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Antioxidant Activity of Chitosan Nanoparticles with Chlorhexidine- An In vitro Study

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ABSTRACT

Introduction: Oxygen, an essential ingredient for life, has the ability to both enhance and degrade physical health. The deadly effects of oxygen were unknown until Gershman's free radical hypothesis of oxygen toxicity was published in 1954.OS and RNS are widely known for serving a dual function as both harmful and beneficial species.ROS are extremely reactive molecules produced by the metabolism of oxygen.During oxidative stress, ROS levels rise dramatically, causing severe damage to cell components. ROS are cytotoxic and have been linked to the pathogenesis of a number of human disorders.Chitosan has been shown to effectively scavenge various types of radical species, indicating a broad range of uses.

Materials and Methods: The chitosan was obtained and plain chitosan with chlorhexidine, chitosan nanoparticles with chlorhexidine was prepared.To measure the antioxidant activity 10 µL-50µL (increasing the quantity by 10µL) of the nanoparticles were added to five separate test tubes. To every test tube, DPPH of 1ml quantity was added. Next, a 50% methanol solution containing 10 µL-50µL (increasing the quantity by 10µL) chitosan solution was added to the five test tubes containing chitosan solution respectively and % of inhibition was calculated

Results: Nanochitosan with chlorhexidine shows higher anti-oxidant activity when compared to plain chitosan.Its activity increases with increase in dosage.Antioxidant activity of chitosan nanoparticles and plain chitosan exhibit a pattern of increasing activity with increasing concentration, with the highest percentage of inhibition observed at a concentration of 50 µl. The antioxidant activity of the nanoparticles also increases as the dosage increases.

Conclusion: The use of this novel irrigant in the field of endodontics would reduce the postoperative pain and overall improve the experience of the patients undergoing endodontic treatment in the future.

Keywords: *Endodontic Irrigants, Root Canal Treatment,Chitosan, Chitosan nanoparticles , Natural Irrigant, Anti oxidant, free radical species, oxidative stress*

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INTRODUCTION

Oxygen, an essential ingredient for life, has the ability to both enhance and degrade physical health. The deadly effects of oxygen were unknown until Gershman's free radical hypothesis of oxygen toxicity was published in 1954, which says that oxygen toxicity is caused by partly reduced forms of oxygen.(1,2) Free radicals are produced when cells utilise oxygen to generate energy in the form of ATP in the mitochondria.(3,4)(5) Free radicals are chemically active atoms with a charge caused by an excess or deficiency of electrons. They can be reactive oxygen or reactive nitrogen species (ROS or RNS).(6,7)(8)

OS and RNS are widely known for serving a dual function as both harmful and beneficial species.(9,10) ROS are extremely reactive molecules produced by the metabolism of oxygen.(11–14) Some of these ROS serve constructive functions in cell physiology in vivo; nevertheless, they may also cause significant damage to cell membranes and DNA by producing membrane lipid peroxidation, reduced membrane fluidity, and DNA alterations that lead to cancer, degenerative, and other disorders.(15,16)(17)

During oxidative stress, ROS levels rise dramatically, causing severe damage to cell components. ROS are cytotoxic and have been linked to the pathogenesis of a number of human disorders.(9,10) Thus, ROS can kill bacteria while also destroying infected host tissues.(18) ROS scavengers/antioxidants are critical for preventing and regulating human illnesses by preventing ROS production. By interacting with oxygen, antioxidants assist to avoid the damaging effects of ROS.(19,20) Antioxidants stop free radicals from stealing electrons by giving one of their electrons. Because antioxidants are stable in any form, giving an electron does not cause them to become free radicals. (21)

