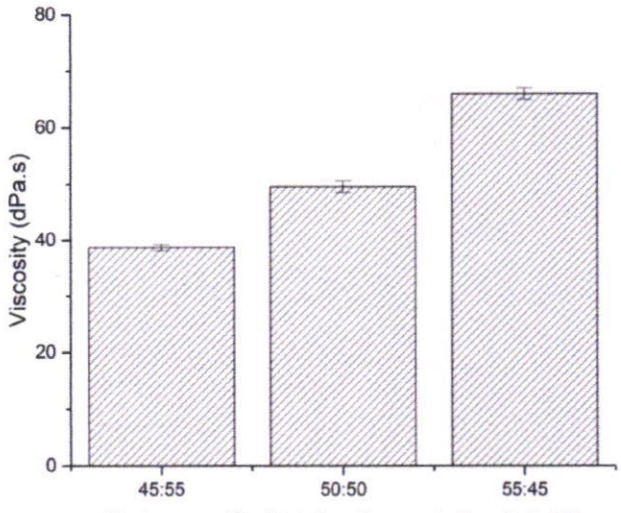
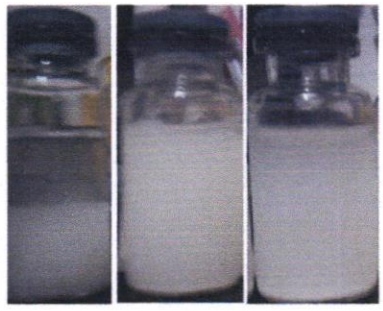


Figure 2. The Average Viscosity of Hydroxyapatite-Gelatin Based IBS Suspension with Addition of Alendronate.

It showed the viscosity of IBS suspension from different hydroxyapatite-gelatin composition variation in five replications. The results of hydroxyapatite-gelatin composition variation viscosity 45:55, 50:50 and 55:45 were (38.7 ± 0.53) dPa.s, (49.6 ± 1.02) dPa.s, and (66 ± 1) dPa.s, respectively. The One-way ANOVA test showed that the result were significantly different with p value < 0.05.

Resuspension test. There were two parameters observed in the resuspension test, which were time and pH to show that the IBS suspension was able to reform and the sediment part could disperse again, did not harden and did not change the pH of Simulated Body Fluid (SBF). The test was performed by applying SBF solution to the suspension samples and measuring the pH and time to form suspension again after mixing or shaking the suspension. The result of resuspension test showed in Figure 3, 4 and 5. Figure 4 showed the results of resuspension test based on pH. The pH was around 7 to 7.9. All samples indicated a stable result and above 7.



(a) (b) (c)

Figure 3. The resuspension test of hydroxyapatite-gelatin based IBS suspension with addition of alendronate (a) Before mixing with SBF (b) After mixing (c) After sedimentation.

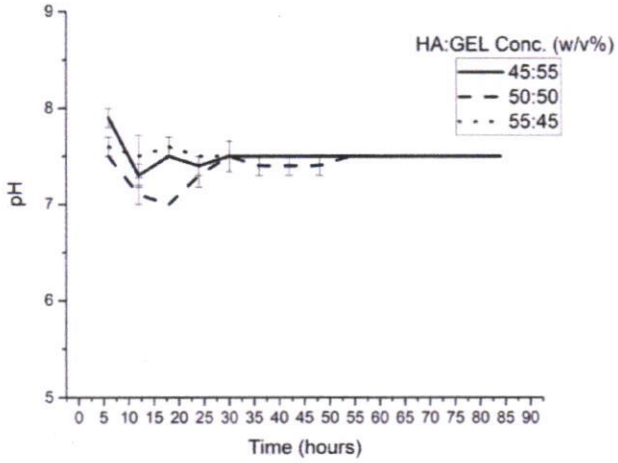


Figure 4. Resuspension test result of hydroxyapatite-gelatin based IBS suspension with addition of alendronate based on pH.

