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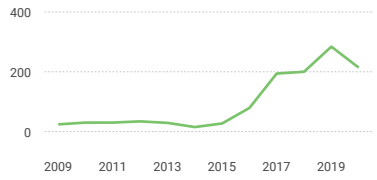

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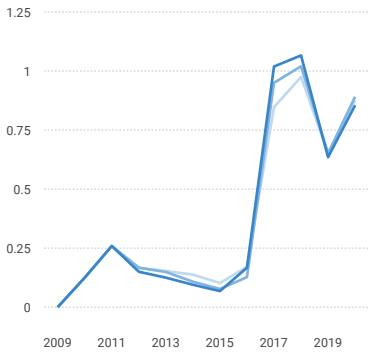

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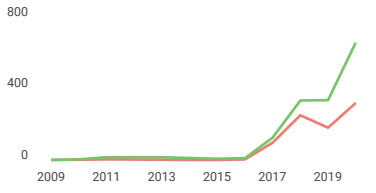

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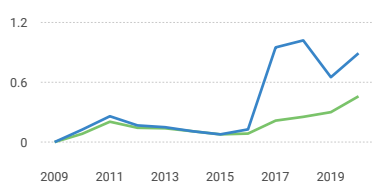

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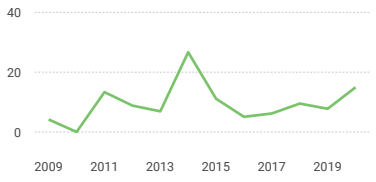

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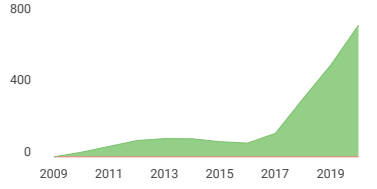

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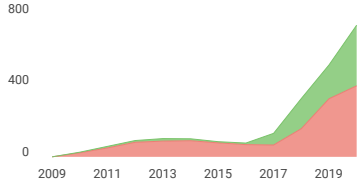

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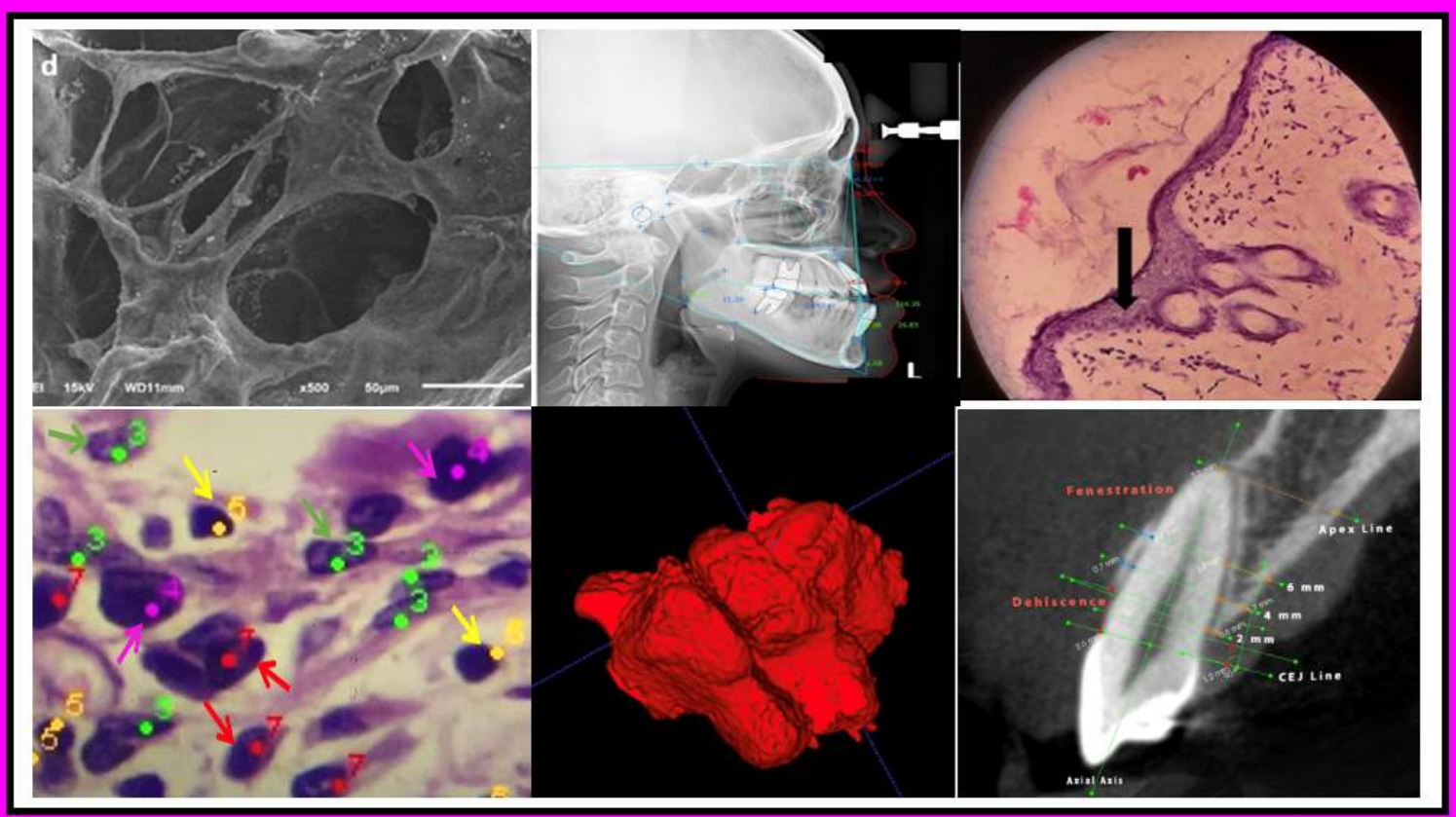

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TABLE OF CONTENTS / 2021; 14 (4)

DENTISTRY

EXPERIMENTAL ARTICLE

1. **Comparative evaluation of the remineralization potential of Theobromine and Fluoride containing dentifrices using Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis: An in-vitro Study**
   Nithya Annie Thomas, Priya Shetty, Charisma Thimmaiah, Sowmya B. Shetty, Nimmy Sabu, Kavita Bekal Kripalani
   Pages 1314-1320

EXPERIMENTAL ARTICLE

2. **Attachment of Streptococcus Mutans to Intraoral Suture Materials: An in Vitro Study**
   Denta Aditya Prasetya, Poerwati Soetji Rahajoe, Bambang Dwirahardjo, Michael Haryadi Wibowo
   Pages 1321-1326

EXPERIMENTAL ARTICLE

3. **Erythrocyte Sedimentation Rate as an Alternative to C-Reactive Protein in Rheumatoid Arthritis Patients with Periodontitis**
   Anirudh B. Acharya, Ibbani Padakannaya, Srinath Thakur
   Pages 1327-1333

EXPERIMENTAL ARTICLE

4. **Effect of Platelet Rich Plasma Incorporated to Autologous Bone Graft on Collagen Production in vivo**
   Vera Julia1, Fitriana, Benny Sjariefsjah Latief, Lilies Dwi Sulistyani, Bambang Pontjo, Tri Isyani Tungga Dewi
   Pages 1334-1338

EXPERIMENTAL ARTICLE

5. **An Invitro Study to Evaluate and Compare the Effect of Surface Treatment of Implant Abutments on the Retentivenesss of Three Commercially Available Provisional Cements**
   Ayesha Shaziya Jubapu, Brilvin Pinto
   Pages 1339-1350

EXPERIMENTAL ARTICLE

6. **Microvascular Activity from the Wound Healing Process in Wistar Rats Due to Administration of Anredera Cordifolia (Ten.) Steenis**
   Christian Khoswanto
   Pages 1351-1356

EXPERIMENTAL ARTICLE

7. **The Effect of Polyvinyl Alcohol on the Physico-Chemical Properties of Collagen-Chitosan Membranes**
   Agus Susanto, Ira Komara, Arief Cahyanto, Basril Abbas, Fajar Lukitowati, Yessy Warastuti
   Pages 1357-1362

EXPERIMENTAL ARTICLE

8. **Efficacy of Bidara Leaf (Ziziphus Mauritiana) Viscous Extract to Gingival Wound Healing in Wistar Rats**
   Mochammad Taha Ma'ruf, Putu Sulistiawati Dewi, Dewi Farida Nurlitasari
   Pages 1363-1372

EXPERIMENTAL ARTICLE

9. **Antibiofilm Efficacy of Myrmecodia Pendens Methanol Extract and NaOCl Against Enterococcus Faecalis ATCC 29212**
   Faisal Kuswandani, Mieke H. Satari, Ani M. Maskoen
   Pages 1373-1378

EXPERIMENTAL ARTICLE

10. **In Vitro Wound Healing Potential of Stem Extract of Spatholobus littoralis Hassk**
    Yessy Ariesanti, Wiwiek Poedjiastoeti, Komariah, Amalia Fauzana Wijaya
    Pages 1379-1385