Metabolic reactions in the human body produce reactive free radicles which can cause oxidative stress by damaging biomolecules like lipids, proteins, carbohydrates, and DNA.(22)(23),(24) The sources of ROS include oxygen-derived free radicals (like superoxide, hydroxyl radical, and nitric oxide) and catalytically oxygen compounds (like hydrogen peroxide, peroxynitrite singlet oxygen and hypochlorite), which are primarily generated by mitochondria during physiological and pathological states. ROS can also be produced externally such as, exposure to ozone, hyperoxia, ionising radiation, and heavy metal ions(22,25)

Several enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, play a role in the cellular defence system against ROS-mediated cellular damage during cell metabolism.(26) However, excessive production of ROS can overwhelm the defence mechanism and lead to oxidative stress.(27) Oxidative stress is associated with a range of health conditions, including aging, cancer, inflammation, hypertension,myocardial infraction, Alzheimer's disease, Parkinson's disease. (28–31)) Therefore, the interest in antioxidant agents is increasing as a potential solution to combat oxidative stress

As a result, numerous scientists have been paying a lot of attention to chitosan's antioxidant function. Chitosan has been shown to effectively scavenge various types of radical species, indicating a broad range of uses. Therefore, the aim of this study is to understand the antioxidant activity of plain chitosan with Chlorhexidine and chitosan nanoparticles with Chlorhexidine.

MATERIALS AND METHODS

Chitosan Synthesis

For this study the chitosan powder was obtained from dried exoskeleton of marine shrimps.

Preparation of Chitosan Nanoparticles

To prepare a solution of chitosan for use in a coating process,the dissolution mixture (500 mg of chitosan and 50 ml of 1% acetic acid solution) is stirred to get a clear solution at room temperature (1000 rpm for 25 minutes). To achieve a neutral pH of 5,the prepared solution was sonicated and titrated by adding either NaOH or HCl. The solution was then filtered using a 0.2 µ mesh. For the coating process, a solution of 5 ml of nano-magnetic solution was added to 75 mL of deionized water and sonicated for 10 minutes.The nanoparticle solution is further sonicated for 5mins until a clear solution is obtained.

Preparation of Nanochitosan with Chlorhexidine solution

50ml of 2% Chlorhexidiene was added to 50 ml of the prepared nano chitosan solution. The

resulting solution was sonicated for 10 mins until the solution was clear.

Preparation of plain chitosan nanoparticles with chlorhexidine

To prepare a solution of chitosan and chlorhexidine, the dissolution mixture (500 mg of chitosan and 50 ml of 1% acetic acid solution) is stirred to get a clear solution at room temperature (1000 rpm for 25 minutes). The prepared solution was titrated and sonicated by adding either NaOH or HCl solution until a pH of 5 was achieved. The solution was then filtered using a 0.2 µ mesh. Next, 50 ml of 2% chlorhexidine was added to 50 ml of the prepared chitosan solution, and the resulting solution was sonicated for 10 minutes until it became clear.

Antioxidant Activity

Test Group 1

To measure the antioxidant activity of the nanoparticles, 10 µL-50µL (increasing the quantity by 10µL) of the nanoparticles were added to five separate test tubes. To every test tube, DPPH of 1ml quantity was added. Next, a 50% methanol solution containing dilutions from 10- 50 µL of nanoparticles was incorporated to the five test tubes containing chitosan nanoparticle solution respectively

Test Group 2

To measure the antioxidant activity of the nanoparticles, 10 µL-50µL (increasing the quantity by 10µL) of the nanoparticles were added to five separate test tubes. To every test tube, DPPH of 1ml quantity was added. Next, a 50% methanol solution containing 10 µL-50µL (increasing the quantity by 10µL) chitosan solution was added to the five test tubes containing chitosan solution respectively

For the control

A 2 mL methanol solution was added to a 1 mL solution of DPPH.

Standard Group

As a control, ascorbic acid was utilised.