Besides that, it did not show any abnormality and did not change pH of SBF solution drastically which had pH around 7.4. Figure 5 showed the resuspension test result based on IBS suspension time of forming suspension again. In the first to second day, the time of resuspension was not stable because of the particles in the suspension was still spread in the suspension. After the third to seventh day, the result showed the stable line of resuspension time.

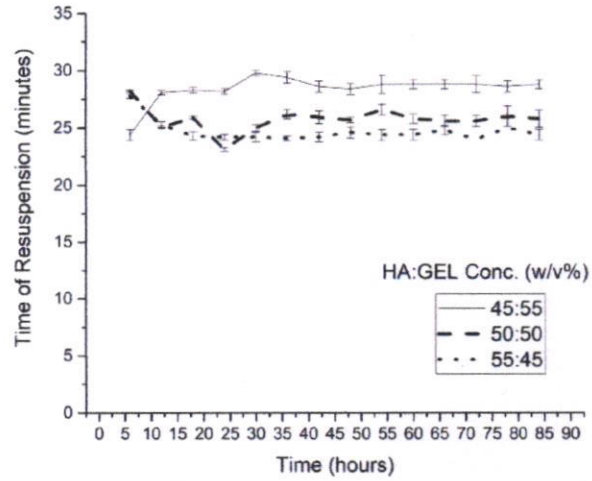


Figure 5. The Resuspension test result of hydroxyapatite-gelatin based IBS suspension with addition of alendronate based on time.

Cytotoxicity test. The cytotoxicity test in this study was using MTT assay method to observe the viability of Baby Hamster Kidney-21 (BHK-21) fibroblast cells towards the IBS suspension. This method was evaluating the change from the tetrazolium rings from MTT due to the activity of mitochondria of the living cells. The amount of the change could be measured by using Elisa reader to obtain the optical density which indicated the viability of the cells. The result showed in Figure 6 and in indicated that all samples were non-toxic since the cell viability exceeded 50%. The sample with a hydroxyapatite-gelatin ratio of 55:45 exceeded 100%.[13] Based on the One-way ANOVA test, the result was significantly different with p value < 0.05.

Morphology test. The setting time test was performed to obtain the time of hardening process of IBS suspension. This test was conducted in a petri dish. The result showed that the suspension could not harden until seven days. This IBS suspension needed a suitable substrate for hardening process with a similar component to the human bone. Because of that, a freeze-dried hydroxyapatite-collagen scaffold was used

in this research as a substrate media. That scaffold was immersed in IBS suspension for an hour and was dried at room temperature. The scaffold needed 5-6 hours for setting and hardening process.

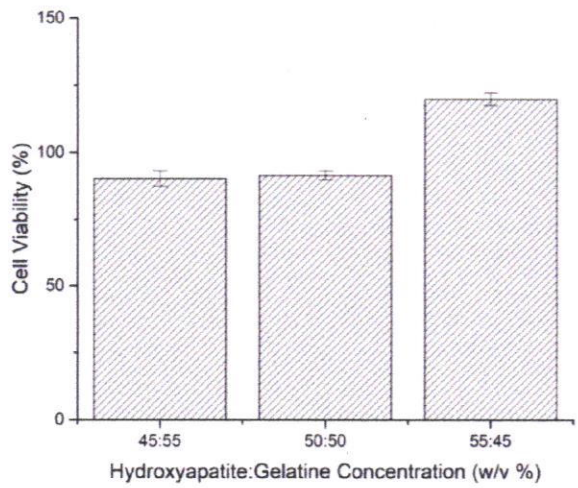


Figure 6. The cytotoxicity test result of hydroxyapatite-gelatin based IBS suspension with addition of alendronate.

