



COMPARATIVE ASSESSMENT OF RESINTAG AND SURFACE TENSION OF HYDROPHILIC PIT AND FISSURE SEALANTS REINFORCED WITH SILVER NANOPARTICLES WITH HYDROPHILIC AND CONVENTIONAL SEALANTS ON PERMANENT MOLARS – AN INVITRO STUDY

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Abstract:

Introduction: The failure of hydrophobic pit and fissure sealants are due to saliva contamination and poor mixture control. This disadvantage can be eliminated or reduced to a greater extent by hydrophilic materials. By incorporating silver nanoparticles by green synthesis to the hydrophilic sealants, it enhances its properties. This study aims to compare the resin tag length formed and surface tension of hydrophilic sealant, UltraSeal XT® hydro™ reinforced with silver nanoparticles with conventional hydrophobic sealant in vitro.

Materials and Methods: Green synthesis of silver nanoparticles was derived by using beetroot as a reducing agent. It was mixed with sealant in 1:10 ratio and applied to the teeth. Ultra seal and Clinpro sealants are also applied to the teeth. Resin tag length is assessed in Scanning electron Microscope and Surface tension is assessed using Goniometer.

Results: Results revealed that with regard to surface tension of the sealants, Reinforced Ultraseal had a statistically significant lower surface tension than Clinpro and UltraSeal. Post hoc tukey's revealed that reinforced UltraSeal had a statistically significant difference of 4.782 and 9.868 lesser surface tension than Clinpro and UltraSeal respectively.

With the resin tag formation, Reinforced Ultraseal had a statistically significant higher resin tag than Clinpro and UltraSeal.

Conclusion: Wettability of silver reinforced UltraSeal is better than the comparator and the resin tag length is more in silver reinforced UltraSeal, thus adding silver enhances the micromechanical retentive properties and wettability of the sealant.

Keywords: Hydrophilic sealants, Nanoparticles, Green synthesis, resintag, pit and fissure sealants.

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1. Introduction

Dental caries is one of the most chronic intraoral diseases, with major medical, social, and economic ramifications for both the individual patient and the general society, as it is depended on multiple factors such as presence of cariogenic microbial flora, amount of fermentable sugar present, host factors and also the associated environmental factors.⁽¹⁾

The occlusal surfaces of posterior teeth are the most vulnerable to caries development due to pits and fissures with complex morphologies that provide the ideal environment for caries to thrive.⁽²⁾ Pit and fissure sealants have consistently been suggested as a way to prevent pit and fissure caries in permanent molars.^{(3) (4) (5) (6)}

Sealants mechanically seal off susceptible fissures and reduce plaque stagnation. The occlusal surfaces of molars can be protected from demineralization and caries formation by preventing the invasion of cariogenic bacteria and obtaining nutrients from fissures.⁽⁷⁾ Pit and fissure sealants establish a micro mechanically bonded protective barrier in the pits and fissures of caries-prone teeth, preventing caries-producing bacteria from accessing their source of nutrients.⁽⁸⁾ Despite the efficacy and caries-preventive effect of pit and fissure sealants have been thoroughly demonstrated in the literature, they are still regarded to be underutilised globally.^{(9) (10) (11) (12)}

Sealants have been developed to treat active early fissure caries and even to arrest non cavitated fissure caries as the popularity of minimal intervention dentistry has grown.⁽¹³⁾ Most of the commercially available resin-based pit and fissure sealants are hydrophobic material and majority of the failure of pit and fissure sealants are due to saliva contamination and poor mixture control. This disadvantage can be eliminated or reduced to a greater extent by hydrophilic materials.⁽⁹⁾ The traditional sealants are hydrophobic, the unique moisture tolerant sealants are hydrophilic in nature. Hence, they are miscible with water and flow into moisture-containing etched enamel creating a strong bond for better retention^{(10) (11) (12)} Hydrophilic sealants, which easily adhere to damp enamel surfaces, have just been introduced and offer a special advantage in paediatric dentistry, where patient compliance, isolation, and moisture management can be particularly difficult.⁽¹⁴⁾

However, numerous advancements have been made in restorative dental materials, the widely used dental resins including pit and fissure sealants remain bio inert with minimal interaction with biofilms in the oral cavity. This leads to the development of new materials which possess extra functional and therapeutic functions using

nanotechnology.⁽¹⁵⁾ The use of nanotechnology in dentistry has attracted much interest in recent years, with new ways for caries prevention and treatment, plaque-related biofilm control, and primary dental caries remineralization.⁽¹⁶⁾ Silver nanoparticles have been shown to be effective antimicrobial components in prosthetic materials, adhesives, and implants, promoting capillary arrest, preventing biofilm formation, and inducing osteogenesis.^{(17) (18) (19)} With all these advancement, silver nanoparticles extraction by green synthesis seems to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimised conditions for translational research.