% Inhibition was calculated using the following formula

% of inhibition is Control Absorbance − Sample Absorbance /Control Absorbance x 100

RESULTS

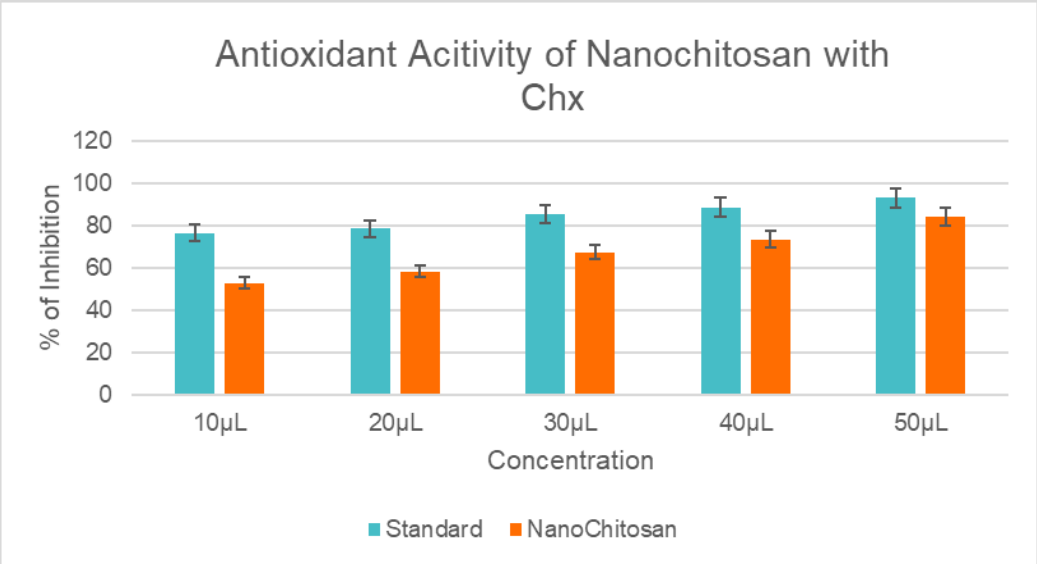

FIG 1: Antioxidant activity of chitosan nanoparticles exhibit a pattern of increasing activity with increasing concentration, with the highest percentage of inhibition observed at a concentration of 50 µl. The antioxidant activity of the nanoparticles also increases as the dosage increases.

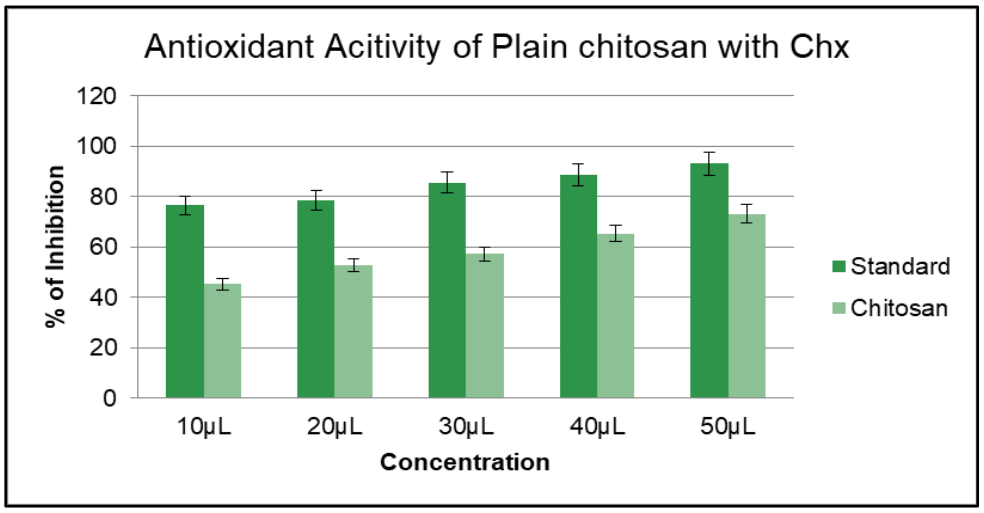

FIG 2: Antioxidant activity of plain chitosan shows that as the concentration of nanoparticles increases, their antioxidant activity also increases in a gradual manner. The highest percentage of inhibition, which refers to the ability of the nanoparticles to neutralize the DPPH radical, is observed at a concentration of 50 µl. Additionally, the antioxidant activity of the nanoparticles also increases as the dosage, or the amount of nanoparticles used, increases.