The change in scaffold mass was measured before and after the immersion in IBS suspension. The result showed that the mass increased from 0.12 gram to 0.26 gram in wet condition and 0.21 gram in dry condition. The height and diameter of the scaffold also changed from 9.2 mm and 4.2 mm to (9.178 ± 0.053) mm and (3.824 ± 0.156) mm. This result showed that the IBS suspension was able to set in the suitable substrate. Hydroxyapatite-collagen that was synthesized in the freeze-dried process produced some pores that facilitated the IBS suspension to infiltrate the scaffold. The dried scaffold after immersion was characterized by Scanning Electron Microscope (SEM). The SEM image of dried hydroxyapatite-collagen scaffold after immersion was shown in Figure 7. The average of pore size was (19.125 ± 0.201) µm. This size was bigger than scaffold pore size before immersion. This size indicated that alendronate in this IBS suspension could bind the calcium ion of hydroxyapatite and formed some pores with increasing its size. This could occur because alendronate had high electron affinity to Ca^{2+} ion.[8]

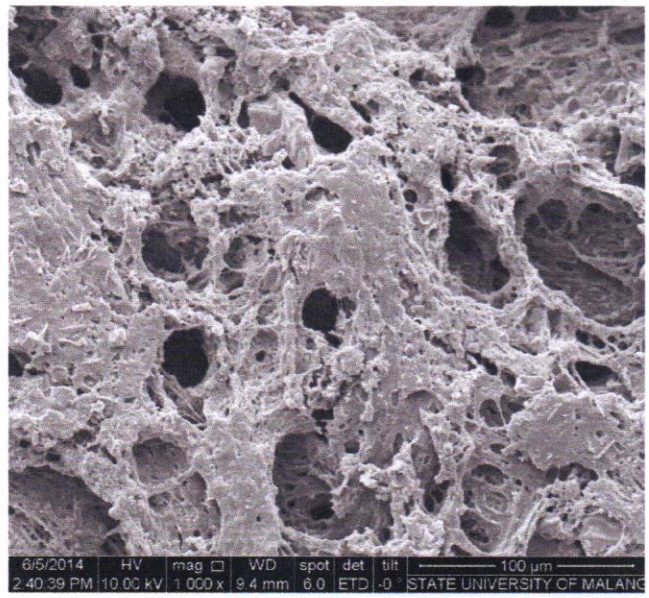


Figure 7. SEM image of dried hydroxyapatite-collagen scaffold after immersion in hydroxyapatite-gelatin based IBS suspension with addition of alendronate.

Discussion

In this study, an Injectable Bone Substitute (IBS) based on hydroxyapatite and gelatin with the addition of alendronate was developed. The presence of HPMC aimed to form the composite as a suspension. The Fourier Transform Infrared (FTIR) study of Injectable Bone Substitute (IBS) showed that there was a new bond between Ca^{2+} ion from hydroxyapatite and carboxyl group (COO^-) from gelatin, as mentioned by Narbat et al. and Wang et al. in their studies.[11,12] This bond was a covalent bond which presented in the composite. The presence of HPMC would provide more interaction between the Ca^{2+} ion from hydroxyapatite and the carboxyl group from gelatin. The alendronate was also still present in the composite as shown in absorbance peak at 1627.82 cm^{-1} in the FTIR test result.[14]

The standard viscosity for IBS application based on Bourges et al. study was 40 dPa.s.[15] The IBS composition that has viscosity value around the standard was the combination of hydroxyapatite and gelatin at 45:55 which was 38.7 dPa.s. The viscosity of IBS suspension had an important role in its application because it determined the injectability. The sample in suspension form was able to sediment and need a mixing or shaking to make it in homogeneous

state again.[6] The samples that were characterized by viscosity test were also tried in 10 ml syringe with 2 mm inner diameter tip.[16] All the IBS suspensions can be injected from that syringe easily. It showed that the samples had good injectability.

The resuspension test observed the change in pH and time to form suspension again. It indicated that the alendronate addition in the IBS suspension could not change the pH of the body when it applied in the bone fracture. Alendronate had low pH or prominent acidity around 4 – 4.5, and it could affect human body when it applied to the body directly. The pH of IBS solution also affected the material ability in setting time when applied to the bone. The average pH of hydroxyapatite for an application that could set in the bone was 6.[16] The amount of hydroxyapatite used in IBS solution increased the resuspension time because hydroxyapatite powder was affected by gravitation although there were still hydroxyapatite powders that flew around in the IBS suspension.[6] Its characteristic as suspension caused its particle sediment less than an hour and could form suspension again after shaking and did not harden at the bottom of the glass vial.