JIDMR
Journal of International Dental and Medical Research / ISSN: 1309-100X

TABLE OF CONTENTS / 2021; 14 (4)

EXPERIMENTAL ARTICLE

11. **Osteoinduction Ability of Human Adipose-Derived Mesenchymal Stem Cell with Chitosan Scaffold Combination Towards Blood Serum Phosphorus Levels**
Dian Agustin Wahjuningrum, Nindya R A Marhendra, M. Roelianto, Ari Subiyanto, Irmaleny, Fery Setiawan, Syania E Febriyanti, Setyabudi, Tamara Yuanita, Swadheena Patro, Anuj Bhardwaj
Pages 1386-1393

EXPERIMENTAL ARTICLE

12. **The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release**
Jackson Dipankara, Joko Kusnoto, Rosalina Tjandrawinata, Rahmi Amtha
Pages 1394-1400

EXPERIMENTAL ARTICLE

13. **Cellulose Fiber from Coconut Coir for Development of Dental Composite Filler**
Twi Agnita Cevanti, Nur Shiyama Purnama Sari, Steella Ilham Isnaini, Mahardika F. Rois, Heru Setyawan, Adioro Soetojo, Ira Widjiastuti
Pages 1401-1406

EXPERIMENTAL ARTICLE

14. **Mefenamic Acid Induces Apoptosis in Oral Malignant Burkitt's lymphoma Through Caspase-3 and -9 Pathways Followed by Down-Regulation of Cox-2 and Overexpression of p27Kip-1**
Supriatno, Fauzi Adityawan, Faizal Dentawan Pritama, Muhammad Arindra Saka, Sartari Entin Yuletnawati, Faisal Fikri Hakim
Pages 1407-1412

EXPERIMENTAL ARTICLE

15. **The Effect of the Application of (Garcinia mangostana L.) towards PDGF-B Expression on Human Gingival Fibroblast Cell Culture After Wound Healing Scratch Test Assay (In-Vitro Study)**
Felicia Laurens Lesmana, Andra Rizqiawan, Indra Mulyawan, Ni Putu Mira Sumarta, David B. Kamadjaja, Coen Pramono D, Tobiumei Kei, Gde Djodi Satria Rurus, Naura Athiyyah Sativa, Rozhaline Apriliany Fanddhy
Pages 1413-1418

EXPERIMENTAL ARTICLE

16. **The Effects of Ag+ Ion in Osteoblast Cell Proliferation (In Vitro Study)**
R. Aries Muharram, Coen Pramono D, Pratiwi Soesilawati, Muhammad Subhan Amir, Aisyah Rachmadani Putri Gofur, Ajeng Hayyuning Citrasari
Pages 1419-1424

EXPERIMENTAL ARTICLE

17. **Physical Modification of Bovine Amniotic Membrane for Dental Application**
Octarina, Elly Munadziroh, Fathilah Abdul Razak
Pages 1425-1428

EXPERIMENTAL ARTICLE

18. **Effects of Centella asiatica Leaves Extract on Dimethyl Benz(A) Anthracene (DMBA) Induced Oral Epithelial Dysplasia in Rats**
Ahyar Riza, Gostry Aldica Dohude, Anisa Fitri
Pages 1429-1434

EXPERIMENTAL ARTICLE

19. **Spectroscopy Structure Analysis of Ellagic Acid and Calcium Phosphate**
Debby Saputera, Intan Nirwana, Michael Josef Kridanto Kamadjaja
Pages 1435-1441

JIDMR
Journal of International Dental and Medical Research / ISSN: 1309-100X

TABLE OF CONTENTS / 2021; 14 (4)

EXPERIMENTAL ARTICLE

20. **The Effect of Titanium Oxide (Tio2) Nanoparticles Addition on Polymethyl Methacrylate Denture Base Impact Strength, Tensile Strength, and Hardness**
Sanggry Mutiara, Hubban Nasution, Ricca Chairunnisa, Kholidina Harahap, Sefty Aryani Harahap, Astrid Yudhit, Febby Revita Sari, Slamat Tarigan
Pages 1442-1446

EXPERIMENTAL ARTICLE

21. **The Pattern of Collagen, Col1a, Bsp and Mmp-8 in Alveolar Bone Socket Post Tooth Extraction of Rattus Norvegicus Strain Wistar After Induced With Hydroxyapatite Bovine Tooth Graft**
Nanik Zubaidah, Yosefin Adventa, Dian Dwi Pratiwi, Latief Mooduto, Ernie Maduratna Setiawati, Sri Kunarti
Pages 1447-1452

EXPERIMENTAL ARTICLE

22. **The Benefits of the Combination of Vitamin D3, K2 Supplements, and UV-B Exposure for Increasing Bone Density: A Simple Solution for Bone Health**
Sindy Cornelia Nelwan, Udijanto Tedjosasongko, Satiti Kuntari, Daniel Haryono Utomo, Tania Saskianti, Mega Moeharyono Puteri, Devi Dharmawan, Yufita Fitriani, Pradita Agung Kurnia, Amalia Ramadhani Mufida, Nadhia Zahria Fajrin, Retno Pudji Rahayu, Nunthawan Nowwarote
Pages 1453-1458

EXPERIMENTAL ARTICLE

23. **The Effect of Chitosan and Acrylate Acid Complex into Acrylic Resin as Denture Material Against Fibroblast and Inflammatory Cells**
Titik Ismiyati, Ananto Ali Alhasyimi, Widowati Siswomihardjo, Supriatno
Pages 1459-1464

EXPERIMENTAL ARTICLE

24. **Comparison of the Effect of Calcium Hydroxide Combination with Cocoa Pod Husk Extract and Green Tea Extract on Fibroblast and Alp Activation**
Tamara Yuanita, Lailatun Tedja, Debby Suryani, Irma Drismayanti
Pages 1465-1471

CLINICAL ARTICLE

25. **To assess the usefulness of the Mandibular Ramus in determining Age and Gender among Malaysians in digital OPGs**
Ranjana GARG, Tiew JACKY, Timothy Gan Hwa YUNG, Young Wen LI, Tengku Mariam Batrisyia Tengku BRAHANUDIN
Pages 1472-1477

CLINICAL ARTICLE

26. **The Relationship Between Parenting Stress in Parents and Oral Health-Related Quality of Life (OHRQoL) Children with Down Syndrome**
Masayu Sesiliana, Willyanti Soewondo, Inne Suherna Sasmita
Pages 1478-1484

CLINICAL ARTICLE

27. **Accuracy of Sphenoidal Sinus Morphometry in Forensic Identification Using Cone Beam Computed Tomography**
Asmaa T Uthman, Abdullah Alomar, Ali Almukhtar, Rama Jaber, Raneen Essale, Rifqa Abdulsalam, Samsam Warsame, Walid Shaaban Elsayed, Natheer H Al-Rawi
Pages 1485-1491

CLINICAL ARTICLE

28. **Relationship Between the Satisfaction of Removable Denture Patients and Nutritional Status**
Rifka Dennisa, Lia Kartika Wulansari, Fakhrana Ariani Ayub
Pages 1492-1497

JIDMR
Journal of International Dental and Medical Research / ISSN: 1309-100X

TABLE OF CONTENTS / 2021; 14 (4)

CLINICAL ARTICLE

29. **Fruits and Vegetables: A Cost-effective Practical Solution in Periodontal Pre-Clinical Surgical Training for Postgraduate Students**
Fouad H AL-Bayaty, MFH Hidayat, Farha Ariffin, Erni Noor, Mahyunah Masud, Muhammad Hilmi Bin Zainal Ariffin, Hafizul Izwan Mohd Zahari, Fara Azwin Adam
Pages 1498-1502

CLINICAL ARTICLE

30. **Lifestyle as a Risk Factor of High Periodontitis Prevalence with and without Type 2 Diabetes Mellitus in Surabaya**
Titiek Berniyanti, Retno Palupi, Dini Setiyowati, Aulia Rahmadhani, Dinda Novia, Novitasari Mira Afanda, Nadya Innasa Khairani, Annisa Zahra Nurlaili, Fidelia Kartikasari
Pages 1503-1508

CLINICAL ARTICLE

31. **Effects of Sucking Exercise using Straws on Mouth Rinsing Ability in Children with Down syndrome**
Megananda Hiranya Putri, Susi Sukmasari, Eliza Herijulianti, Hetty Anggrawati, Neneng Nurjanah, Arlette Suzy Setiawan
Pages 1509-1516

CLINICAL ARTICLE

32. **Effect of Carbonate Apatite Membrane as Adjunctive Therapy of Scaling and Root Planing on Gingival Crevicular Fluid Matrix Metalloproteinase-8 in Chronic Periodontitis Patient**
Ira Komara, Siti Sopiatin, Ina Hendiani, Nunung Rusminah, Agus Susanto
Pages 1517-1522