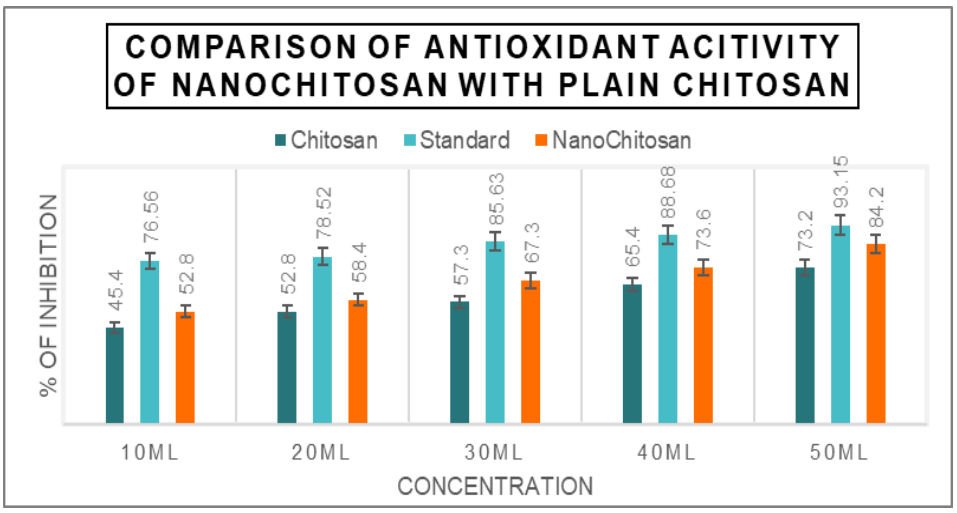

FIG 3: Nanochitosan with chlorhexidine shows higher anti-oxidant activity when compared to plain chitosan.Its activity increases with increase in dosage.

DISCUSSION

Chitosan has been found to effectively neutralise various types of free radicals, which implies that it has a wide range of potential applications. Xie et al. suggested many hypotheses to explain how derivatives scavenge the free radicals by donating hydrogen atoms. The DDA and MW of chitosan are also important parameters in determining its scavenging capacity.(32,33) Unlike chitosan,Chitin is a type of polymer that is not soluble in water, which is a fundamental constraint for it to be a viable antioxidant agent.

Chitosan has amine groups which have the ability to scavenge free radicals, and these groups can be protonated in acidic solutions. There have been several studies on the influence of DDA and MW on the capability to scavenge free radicals.(34) Mahdy Samar et al investigated the antioxidant ability of several chitosan samples having varying MW and DDA and discovered that chitosan with a high DDA rate and a low MW had stronger antioxidant ability.(35)

Hajji and colleagues conducted research on strains of chitosan obtained from marine sources

in Tunisia: Penaeus kerathurus waste (88% DDA), Carcinus mediterraneus(crab) shells (83% DDA), and Sepia officinalis(cuttlefish) bones (95% DDA). (36,37).95% DDA showed the most effective DPPH-free radical scavenging ability in an experiment to assess antioxidant activity of chitosan from cuttlefish.Thomas and Kim investigated chitosan's antioxidant activity with varying molecular weights (MW) of 30, 90, and 120 kDa and found that chitosan with lower MW (30 kDa) exhibited better antioxidant activity.(38)(14)(39)