The setting time test result indicated that the IBS suspension needs a suitable substrate to set. In the application case, the bone is the most ideal substrate. Thus, the IBS suspension would set in the bone. The use of hydroxyapatite-collagen scaffold with the application of IBS suspension showed that it could set and also infiltrate the scaffold which represented the bone. The dimensional change of the scaffold also emphasized the result of the FTIR test which showed that there was an interaction between the hydroxyapatite and gelatin.

All of the results mentioned above was also strengthened by the result of the cytotoxicity test that all the samples were non-toxic with the cell viability of around 100% and even stimulate the proliferation of the fibroblast cells.[10] The other studies need to be conducted to investigate the behavior of this IBS suspension thoroughly, especially in the injectability, alendronate release, the stability, and also in vivo study.

Conclusions

The hydroxyapatite-gelatin based injectable bone substitute with addition of alendronate can be synthesized and showed good characteristics in FTIR, viscosity, resuspension and setting time.

Acknowledgements

The authors would like to thank the Indonesian Ministry of Research, Technology and Higher Education for funding this research.

Declaration of Interest

The authors declare there is no conflict of interest.

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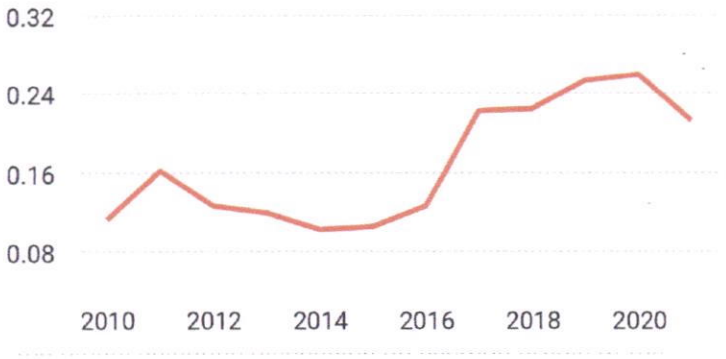

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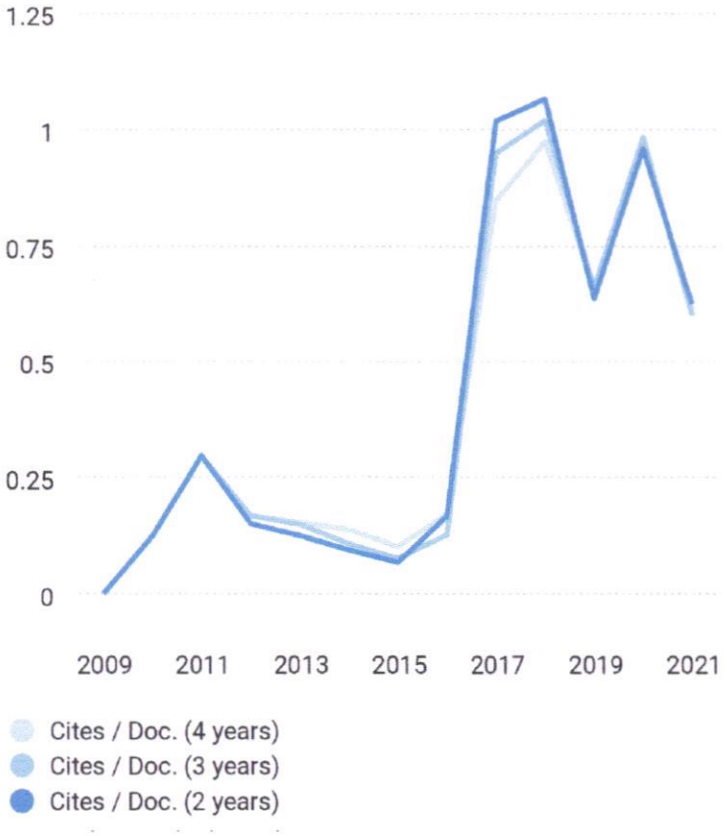