CLINICAL ARTICLE

33. **Position of Unilateral / Bilateral Permanent Canine Impaction on the Prognosis of Treatment with KPG Index: 3D Cone Beam Computed Tomography Analysis**
Nina Agustin Chrystinasari, Ida Bagus Narmada, Ari Triwardhani
Pages 1523-1530

CLINICAL ARTICLE

34. **Development of the Indonesian version of the Oral Health Impact Profile in Edentulous Prosthodontic Patients**
Muslita Indrasari, Lindawati S. Kusdhany, Diah Ayu Maharani, R. Irawati Ismail
Pages 1531-1536

CLINICAL ARTICLE

35. **Correction of Gummy Smile using Botulinum Toxin**
Awad Ashekhi, Maher Al Shayeb, Danyah Ashekhi, Amany Ghazy, Aiman Abu-fanas, Syed Kuduruthullah, Ahmed Taha, Ibrahim Taher
Pages 1537-1541

CLINICAL ARTICLE

36. **Surface Electromyography Reveal Association between Masticatory Muscles with Malocclusion Class I And Class III Skeletal in Javanese Ethnic Patient**
Dwi Rahmawati, I Gusti Aju Wahju Ardani, Thalca hamid, Irina Fardhani, Haydar Taftazani, Alexander Patera Nugraha, Martha Kurnia Kusumawardani
Pages 1542-1546

CLINICAL ARTICLE

37. **Factors Associated with Dental Attendance Among Indonesian Adults: A Cross-Sectional Study**
Latifah Fitriani Rakhman, Atik Ramadhani, Diah Ayu Maharani
Pages 1547-1551

TABLE OF CONTENTS / 2021; 14 (4)

CLINICAL ARTICLE

38. **Knowledge, Attitude, Practice Towards Plaque Disclosing Agent Among Dentists in West Java, Indonesia**
Giannissah Fathina Fairuz, Siti Sopiatin, Amaliya Amaliya
Pages 1552-1560

CLINICAL ARTICLE

39. **Prevalence of Dehiscence and Fenestration on Incisors after Orthodontic Treatment in High-Angle Patients using Cone Beam Computed Tomography**
Yoshua Christian Hendrik, Retno Widayati, Menik Priaminiarti, Miesje Karmiati Purwanegara
Pages 1561-1568

CLINICAL ARTICLE

40. **Prevalence of Most Common Tongue Lesions Related to Degenerative Diseases in the Elderly**
Adiastuti Endah Parmadiati, Diah Savitri Ernawati, Fatma Yasmin Mahdani, Nurina Febriyanti Ayuningtyas, Meircurius Dwi Condro Surboyo, Aulya Setyo Pratiwi, Riyan Iman Marsetyo, Candrika Ramya Inastu, Vint Erawati
Pages 1569-1572

CLINICAL ARTICLE

41. **Associated Factors of Early Childhood Caries (ECC) Among 24–42-Month-Old-Children in Jakarta: A Cross-Sectional Study**
Febriana Setiawati, Iwany Amalliah, Preticia, Atik Ramadhani, Diah Ayu Maharani
Pages 1573-1579

CLINICAL ARTICLE

42. **Elements Affecting Toothbrushing Parenting among Mothers in Banjarbaru City**
Widodo, R. Darmawan Setijanto, Taufan Bramantoro
Pages 1580-1586

CASE REPORT

43. **The First Case Report about Noninvasive Impression Taking in Orthodontic Patient with Epidermolysis Bullosa**
Oleg Valovikov, Ellina Velichko, Svetlana Razumova, Olga Bait Said
Pages 1587-1591

CASE REPORT

44. **Oral Hygine, Periodontal Condition and Their Treatment Need of Teaching Faculty in Higher Schools**
Sunitha.S, Aruna.G, Vidya Doddawad, Arunpriya Srinivasan
Pages 1592-1598

CASE REPORT

45. **Dental Management of Severe Malocclusion and Syndromic Multiple Odontogenic Keratocysts**
Nabeel Almotairy
Pages 1599-1603

REVIEW

46. **Intraosseous Anesthesia of the Mandibular Molars: A Critical Literature Review**
Emiliya Simeonova, Valeriya Aleksandrova, Svetlin Aleksandrov
Pages 1604-1610

REVIEW

47. **Role of Salivary Nitric Oxide on Caries Status of Children with Down Syndrome**
Nita Naomi, Tania Saskianti, Ardianti Maartrina Dewi, Barnabas Bonardo, Alit Rahma Estu, Brian Maulani
Pages 1611-1616

TABLE OF CONTENTS / 2021; 14 (4)

REVIEW

48. Minimally Invasive Posterior Full Crown Competitors: Onlays, Occlusal Veneers, Vonlays and Endocrowns: A Review and Proposed Classification
Sherif Sultan, Hmoud Al Garni, Meshal Al Onazi, Kiran Gangi, Salah Al Otha, Fahad Al Ruwaili, Saif Al Anazi, Sultan Al Shammari, Abdul Aziz Fandi, Mostafa Fayad
Pages 1617-1622

REVIEW

49. Periodontitis Affects Skeletal Muscle Metabolism Through an Increase in Proinflammatory Cytokines
Risma Aprinda Kristanti, Taufan Bramantoro, Pratiwi Soesilawati, Erni Maduratna Setiawatie, Bambang Purwanto
Pages 1623-1628

REVIEW

50. Dental Articulators
Rawan Abu Zaghlan, Jamal Aqrabawi, Omar Al-Fatyan, Basmah AbuZaghllan
Pages 1629-1638

REVIEW

51. Teachers' Role in Regular and Special Need Students' Oral Health: A Narrative Review
Tania Saskianti, Mega Moeharyono Puteri, Barnabas Bonardo, Brian Maulani, Nita Naomi, Alit Rahma Estu
Pages 1639-1647

REVIEW

52. Strategically Important Features of the Influence of Sodium Hypochlorite on the Mechanical Properties of Dentin: A Systematic Review
Zurab Khabadze, Alexandra Kotelnikova, Mikhail Protsky, Oleg Mordanov, Ekaterina Faustova, Irina Nikolskaya, Shushanik Minosyan, Khadizhat Omarova, Ekaterina Shilyaeva, Daria Nazarova, Alena Kulikova
Pages 1648-1655

REVIEW

53. Comparison of Screw- and Cement-Retained Dental Implant from Biological, Clinical, and Technical Complications: A Systematic Review
Margaretha Elfridamanuela Samosir, Nada Fairuzia Soadi, Sheila Indrisavira, Hubban Nasution
Pages 1656-1663

REVIEW

54. Clinical Dental Risk Management: The Needs and Challenges
Didin Mirandani, Taufan Bramantoro, Dini Setyowati
Pages 1664-1666

MEDICINE

EXPERIMENTAL ARTICLE

55. Mangiferin Attenuates Doxorubicin-Induced Nephrotoxicity in Rats Through Reduction of Oxidative Stress
W. Arozal, A.J. Barinda, E.R. Monayo, R. Aulia
Pages 1667-1674

CLINICAL ARTICLE

56. Evaluation of 900 and 1800 Mhz Radiofrequency Radiation Emitted from Mobile Phones on Pregnant Women
Hava Bektas, Suleyman Dasdag, Mehmet Selcuk Bektas
Pages 1675-1683

JIDMR
Journal of International Dental and Medical Research / ISSN: 1309-100X

TABLE OF CONTENTS / 2021; 14 (4)

CLINICAL ARTICLE

57. Infrared Thermography as a Evaluation Metod an Athlete's Emotional Readiness
Kozhevnikova I. S., Anikina N.Yu., Pankov M. N., Plaksin V.A., Startseva L. F.
Pages 1684-1687

CLINICAL ARTICLE

58. Effect of Nutritional Literacy on Mother's Self Efficacy in Child Feeding (Effect of Nutritional Literacy on Mother's)
Maula Mar'atus Solikhah, Lita Heni Kusumawardani, Nurul Devi Ardiani, Annisa Cindy Nurul Afni, Atiek Murharyati1, Siti Nurjanah, Erinda Nur Pratiwi
Pages 1688-1693

CLINICAL ARTICLE

59. The Implementation of Theory of Planned Behavior in Identifying Behavioral Models of Nursing Documentation in "X" Hospital
Erna Dwi Wahyuni, Nursalam, Yulis Setiya Dewi, Amel Dawod Kamel
Pages 1694-1700

CLINICAL ARTICLE

60. Overview of Self-Care of Patients with Chronic Kidney Disease based on a Family Perspective
Virgianti Nur Faridah, Nursalam Nursalam, Ninuk Dian Kurniawati, Trijati Puspita Lestari, Nurul Hikmatul Qowi, Arifal Aris
Pages 1701-1704

CLINICAL ARTICLE

61. Factors Affecting Anemia Prevention Behavior in Pregnant Women based on Lawrence Green's Theory
Mira Triharini, Ayu Rahmawati, Aria Aulia Nastiti, Yulis Setiya Dewi, Smriti Kana Mani
Pages 1705-1708