Sun and colleagues investigated various MW chitosan oligomers and examined their scavenging ability against hydroxyl radical and superoxide anion[88]. Chitosan oligomers with lower MW shown relative greater scavenging efficacy in both superoxide anion and hydroxyl radical. Chang et al.(40) discovered that enzymatically degraded chitosan had antioxidant action against 2, 2-diphenyl-1-picrylhydrazyl radical, hydrogen peroxide and ferrous ion chelation.(41) The study results revealed that chitosan with a lower 2.2 kDa MW has the greatest influence on its scavenging capacity.Another study by Li et al. used hydrogen peroxide to synthesise low MW chitosan and investigated its scavenging ability against hydroxyl radicals.(42) The results showed that chitosan MW (greater activity seen in lower MW) and concentration was related to the free radical scavenging action.(12) Although numerous studies have demonstrated chitosan's antioxidant activity, the level of activity is not particularly satisfying due to the absence of a donor H-atom to function as a suitable (chain-breaking) antioxidant.(43,44) The ability of free radicals to scavenge is in relation to the stability of the generated radicals and the bond dissociation energy of N–H or O–H. The strong hydrogen bonding of the OH and amine groups in chitosan molecules makes it difficult to dissociate them and react with hydroxyl radicals(45). The molecular structure of chitosan was modified to increase its activity by incorporating functional groups into it. The addition of the functional group of polyphenols onto chitosan was the most actively researched among the many attempts.(46)(39)

Chitosan and its metabolites are being studied extensively for medical and pharmacological applications. In situ and in vitro testing have shown their distinct and intriguing bioactivities. They are easily synthesised by natural products through low-cost alkaline deacetylation of chitin mechanisms (47). Our team has extensive knowledge and research experience that has translated into high quality publications (48–57) Given their multiple advantages, chitosan and its derivatives may continue to pique people's interest. The commercial utilisation of derivatives of chitosan is not yet common or easy to get by (58). More study on natural polysaccharides with beneficial bioactivities, including the mechanism of chitosan molecules' bioactivities, may be conducted in the future.

CONCLUSION

The use of this novel irrigant in the field of endodontics would reduce the postoperative pain and overall improve the experience of the patients undergoing endodontic treatment in the future.

REFERENCES

1. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. Science. 1954 May 7;119(3097):623–6.
2. Tučkutė S, Pranevičius L, Pranevičius L, Urbonavičius M. On the Oxygen Transport Mechanism in Titanium Thin Films under Irradiation by Molecular Water Ions [Internet]. Vol. 19, Materials Science. 2013. Available from: http://dx.doi.org/10.5755/j01.ms.19.1.3822
3. Stone B. Free Radicals: The Role of Antioxidants and Pro-Oxidants in Cancer Development. 2014. 185 p.
4. Rivas-Domínguez A, Pastor N, Martínez-López L, Colón-Pérez J, Bermúdez B, Orta ML. The Role of DNA Damage Response in Dysbiosis-Induced Colorectal Cancer. Cells [Internet]. 2021 Jul 29;10(8). Available from: http://dx.doi.org/10.3390/cells10081934
5. J LTH, Hercogova J LT. New Therapeutic Opportunities in Dermatology: Low Dose Cytokines Treatment for Vitiligo [Internet]. Vol. s3, Journal of Pigmentary Disorders. 2015. Available from: http://dx.doi.org/10.4172/2376-0427.s3-001
6. Nagendrappa G. An appreciation of free radical chemistry 3. Free radicals in diseases and health [Internet]. Vol. 10, Resonance. 2005. p. 65–74. Available from: http://dx.doi.org/10.1007/bf02834649
7. Vallyathan V, Shi X. The Role of Oxygen Free Radicals in Occupational and Environmental Lung Diseases [Internet]. Vol. 105,