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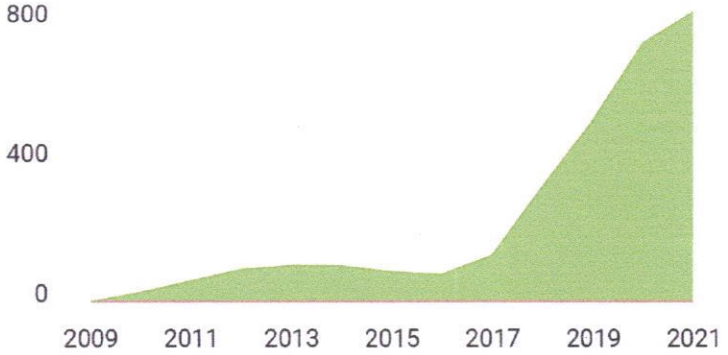

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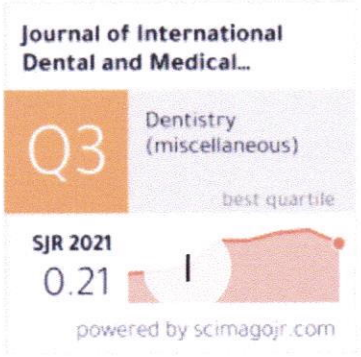

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SCImago Team

Melanie Ortiz 2 years ago

Dear Shivani,
Thank you very much for your comment.
All the metadata have been provided by Scopus /Elsevier in their last update sent to SCImago, including the Coverage's period data. The SJR for 2019 was

released on 11 June 2020. We suggest you consult the Scopus database directly to see the current index status as SJR is a static image of Scopus, which is changing every day.
Best Regards, SCImago Team

W **Warkaa mahmood** 3 years ago

dear sir
we found this journal in jevry Bill list which means its a peditory journal
how can you explain this?

reply

N **Nasution** 1 year ago

Dear Warka Mahmood

I've been looking Beall List and did not find this Journal to be predatory. Maybe I read wrong link. So could pease send me the right one? I also the journal's management should answer this accusation

Many thanks

W **Warkaa alwattar** 3 years ago

dear sir:
is this journal(journal of International Dental and Medical Research) still active in scopus or not. if not when did it became inactive.

reply

SCImago Team

Melanie Ortiz 3 years ago

Dear Warkaa, thank you very much for your comment, unfortunately we cannot help you with your request. We suggest you to consult the Scopus database directly. Remember that the SJR is a static image of a database (Scopus) which

is changing every day. Best regards, SCImago Team

D **Dr. Hawrra Khalid** 3 years ago

Good day
Iwant to publish in the jouranl
1.what are the cost of publication
2.is index in scopua ?

reply

M **mohamed motaz mohamed** 3 years ago

hello, what is the cost for publishing in the journal?? what is the time required for approval of the publishing request ??

SCImago Team

Melanie Ortiz 3 years ago

Dear Mohamed,
thank you for contacting us.
Sorry to tell you that SCImago Journal & Country Rank is not a journal. SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus.
Unfortunately, we cannot help you with your request, we suggest you to visit the journal's homepage or contact the journal's editorial staff , so they could inform you more deeply. Best Regards, SCImago Team

Leave a comment

Name

Email
(will not be published)

Submit

The users of Scimago Journal & Country Rank have the possibility to dialogue through comments linked to a specific journal. The purpose is to have a forum in which general doubts about the processes of publication in the journal, experiences and other issues derived from the publication of papers are resolved. For topics on particular articles, maintain the dialogue through the usual channels with your editor.

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EST MODUS IN REBUS
Horatio (Satire 1,1,106)