CLINICAL ARTICLE

62. Cinematherapy-based Group Reminiscence on Older Adults' Quality of Life
Intan M. S. Batubara, Niken Y. Sari, Febriana S. Sari, Megan Eagle, Erlina Windyastuti, Erlyn Hapsari, Desy Widyastutik, Joko Santoso
Pages 1709-1714

CLINICAL ARTICLE

63. Comparison of Urogynecological Care in Hospitals Before and During the SARS CoV-2 Infection: The Case Approach in Dr. Soetomo Hospital Indonesia
Eighty Mardiyan Kurniawati, Hari Paraton, Gatut Hardiyanto, Azami Denas Azinar,Tri Hastono Setyo Hadi, Rizqy Rahmatyah, Nur Anisah Rahmawati
Pages 1715-1721

CLINICAL ARTICLE

64. Effectiveness of the "Emotion Recognition" Music Therapy Module in Schizophrenia Patients: A Quasi Experimental Study
Pangeran Ericson Arthur Siahaan, AAAA Kusumawardhani, Raden Irawati Ismail, Khamelia Malik
Pages 1722-1726

CLINICAL ARTICLE

65. Suboptimal Care on Maternal Near-Miss Cases: A Study from s Tertiary Referral Hospital in East Java, Indonesia
Hendy Hendarto, Hanifa Erlin Dharmayanti, Baksono Winardi, Budi Prasetyo, Muhammad Ardian Cahya Laksana, Muhammad Yusuf, Rizki Pranadyan, Pandu Hanindito Habibie, Bambang Trijanto, Erni Rosita Dewi, Alifina Izza, Mohammad Afzal Mahmood
Pages 1727-1735

JIDMR
Journal of International Dental and Medical Research / ISSN: 1309-100X

TABLE OF CONTENTS / 2021; 14 (4)

CLINICAL ARTICLE

66. Natural Killer Cell in Mild and Severe Systemic Lupus Erythematosus
Wita Kartika Nurani, Gatot Soegiarto, Yuliasih
Pages 1736-1742

CASE REPORT

67. Case Study: Health Workers' Perspective on Treatment of People with Post-Pasung Mental Disorder
Febriana S. Sari, Novy H.C. Daulima, Ice Y. Wardani, Intan M.S. Batubara, Ariyanti, Heni N. Kusumawati, Wahyuningsih Safitri, Yunita Wulandari
Pages 1743-1747

REVIEW

68. Diabetes and Metabolic Syndrome – Risk Factors for Covid-19 (literature review)
Nartikoeva M.I., Dzampaeva Zh.V., Takoeva E.A.
Pages 1748-1752

REVIEW

69. The Effectiveness of Tai Chi on Increasing Exercise Capacity and Quality of Life in Patients with Chronic Obstructive Pulmonary Disease: A Systematic review. (Taichi Effectivenes in COPD Patients)
Yosin Herloheti Pella, Hasanudin, Yoyok Bekti Prasetyo, Joel Rey Ugsang Acob, Yulis Setiya Dewi
Pages 1753-1759

REVIEW

70. Analysis Factor Affecting Continuous Learning Based Transformative Learning Theory and Digitalization to Improve Nurse Competencies: Literature Review
Domingos Soares, Nursalam
Pages 1760-1764

REVIEW

71. Energy-Drink and Adverse Kidney Function: A Review of Public Health Concern and Ethical Issue
Ira Suarilah, Chiu-Chu Lin, Ika Yuni Widyawati
Pages 1765-1770

The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

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Abstract

The use of acrylic and composite resin in daily dental practice is an essential material. Both contain plastic particles and are released by several oral activities. To assess the effect of Tooth-brushing activity, temperature, and pH changes of acrylic and composite resin microplastic release.

The samples were designed in a particular size and divided into three groups allocated randomly, soaked with and without artificial saliva for the above activities. The total time for each treatment was designed for 120 hours. The identification of microplastic released after treatment used a 10 µg/mL working concentration of Nile red and weighed in grams. The result was analyzed using one-way ANOVA (P<0.05) and paired t-test.

The study found that the acrylic and composite resin microplastics were identified differently in total artificial saliva after the treatment. The paired t-test showed significant differences for acrylic resin (p=0.015) in pH changes treatment and composite resin (p=0.035) in brushing activity. No significant differences were found between brushing activity, temperature, and pH changes treatment for acrylic (p=0.298) and composite resin (p=0.293) microplastic release. The result concluded that brushing activity, temperature, and pH changes might cause acrylic and composite resin microplastic release.

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Introduction

Microplastics are synthetic solid particle or polymeric matrix, with regular or irregular shape with size ranging from one µm to five mm, of either primary or secondary manufacturing origin, and water insoluble. A recent study has been included the nano size into the microplastic definition.[1] In dentistry, materials containing plastic are usually used, such as dental polymers for cavity filling, sealants, dentures, and abrasion in dental polish.[2]

Dental health care behavior is closely related to tooth-brushing and consumption of food and beverages.[3] Tooth-brushing activity was one of the most oral daily routines for maintaining oral hygiene. On the other side, tooth-brushing activity has an abrasive effect due to several factors such as inappropriate frequency, duration, and technique of tooth-brushing.[4] Some food and beverages might also cause pH and temperature changes that may influence the tooth surface.[5,6] When dental materials such as composite resin are placed as a tooth restoration and acrylic resin for dental prostheses, they are constantly exposed by tooth-brushing activity, pH, and temperature changes, and other factors that lead to an unfavorable effect on dental material.[6,7] The dental material may undergo degradation caused by various factors in the oral cavity, such as mechanical load, temperature, pH, and tooth-brushing activity.[8] The degradation of dental material assumed it may release microplastics. Microplastics can be carried into the human body through inhalation and ingestion from the oral cavity. Microplastics particles (<130 µm) can translocate into human tissues then trigger a localized immune response.[9]

Chronic inhalation of composite microparticles

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(<5 μm) and nanoparticles may provoke local and systemic toxicity. Nano-particles (<100nm) may also enter the blood or lymphatic system resulting in systemic toxicity.[10] Ingested microplastics can cause physical damage such as erosion or ulcers in the digestive tract.[11] Prata investigates the risk of airborne microplastics to human health then concluded that airborne microplastics could cause airway and interstitial lung diseases.[12] Mak et al. investigated the effect of microplastics in the intestine, and the result showed that microplastics accumulated in the intestine could increase the expression of cytochrome p450. Increased expression of cytochrome p450 is associated with modulation of metabolism when exposed to microplastics.[13]

Various methods for microplastics identification have been developed, ranging from the simplest method visually and separated manually without a microscope to the aid of fluorescent dye. Nile red was the commonly used fluorescent dye for microplastic identification.[14] Recent studies have supported the use of Nile red as an accurate stain for the rapid detection and quantification of microplastics. Maes *et al*. validated the use of Nile red with analysis using Fourier-Transform Infrared Spectrometer (FTIR) to verify the polymeric content of fluorescing particles, then concluded that Nile red might be used for rapid detection microplastic without the need for additional spectroscopic analysis. Maes *et al*. also suggested that Nile red alone is sufficient to identify a particle as polymeric.[15,16]

The degradation of dental material assumes may release microplastics, but to date, there is still a lack of evidence acrylic and composite resin release microplastic after tooth-brushing, temperature, and pH treatment. Therefore, this study was conducted to assess the effect of between tooth-brushing activity, temperature, and pH to acrylic and composite resin microplastic release.

Materials and methods

An experimental laboratory study was carried out with a sample size of 20, divided equally between acrylic and composite resins groups. Tooth-brushing activity simulated with toothbrush simulator, temperature simulated with 5°C and 55°C cycle, and pH simulated with pH 4 and pH 7 cycle. In this study, a brushing simulator carried out using Oral-B 3D White electric toothbrush (Oral-B, USA). Temperature simulation carried out using refrigerator (LG, Korea) for 5°C and incubator (JISICO, Korea) for 55°C. pH simulation carried out using buffer solution pH 4 and pH 7 (Merck, Germany)

Sample preparation

Both acrylic and composite resin were made in sizes 10 mm x 10 mm x 3 mm. Three specimens of acrylic and composite resin were mounted with dental stone into a beaker glass for fixation, then pouring 10 mL of artificial saliva for brushing activity treatment. Three specimens of acrylic and composite resin were put in beaker glass then pouring 10 mL of artificial saliva for temperature treatment. Three specimens of acrylic and composite resin were put in beaker glass for pH treatment.

The remaining samples of acrylic and composite resin were scraped using a scalpel. The particles obtained were then kept into a beaker glass before adding 10 mL of artificial saliva, and was treated as a positive control.[18]

Tooth-brushing experiment

Seven Oral-B 3D White electric toothbrushes (Oral-B, USA) used. Each toothbrush work on one specimen. The toothbrush's handle holds a universal table vise (Krisbow, Indonesia) to ensure the toothbrush stays in place. A force of two Newton was applied for tooth-brushing. The cleaning force was generated using a 200 g Chrome Plating Calibration tied with dental floss (P&G, USA).