Environmental Health Perspectives. 1997. p. 165. Available from: http://dx.doi.org/10.2307/3433405
8. Murugaboopathy V, Saravankumar R, Mangaiyarkarasi R, Kengadaran S, Samuel SR, Rajeshkumar S. Efficacy of marine algal extracts against oral pathogens - A systematic review. Indian J Dent Res. 2021 Oct-Dec;32(4):524–7.
9. Chapple ILC. Reactive oxygen species and antioxidants in inflammatory diseases [Internet]. Vol. 24, Journal of Clinical Periodontology. 1997. p. 287–96. Available from: http://dx.doi.org/10.1111/j.1600-051x.1997.tb00760.x
10. Reactive Oxygen Species and Programmed Cell Death [Internet]. Reactive Oxygen Species and Antioxidants in Higher Plants. 2010. p. 81–94. Available from: http://dx.doi.org/10.1201/9781439854082-8
11. Nishanthine C, Miglani R, R I, Poorni S, Srinivasan MR, Robaian A, et al. Evaluation of Fluoride Release in Chitosan-Modified Glass Ionomer Cements. Int Dent J. 2022 Dec;72(6):785–91.
12. Pandiyan I, Sri SD, Indiran MA, Rathinavelu PK, Prabakar J, Rajeshkumar S. Antioxidant, anti-inflammatory activity of -mediated selenium nanoparticles: An study. J Conserv Dent. 2022 Jun 13;25(3):241–5.
13. Ramamurthy S, Thiagarajan K, Varghese S, Kumar R, Karthick BP, Varadarajan S, et al. Assessing the Antioxidant and Anti-inflammatory Activity of Crude Extract. J Contemp Dent Pract. 2022 Apr 1;23(4):437–42.
14. Janani K, Teja KV, Ajitha P. Cytotoxicity of oregano essential oil and calcium hydroxide on L929 fibroblast cell: A molecular level study. J Conserv Dent. 2021 Sep-Oct;24(5):457–63.
15. From Reactive Oxygen Species to Reactive Brominating Species: Fenton Chemistry for Oxidative Bromination [Internet]. Available from: http://dx.doi.org/10.1021/acssuschemeng.1c01709.s001
16. Kong H, Chandel NS. Reactive oxygen species and cancer [Internet]. Oxidative Stress. 2020. p. 619–37. Available from: http://dx.doi.org/10.1016/b978-0-12-818606-0.00030-4
17. Kamath KA, Nasim I, Rajeshkumar S. Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An study. J Conserv Dent. 2020 Jul-Aug;23(4):390–4.
18. Website [Internet]. Available from: https://www.researchgate.net/publication/351419001_Ecofriendly_Synthesis_Characterisation_and_Antibacterial_Activity_Of_Curcumin_Mediated_Silver_Nanoparticles
19. Cristiana F, Elena A. Reactive Oxygen Species (ROS) in Living Cells. BoD – Books on Demand; 2018. 218 p.
20. Schmidt HHH, Ghezzi P, Cuadrado A. Reactive Oxygen Species: Network Pharmacology and Therapeutic Applications. Springer Nature; 2021. 425 p.
21. Ahmad SI. Reactive Oxygen Species in Biology and Human Health. CRC Press; 2017. 543 p.
22. Patlevič P, Vašková J, Švorc P, Vaško L, Švorc P. Reactive oxygen species and antioxidant defense in human gastrointestinal diseases [Internet]. Vol. 5, Integrative Medicine Research. 2016. p. 250–8. Available from: http://dx.doi.org/10.1016/j.imr.2016.07.004
23. Madkour LH. Reactive Oxygen Species (ROS), Nanoparticles, and Endoplasmic Reticulum (ER) Stress-Induced Cell Death Mechanisms. Academic Press; 2020. 780 p.
24. Gowder SJT. Basic Principles and Clinical Significance of Oxidative Stress. BoD – Books on Demand; 2015. 330 p.
25. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense [Internet]. Vol. 5, World Allergy Organization Journal. 2012. p. 9–19. Available from: http://dx.doi.org/10.1097/wox.0b013e3182439613
26. Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci O. Oxidative Stress in Asthma [Internet]. Vol. 4, World Allergy Organization Journal. 2011. p. 151–8. Available from: http://dx.doi.org/10.1097/wox.0b013e318232389e
27. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases [Internet]. Vol. 94, Physiological Reviews. 2014. p. 329–54. Available from: http://dx.doi.org/10.1152/physrev.00040.2012
28. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis [Internet]. Vol. 17, International Journal of Molecular Sciences. 2016. p. 868. Available from: http://dx.doi.org/10.3390/ijms17060868
29. Hosoda S, Meephansan J, Karakawa M, Oshio T, Tsuda H, Komine M, et al. Retinoids regulates the production of matrix metalloproteinases in the human epidermal keratinocyte cell line HaCaT [Internet]. Vol. 69, Journal of Dermatological Science. 2013. p. e24. Available from: http://dx.doi.org/10.1016/j.jdermsci.2012.11.372
30. Yoo SJ, Go E, Kim YE, Lee S, Kwon J. Roles of Reactive Oxygen Species in Rheumatoid