Specimen were randomly allocated. Three samples of acrylic and composite resin were assigned to each toothbrush. The remaining toothbrush was assigned to be the negative control sample. Negative control was the mounted dental stone into a beaker glass filled with 10 mL of artificial saliva without any acrylic or composite resin sample. The total of tooth-brushing stroke was equivalent to five years of tooth-brushing, 120 seconds twice a day of all teeth.[17] The total-brushing time was then designed as 120 hours.

Temperature treatment

Specimen were randomly allocated. Three samples of acrylic and composite resin were put in the refrigerator at 5°C treatment for 60 hours along with the negative control sample. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample. After 60 hours, the sample was moved into the incubator set at 55°C

for 60 hours.

pH treatment

Specimen were randomly allocated. Buffer solution with pH 4 was poured 10 mL into the prepared three samples of acrylic and composite resin for 40 hours. After 40 hours, move the sample into another beaker glass then pour 10 mL pH 7 buffer solution for 80 hours. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample.

Microplastic identification and measurement

After the treatment is completed, the artificial saliva of three acrylic resin samples was collected and done the same thing as composite resin samples for each treatment. The microplastics identification was conducted by dripping Nile red solution (TCi, Japan) into the artificial saliva (working concentration 10 g/mL using n-Hexane solvent) and then incubated for 30 minutes. After the incubation period, artificial saliva contained Nile red is filtered using filter paper (Whatman, grade 934-AH, 55 mm diameter, 1,5 mm pore, GE Healthcare, USA), and the filter paper was air-dried for five minutes and examined under a fluorescent microscope (Zeiss Axio Vert A1, Germany) [Figure 1].[16,19] Microplastic measurement was done by weighing the filter papers in grams.

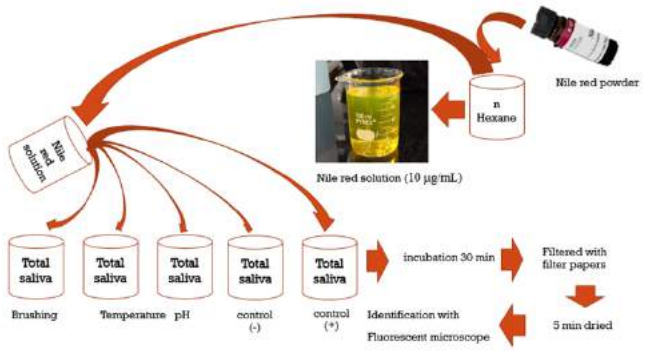

Figure 1. Microplastic identification steps.

Statistical Analysis

All statistical analysis was performed using statistical software SPSS for Windows version 22 (IBM, USA). After treatment, the among treatments were compared using a one-way ANOVA test. Inter-treatment comparison was conducted using a pair sample t-test. For all test, the level of significance chosen was $p< 0.05$.

Result

Identification microplastics was carried out using a fluorescent microscope on blue light filters (excitation wavelength 365 nm and emission wavelength 445 nm) and green light filters (excitation wavelength 450 nm and emission wavelength 515 nm). First, identification was conducted for the positive control sample and negative control sample in artificial saliva. The result showed that microplastics are found on the positive control sample, while no microplastics are found on the negative control sample. The Nile red-stained acrylic and composite microplastics particles were visible in both blue and green fluorescence [Figure 2].

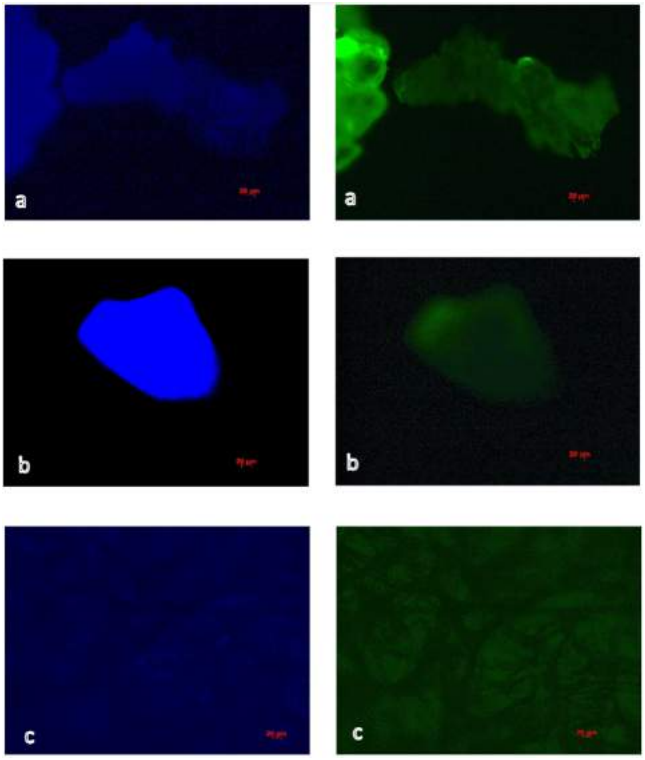

Figure 2. Microplastics identification using a fluorescence microscope with different filters (blue and green light) on positive and negative control. (a) positive control acrylic resin, (b) positive control composite resin, (c) negative control.

Microplastics found on positive control confirmed that Nile red could identify the acrylic and composite resin in artificial saliva. Furthermore, acrylic and composite resin samples of total artificial saliva were conducted after the tooth-brushing, temperature, and pH treatment. The result has shown that acrylic and

composite resin microplastics were found in total artificial saliva after the tooth-brushing, temperature, and pH treatment [Figure 3].

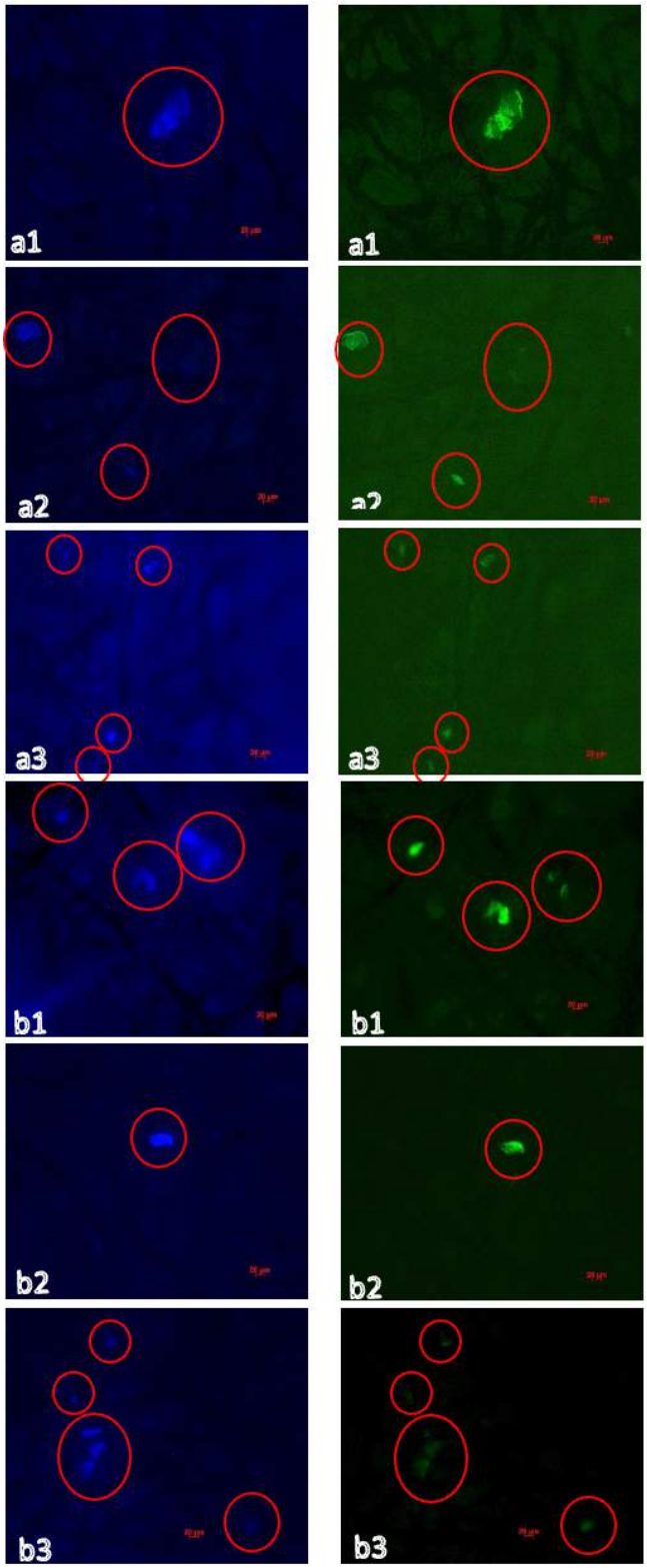

Figure 3. Microplastics identification using fluorescence microscope on samples with different filters (blue and green light). (a1) acrylic resin microplastics after brushing treatment, (a2) acrylic resin microplastics after temperature treatment, (a3) acrylic resin microplastics after pH treatment, (b1) composite resin microplastics after brushing treatment, (b2) composite resin microplastics after brushing treatment, (b3) composite resin microplastics after brushing treatment.