Arthritis Pathogenesis [Internet]. Vol. 23, Journal of Rheumatic Diseases. 2016. p. 340. Available from: http://dx.doi.org/10.4078/jrd.2016.23.6.340
31. Mirshafiey A, Mohsenzadegan M. The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis. Iran J Allergy Asthma Immunol. 2008 Dec;7(4):195–202.
32. Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, et al. Functional Characterization of Chitin and Chitosan [Internet]. Vol. 3, Current Chemical Biology. 2009. p. 203–30. Available from: http://dx.doi.org/10.2174/187231309788166415
33. Aranaz I, Mengibar M, Harris R, Miralles B, Acosta N, Calderon L, et al. Role of Physicochemical Properties of Chitin and Chitosan on their Functionality [Internet]. Vol. 8, Current Chemical Biology. 2014. p. 27–42. Available from: http://dx.doi.org/10.2174/22127968080114112095704
34. El-Naggar MM, Abou-Elmagd WSI, Suloma A, El-Shabaka HA, Khalil MT, Abd El-Rahman FA. Optimization and Physicochemical Characterization of Chitosan and Chitosan Nanoparticles Extracted from the Crayfish Procambarus clarkii Wastes [Internet]. Vol. 38, Journal of Shellfish Research. 2019. p. 385. Available from: http://dx.doi.org/10.2983/035.038.0220
35. Samar MM, Mahdy Samar M, El-Kalyoubi MH, Khalaf MM, Abd El-Razik MM. Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique [Internet]. Vol. 58, Annals of Agricultural Sciences. 2013. p. 33–41. Available from: http://dx.doi.org/10.1016/j.aoas.2013.01.006
36. Harding D, Sashiwa H. Advances in Marine Chitin and Chitosan. MDPI; 2018. 485 p.
37. Hajji S, Younes I, Rinaudo M, Jellouli K, Nasri M. Characterization and In Vitro Evaluation of Cytotoxicity, Antimicrobial and Antioxidant Activities of Chitosans Extracted from Three Different Marine Sources [Internet]. Vol. 177, Applied Biochemistry and Biotechnology. 2015. p. 18–35. Available from: http://dx.doi.org/10.1007/s12010-015-1724-x
38. Younes I, Hajji S, Frachet V, Rinaudo M, Jellouli K, Nasri M. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan [Internet]. Vol. 69, International Journal of Biological Macromolecules. 2014. p. 489–98. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2014.06.013
39. Nandakumar M, Nasim I. Effect of intracanal cryotreated sodium hypochlorite on postoperative pain after root canal treatment - A randomized controlled clinical trial. J Conserv Dent. 2020 Nov 5;23(2):131–6.
40. Chang SH, Wu CH, Tsai GJ. Effects of chitosan molecular weight on its antioxidant and antimutagenic properties [Internet]. Vol. 181, Carbohydrate Polymers. 2018. p. 1026–32. Available from: http://dx.doi.org/10.1016/j.carbpol.2017.11.047
41. Chang SH, Lin HTV, Wu GJ, Tsai GJ. pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan [Internet]. Vol. 134, Carbohydrate Polymers. 2015. p. 74–81. Available from: http://dx.doi.org/10.1016/j.carbpol.2015.07.072
42. Božič M, Gorgieva S, Kokol V. Laccase-mediated functionalization of chitosan by caffeic and gallic acids for modulating antioxidant and antimicrobial properties [Internet]. Vol. 87, Carbohydrate Polymers. 2012. p. 2388–98. Available from: http://dx.doi.org/10.1016/j.carbpol.2011.11.006
43. Lišková J, Douglas TEL, Beranová J, Skwarczyńska A, Božič M, Samal SK, et al. Chitosan hydrogels enriched with polyphenols: Antibacterial activity, cell adhesion and growth and mineralization [Internet]. Vol. 129, Carbohydrate Polymers. 2015. p. 135–42. Available from: http://dx.doi.org/10.1016/j.carbpol.2015.04.043
44. Sousa F, Guebitz GM, Kokol V. Antimicrobial and antioxidant properties of chitosan enzymatically functionalized with flavonoids [Internet]. Vol. 44, Process Biochemistry. 2009. p. 749–56. Available from: http://dx.doi.org/10.1016/j.procbio.2009.03.009
45. Xie W, Xu P, Liu Q. Antioxidant activity of water-soluble chitosan derivatives [Internet]. Vol. 11, Bioorganic & Medicinal Chemistry Letters. 2001. p. 1699–701. Available from: http://dx.doi.org/10.1016/s0960-894x(01)00285-2
46. Jose J, Palanivelu A, Subbaiyan H. Cytotoxicity evaluation of calcium hypochlorite and other commonly used root canal irrigants against human gingival fibroblast cells: An in vitro evaluation. Dent Med Probl. 2021 Jan-Mar;58(1):31–7.
47. Rajendran R, Nair KR, Sandhya R, Ashik PM, Veedu RP, Saleem S. Evaluation of remineralization potential and cytotoxicity of a novel strontium-doped nanohydroxyapatite paste: An study. J Conserv Dent. 2020 Jul-Aug;23(4):330–6.
48. Neelakantan P, Grotra D, Sharma S. Retreatability of 2 mineral trioxide aggregate-