Acrylic and composite resin sample were weighed before and after the tooth-brushing, temperature, and pH treatment. The result shown there is differences in the weight of acrylic and composite resin before and after treatment, it indicates acrylic and composite resin sustained degradation after treatments and release the microplastic [Figure 4].

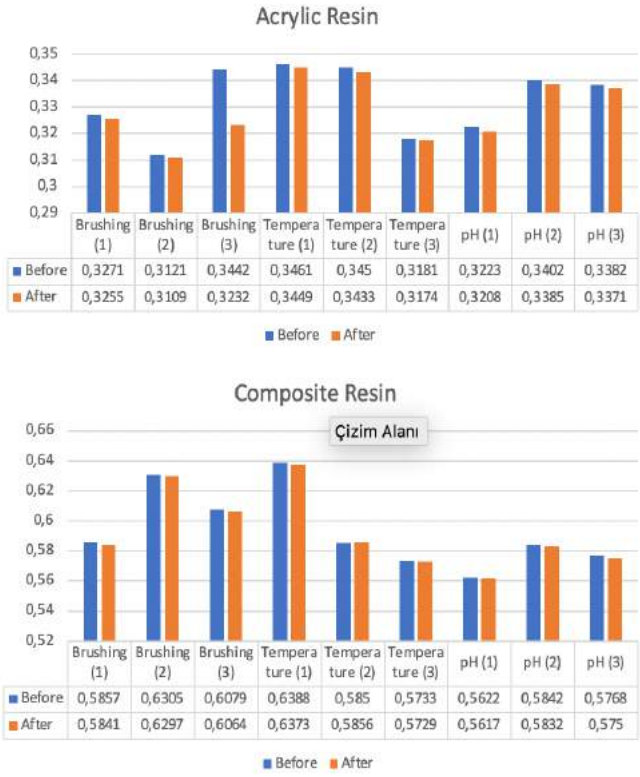

Figure 4. Acrylic and composite resin samples weight (in grams).

Data from each treatment were analyzed using paired t-test and showed significant differences only at pH treatment for acrylic resin and brushing treatment for composite resin (Table 1). The significant differences show there are differences in the amount of microplastic released after the treatment.

Sample	Treatment	Mean		SD		p
		Before	After	Before	After	
Acrylic resin	Brushing	0.3278	0.3198	0.0160	0.0078	0.349
Acrylic resin	Temperature	0.3364	0.3352	0.0158	0.0154	0.053
Acrylic resin	pH	0.3335	0.3321	0.0098	0.0098	0.015*
Composite resin	Brushing	0.6080	0.6067	0.0224	0.0228	0.035*
Composite resin	Temperature	0.5990	0.5986	0.0349	0.0341	0.549
Composite resin	pH	0.5744	0.5733	0.0111	0.0108	0.101

Table 1. Paired t-test among variables.

To determine the significant differences between treatments, the one-way ANOVA test was carried out. It was found that there were no significant differences between the treatments (p=0.298 for acrylic resin and p=0.293 for composite resin). Quantification of the microplastics were done by weighing the dried filter papers to obtain the amount of microplastic release from acrylic and composite resin. The result has showed that acrylic resin released 0.0595 g microplastics after brushing treatment, 0.0419 g microplastics after temperature treatment, 0.0439 g microplastic after pH treatment, Composite resin released 0.0337 g microplastics after brushing treatment, 0.0472 g microplastics after temperature treatment, 0.0442 g microplastics after pH treatment.

Discussion

Acrylic and composite resins were plastics-based dental materials.[2] Social *et al*. state that brushing treatment on composite resin will cause surface degradation.[20] Szczesio-Wlodarczyk *et al*. state that various factors, such as saliva, mastication, brushing activity, temperature, and pH, will cause degradation to acrylic resin and release toxic components resulted from degradation.[8] This study reveals acrylic and composite resin encounter weight loss after the brushing, temperature, and pH treatment in line with the above statement. This finding indicated acrylic and composite resin sustained degradation after brushing treatment and release the microplastic.

This study successfully found microplastic released from the acrylic and composite resin after brushing, temperature, and pH treatment with Nile red identification. The result was confirming our notion that the degradation of plastic base dental material will release microplastic. The microplastics found in this study are secondary. Secondary microplastics are plastics particles that result from wear, tear, abrasion, breakdown, and degradation of large plastic debris.[21]

Utilization of Nile red dyes to identify microplastic in this study in line with the research conducted by Shim *et al*., which identified the microplastic using the Nile red and fluorescence microscope.[22] Generally, microplastics can be identified using a conventional microscope. However, this method has low reliability, especially on small, transparent, and fiber-type particles.[23,24]

Micron-sized microplastics are usually identified using Raman spectroscopy and Fourier transform infrared spectroscopy. Both methods require repeated experiments to obtain reliable results because of the small and wet particle size, expensive equipment, and time-consuming.[20,25] Nile red has good reliability and sensitivity for microplastic identification. Gagne *et al*. stated that Nile red could detect microplastics up to 50-100nm nanoparticles in his research using transparent polystyrene material.[26] Maes *et al*. also suggested that microplastic identification using Nile red alone was sufficient.[15]

Our study found the highest amount of microplastic released after the treatments were 0.0595 g. According to a recent study, an estimated 80 g per day of microplastic entered the human body. Microplastic can also be found in food and drink (either tap water or bottled water). Approximately 0.44 MPs/g of nano and microplastics were found in sugar, 0.11 MPs/g in salt, 0.03 MPs/g in alcohol, and 0.09 MPs/g in bottled water.[27] Furthermore, microplastics could enter the human body through inhalation.[28] Because of the many sources of microplastics that can enter the human body, expectantly microplastics will receive more attention to be evaluated regarding the risks of microplastics to the environment and human health.

The effect of acrylic and composite resin microplastic on the environment and human health is unknown. However, generally microplastics consumed in the body can cause tissue inflammation, cell proliferation, necrosis, and compromise of immune cells.[29] Yong *et al*. stated lack of in vivo data on the effects of microplastics on humans to date, but it is known that microplastics in fish and mice caused inflammation, oxidative stress, and metabolic changes, and in fish, the microplastic could cause changes in the brain.[28] Prietl *et al*. stated that 20 nm microplastic was toxic to the human

monocytic cell line (U937) and human monocytic cell line (THP-1) and stimulated IL-8 and caused an increase in oxidative stress in THP-1.[30] Dong *et al*. stated that microplastics cause cytotoxicity, oxidative stress, and inflammatory responses in human lung epithelial cells (BEAS-2B) and increase the risk of chronic obstructive pulmonary disease (COPD).[31] Poma *et al*. stated that microplastics with a size of 100 nm at 5-75 g/mL can stimulate the production of reactive oxygen species (ROS) are genotoxic and cause DNA damage in human fibroblast cells (Hs27).[32]

The limitation of this study was the brushing treatment conducted in this study significantly depends on the battery lifespan. Therefore, the future specific tools could be used to overcome the limitation.

Conclusion

Based on the result of this study, it can be concluded that the tooh-brushing activity, temperature, and pH changes might cause microplastic release in different number and there were no significant differences among treatment groups in microplastic release from the acrylic and composite resin. Further study to evaluate the acrylic and composite resin microplastics in vitro is recommended.

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Declaration of Interest

The authors report no conflict of interest.

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The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

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The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

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Abstract

The use of acrylic and composite resin in daily dental practice is an essential material. Both contain plastic particles and are released by several oral activities. To assess the effect of Tooth-brushing activity, temperature, and pH changes of acrylic and composite resin microplastic release.

The samples were designed in a particular size and divided into three groups allocated randomly, soaked with and without artificial saliva for the above activities. The total time for each treatment was designed for 120 hours. The identification of microplastic released after treatment used a 10 µg/mL working concentration of Nile red and weighed in grams. The result was analyzed using one-way ANOVA (P<0.05) and paired t-test.

The study found that the acrylic and composite resin microplastics were identified differently in total artificial saliva after the treatment. The paired t-test showed significant differences for acrylic resin (p=0.015) in pH changes treatment and composite resin (p=0.035) in brushing activity. No significant differences were found between brushing activity, temperature, and pH changes treatment for acrylic (p=0.298) and composite resin (p=0.293) microplastic release. The result concluded that brushing activity, temperature, and pH changes might cause acrylic and composite resin microplastic release.

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Introduction

Microplastics are synthetic solid particle or polymeric matrix, with regular or irregular shape with size ranging from one µm to five mm, of either primary or secondary manufacturing origin, and water insoluble. A recent study has been included the nano size into the microplastic definition.[1] In dentistry, materials containing plastic are usually used, such as dental polymers for cavity filling, sealants, dentures, and abrasion in dental polish.[2]

Dental health care behavior is closely related to tooth-brushing and consumption of food and beverages.[3] Tooth-brushing activity was one of the most oral daily routines for maintaining oral hygiene. On the other side, tooth-brushing activity has an abrasive effect due to several factors such as inappropriate frequency, duration, and technique of tooth-brushing.[4] Some food and beverages might also cause pH and temperature changes that may influence the tooth surface.[5,6] When dental materials such as composite resin are placed as a tooth restoration and acrylic resin for dental prostheses, they are constantly exposed by tooth-brushing activity, pH, and temperature changes, and other factors that lead to an unfavorable effect on dental material.[6,7] The dental material may undergo degradation caused by various factors in the oral cavity, such as mechanical load, temperature, pH, and tooth-brushing activity.[8] The degradation of dental material assumed it may release microplastics. Microplastics can be carried into the human body through inhalation and ingestion from the oral cavity. Microplastics particles (<130 µm) can translocate into human tissues then trigger a localized immune response.[9]

Chronic inhalation of composite microparticles

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(<5 µm) and nanoparticles may provoke local and systemic toxicity. Nano-particles (<100nm) may also enter the blood or lymphatic system resulting in systemic toxicity.[10] Ingested microplastics can cause physical damage such as erosion or ulcers in the digestive tract.[11] Prata investigates the risk of airborne microplastics to human health then concluded that airborne microplastics could cause airway and interstitial lung diseases.[12] Mak et al. investigated the effect of microplastics in the intestine, and the result showed that microplastics accumulated in the intestine could increase the expression of cytochrome p450. Increased expression of cytochrome p450 is associated with modulation of metabolism when exposed to microplastics.[13]

Various methods for microplastics identification have been developed, ranging from the simplest method visually and separated manually without a microscope to the aid of fluorescent dye. Nile red was the commonly used fluorescent dye for microplastic identification.[14] Recent studies have supported the use of Nile red as an accurate stain for the rapid detection and quantification of microplastics. Maes *et al*. validated the use of Nile red with analysis using Fourier-Transform Infrared Spectrometer (FTIR) to verify the polymeric content of fluorescing particles, then concluded that Nile red might be used for rapid detection microplastic without the need for additional spectroscopic analysis. Maes *et al*. also suggested that Nile red alone is sufficient to identify a particle as polymeric.[15,16]

The degradation of dental material assumes may release microplastics, but to date, there is still a lack of evidence acrylic and composite resin release microplastic after tooth-brushing, temperature, and pH treatment. Therefore, this study was conducted to assess the effect of between tooth-brushing activity, temperature, and pH to acrylic and composite resin microplastic release.

Materials and methods

An experimental laboratory study was carried out with a sample size of 20, divided equally between acrylic and composite resins groups. Tooth-brushing activity simulated with toothbrush simulator, temperature simulated with 5°C and 55°C cycle, and pH simulated with pH 4 and pH 7 cycle. In this study, a brushing simulator carried out using Oral-B 3D White electric toothbrush (Oral-B, USA). Temperature simulation carried out using refrigerator (LG, Korea) for 5°C and incubator (JISICO, Korea) for 55°C. pH simulation carried out using buffer solution pH 4 and pH 7 (Merck, Germany)

Sample preparation

Both acrylic and composite resin were made in sizes 10 mm x 10 mm x 3 mm. Three specimens of acrylic and composite resin were mounted with dental stone into a beaker glass for fixation, then pouring 10 mL of artificial saliva for brushing activity treatment. Three specimens of acrylic and composite resin were put in beaker glass then pouring 10 mL of artificial saliva for temperature treatment. Three specimens of acrylic and composite resin were put in beaker glass for pH treatment.

The remaining samples of acrylic and composite resin were scraped using a scalpel. The particles obtained were then kept into a beaker glass before adding 10 mL of artificial saliva, and was treated as a positive control.[18]

Tooth-brushing experiment

Seven Oral-B 3D White electric toothbrushes (Oral-B, USA) used. Each toothbrush work on one specimen. The toothbrush's handle holds a universal table vise (Krisbow, Indonesia) to ensure the toothbrush stays in place. A force of two Newton was applied for tooth-brushing. The cleaning force was generated using a 200 g Chrome Plating Calibration tied with dental floss (P&G, USA).

Specimen were randomly allocated. Three samples of acrylic and composite resin were assigned to each toothbrush. The remaining toothbrush was assigned to be the negative control sample. Negative control was the mounted dental stone into a beaker glass filled with 10 mL of artificial saliva without any acrylic or composite resin sample. The total of tooth-brushing stroke was equivalent to five years of tooth-brushing, 120 seconds twice a day of all teeth.[17] The total-brushing time was then designed as 120 hours.

Temperature treatment

Specimen were randomly allocated. Three samples of acrylic and composite resin were put in the refrigerator at 5°C treatment for 60 hours along with the negative control sample. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample. After 60 hours, the sample was moved into the incubator set at 55°C

for 60 hours.

pH treatment

Specimen were randomly allocated. Buffer solution with pH 4 was poured 10 mL into the prepared three samples of acrylic and composite resin for 40 hours. After 40 hours, move the sample into another beaker glass then pour 10 mL pH 7 buffer solution for 80 hours. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample.

Microplastic identification and measurement

After the treatment is completed, the artificial saliva of three acrylic resin samples was collected and done the same thing as composite resin samples for each treatment. The microplastics identification was conducted by dripping Nile red solution (TCi, Japan) into the artificial saliva (working concentration 10 g/mL using n-Hexane solvent) and then incubated for 30 minutes. After the incubation period, artificial saliva contained Nile red is filtered using filter paper (Whatman, grade 934-AH, 55 mm diameter, 1,5 mm pore, GE Healthcare, USA), and the filter paper was air-dried for five minutes and examined under a fluorescent microscope (Zeiss Axio Vert A1, Germany) [Figure 1].[16,19] Microplastic measurement was done by weighing the filter papers in grams.

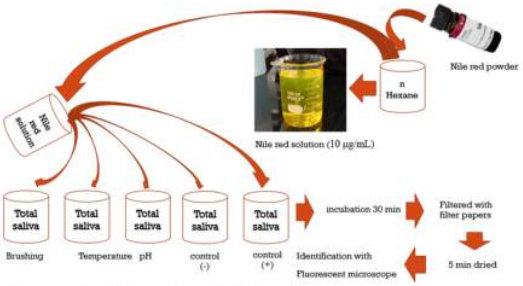

Figure 1. Microplastic identification steps.

Statistical Analysis

All statistical analysis was performed using statistical software SPSS for Windows version 22 (IBM, USA). After treatment, the among treatments were compared using a one-way ANOVA test. Inter-treatment comparison was conducted using a pair sample t-test. For all test, the level of significance chosen was $p< 0.05$.

Result

Identification microplastics was carried out using a fluorescent microscope on blue light filters (excitation wavelength 365 nm and emission wavelength 445 nm) and green light filters (excitation wavelength 450 nm and emission wavelength 515 nm). First, identification was conducted for the positive control sample and negative control sample in artificial saliva. The result showed that microplastics are found on the positive control sample, while no microplastics are found on the negative control sample. The Nile red-stained acrylic and composite microplastics particles were visible in both blue and green fluorescence [Figure 2].

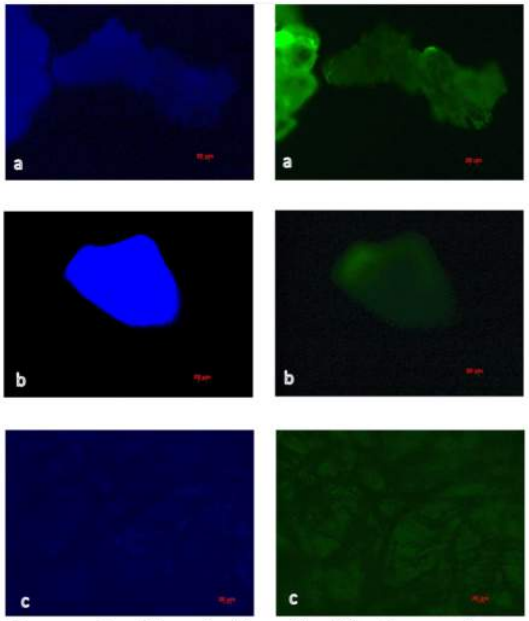

Figure 2. Microplastics identification using a fluorescence microscope with different filters (blue and green light) on positive and negative control. (a) positive control acrylic resin, (b) positive control composite resin, (c) negative control.

Microplastics found on positive control confirmed that Nile red could identify the acrylic and composite resin in artificial saliva. Furthermore, acrylic and composite resin samples of total artificial saliva were conducted after the tooth-brushing, temperature, and pH treatment. The result has shown that acrylic and

composite resin microplastics were found in total artificial saliva after the tooth-brushing, temperature, and pH treatment [Figure 3].