based root canal sealers: a cone-beam computed tomography analysis. J Endod. 2013 Jul;39(7):893–6.
49. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. Risk Manag Healthc Policy. 2021 Jul 7;14:2851–61.
50. Sheriff KAH, Ahmed Hilal Sheriff K, Santhanam A. Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College [Internet]. Vol. 11, Research Journal of Pharmacy and Technology. 2018. p. 543. Available from: http://dx.doi.org/10.5958/0974-360x.2018.00101.4
51. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021 Mar 18;12(1):192.
52. Jayaraj G, Ramani P, Herald J. Sherlin, Premkumar P, Anuja N. Inter-observer agreement in grading oral epithelial dysplasia – A systematic review [Internet]. Vol. 27, Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2015. p. 112–6. Available from: http://dx.doi.org/10.1016/j.ajoms.2014.01.006
53. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.
54. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111773
55. Gan H, Zhang Y, Zhou Q, Zheng L, Xie X, Veeraraghavan VP, et al. Zingerone induced caspase-dependent apoptosis in MCF-7 cells and prevents 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in experimental rats. J Biochem Mol Toxicol. 2019 Oct;33(10):e22387.
56. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res. 2019 Sep;80(6):714–30.
57. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev. 2014 Mar 17;8(1):244.
58. Sairaman S, Nivedhitha MS, Shrivastava D, Al Onazi MA, Algarni HA, Mustafa M, et al. Biocompatibility and antioxidant activity of a novel carrageenan based injectable hydrogel scaffold incorporated with Cissus quadrangularis: an in vitro study. BMC Oral Health. 2022 Sep 5;22(1):377.