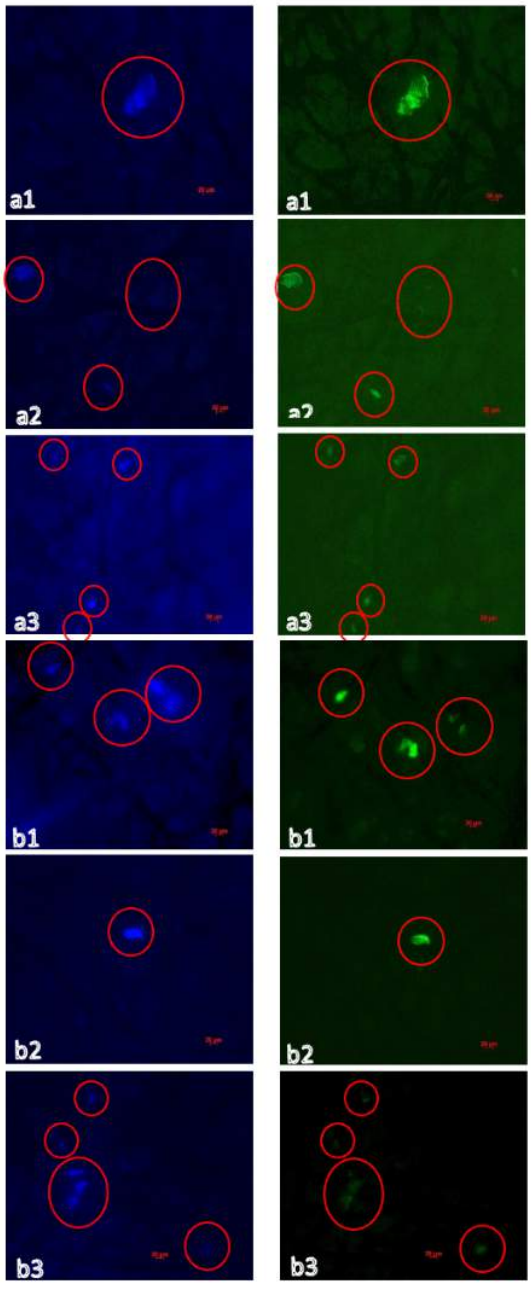

Figure 3. Microplastics identification using fluorescence microscope on samples with different filters (blue and green light). (a1) acrylic resin microplastics after brushing treatment, (a2) acrylic resin microplastics after temperature treatment, (a3) acrylic resin microplastics after pH treatment, (b1) composite resin microplastics after brushing treatment, (b2) composite resin microplastics after brushing treatment, (b3) composite resin microplastics after brushing treatment.

Acrylic and composite resin sample were weighed before and after the tooth-brushing, temperature, and pH treatment. The result shown there is differences in the weight of acrylic and composite resin before and after treatment, it indicates acrylic and composite resin sustained degradation after treatments and release the microplastic [Figure 4].

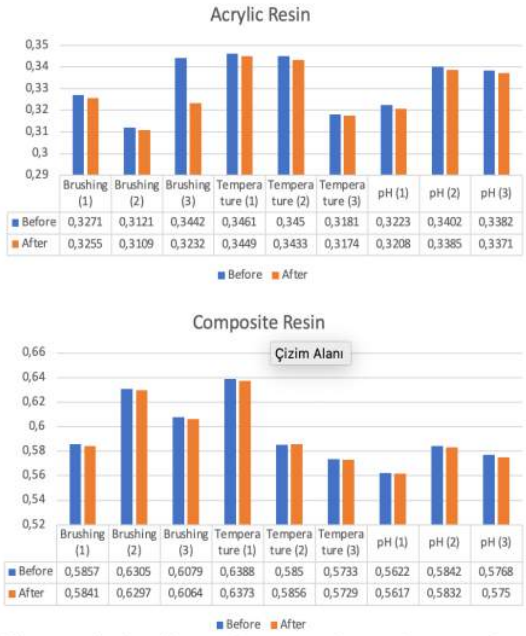

Figure 4. Acrylic and composite resin samples weight (in grams).

Data from each treatment were analyzed using paired t-test and showed significant differences only at pH treatment for acrylic resin and brushing treatment for composite resin (Table 1). The significant differences show there are differences in the amount of microplastic released after the treatment.

Sample	Treatment	Mean		SD		p
		Before	After	Before	After	
Acrylic resin	Brushing	0.3278	0.3198	0.0160	0.0078	0.349
Acrylic resin	Temperature	0.3364	0.3352	0.0158	0.0154	0.053
Acrylic resin	pH	0.3335	0.3321	0.0098	0.0098	0.015*
Composite resin	Brushing	0.6080	0.6067	0.0224	0.0228	0.035*
Composite resin	Temperature	0.5990	0.5986	0.0349	0.0341	0.549
Composite resin	pH	0.5744	0.5733	0.0111	0.0108	0.101

Table 1. Paired t-test among variables.

To determine the significant differences between treatments, the one-way ANOVA test was carried out. It was found that there were no significant differences between the treatments (p=0.298 for acrylic resin and p=0.293 for composite resin). Quantification of the microplastics were done by weighing the dried filter papers to obtain the amount of microplastic release from acrylic and composite resin. The result has showed that acrylic resin released 0.0595 g microplastics after brushing treatment, 0.0419 g microplastics after temperature treatment, 0.0439 g microplastic after pH treatment, Composite resin released 0.0337 g microplastics after brushing treatment, 0.0472 g microplastics after temperature treatment, 0.0442 g microplastics after pH treatment.

Discussion

Acrylic and composite resins were plastics-based dental materials.[2] Social *et al*. state that brushing treatment on composite resin will cause surface degradation.[20] Szczesio-Wlodarczyk *et al*. state that various factors, such as saliva, mastication, brushing activity, temperature, and pH, will cause degradation to acrylic resin and release toxic components resulted from degradation.[8] This study reveals acrylic and composite resin encounter weight loss after the brushing, temperature, and pH treatment in line with the above statement. This finding indicated acrylic and composite resin sustained degradation after brushing treatment and release the microplastic.

This study successfully found microplastic released from the acrylic and composite resin after brushing, temperature, and pH treatment with Nile red identification. The result was confirming our notion that the degradation of plastic base dental material will release microplastic. The microplastics found in this study are secondary. Secondary microplastics are plastics particles that result from wear, tear, abrasion, breakdown, and degradation of large plastic debris.[21]

Utilization of Nile red dyes to identify microplastic in this study in line with the research conducted by Shim *et al*., which identified the microplastic using the Nile red and fluorescence microscope.[22] Generally, microplastics can be identified using a conventional microscope. However, this method has low reliability, especially on small, transparent, and fiber-type particles.[23,24]

Micron-sized microplastics are usually identified using Raman spectroscopy and Fourier transform infrared spectroscopy. Both methods require repeated experiments to obtain reliable results because of the small and wet particle size, expensive equipment, and time-consuming.[20,25] Nile red has good reliability and sensitivity for microplastic identification. Gagne *et al*. stated that Nile red could detect microplastics up to 50-100nm nanoparticles in his research using transparent polystyrene material.[26] Maes *et al*. also suggested that microplastic identification using Nile red alone was sufficient.[15]

Our study found the highest amount of microplastic released after the treatments were 0.0595 g. According to a recent study, an estimated 80 g per day of microplastic entered the human body. Microplastic can also be found in food and drink (either tap water or bottled water). Approximately 0.44 MPs/g of nano and microplastics were found in sugar, 0.11 MPs/g in salt, 0.03 MPs/g in alcohol, and 0.09 MPs/g in bottled water.[27] Furthermore, microplastics could enter the human body through inhalation.[28] Because of the many sources of microplastics that can enter the human body, expectantly microplastics will receive more attention to be evaluated regarding the risks of microplastics to the environment and human health.

The effect of acrylic and composite resin microplastic on the environment and human health is unknown. However, generally microplastics consumed in the body can cause tissue inflammation, cell proliferation, necrosis, and compromise of immune cells.[29] Yong *et al*. stated lack of in vivo data on the effects of microplastics on humans to date, but it is known that microplastics in fish and mice caused inflammation, oxidative stress, and metabolic changes, and in fish, the microplastic could cause changes in the brain.[28] Prietl *et al*. stated that 20 nm microplastic was toxic to the human

monocytic cell line (U937) and human monocytic cell line (THP-1) and stimulated IL-8 and caused an increase in oxidative stress in THP-1.[30] Dong *et al.* stated that microplastics cause cytotoxicity, oxidative stress, and inflammatory responses in human lung epithelial cells (BEAS-2B) and increase the risk of chronic obstructive pulmonary disease (COPD).[31] Poma *et al.* stated that microplastics with a size of 100 nm at 5-75 g/mL can stimulate the production of reactive oxygen species (ROS) are genotoxic and cause DNA damage in human fibroblast cells (Hs27).[32]

The limitation of this study was the brushing treatment conducted in this study significantly depends on the battery lifespan. Therefore, the future specific tools could be used to overcome the limitation.

Conclusion

Based on the result of this study, it can be concluded that the tooh-brushing activity, temperature, and pH changes might cause microplastic release in different number and there were no significant differences among treatment groups in microplastic release from the acrylic and composite resin. Further study to evaluate the acrylic and composite resin microplastics in vitro is recommended.

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Declaration of Interest

The authors report no conflict of interest.

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