This study aims to compare the resin tag length formed and surface tension of hydrophilic sealant, UltraSeal XT® hydro™ reinforced with silver nanoparticles with conventional hydrophobic sealant in vitro.

2. Materials and Methods

An experimental in vitro study was carried out on a sample of 30 permanent molar teeth extracted for orthodontic or surgical reasons. The sample size was estimated based on the study done by Jayashri Prabakar et al⁽²⁰⁾ using G*Power 3.1.2 software with a power of 0.95 and $p \leq 0.05$ and sample size derived was 10 teeth per group and the total sample size will be 30.

Teeth with sound occlusal surfaces were included in the study, while teeth with developmental defects and caries were eliminated. Before the start of the study, ethical clearance was obtained from the institutional ethics committee, Saveetha University. All the molars were randomly allocated to three groups of 10 molars each using computer-generated randomization. Group I is a conventional Clinpro sealant, Group II will be hydrophilic Ultraseal XT hydro and Group III is Ultraseal XT hydro reinforced with silver nanoparticles.

Green synthesis of silver nanoparticles:

To remove any pollution, the beetroots were washed and cleaned with drinking water. The Beetroot was then chopped into little pieces and left to dry at room temperature. Using a grinder mixer, the dried beets were powdered. 20 grams of beetroot powder were mixed in 100 mL distilled water and left to boil for 5 minutes before being filtered.

0.0169gms 1 millimole of silver nitrate was added into a beaker. It was diluted with 50 ml of distilled water. The combination was then given 50 mL of filtered beetroot extract. Following that, the mixture was placed in an orbital shaker for 32 hours before being transferred to six centrifugation

tubes. The tubes were placed in the centrifuge and centrifuged for 20 minutes at 10,000 rpm.

The supernatant liquid from each tube was discarded, and the leftover pellet solution that collected at the bottom of each tube was transferred to a single tube and stored in the refrigerator for later use. The pellet solution was spread out on a petri dish and dried for 8 hours at 100 degrees Celsius in a hot air oven. To obtain silver nanoparticles, the dried solution was scraped off the petri plate.

Incorporating silver nanoparticles into the sealant:

The silver nanoparticles were added to Ultraseal XT hydro sealant in a ratio of 1:10 by weight and mixed in an amalgamator for 10 minutes

Placement of sealant:

Acid etching of the selected teeth was carried out using 37% orthophosphoric acid for a duration of 30 seconds followed by rinsing with water and then dried using a three-way syringe to attain a white frosty appearance of enamel for Clinpro 3M ESPE sealant (group I). With UltraSeal XT Hydro (group II) and Ultraseal XT Hydro reinforced with silver nanoparticles (group III), the tooth should be dried gently and left moderately wet with a shiny semblance. The respective sealants were then applied to the teeth in each of the groups and cured for 30 seconds.

Resin Tag Length Assessment:

Procedure:

Thermocycling

Both the groups were then subjected to thermocycling at a temperature range of 5°C–55°C for 500 cycles, with a dwell time of 30s.

Tooth sectioning

All the molars were mounted on an acrylic block (Figure 1) and sectioned longitudinally in a mesiodistal direction through the centre of the sealant with a diamond wheel measuring 0.02 mm in thickness. (Figure 2) The Root portion of the teeth was then sectioned and removed.

Steps in Resintag length assessment by Scanning Electron Microscope (JSM IT - 300)

Polishing, decalcification, and drying of tooth specimens

The tooth sections were polished using a carbide stone. The polished sections were then decalcified using 37% phosphoric acid for 15 s to etch away any enamel mineral component not protected by sealants and then rinsed and stored in distilled water. The tooth sections were dried thoroughly under the heat lamp.

Mounting of tooth specimens

Tooth specimens were mounted on brass rings using a non-conductor tape made of carbon. This was then applied to the sections, in the areas that did not need scanning.

Gold sputtering

The mountings were then placed inside an ion-sputtering device for 30 min using vacuum evaporation at 200–300Å.

Measurement of resin tag length

The gold-sputtered sections were then placed inside the SEM of 20 kV capacity and photographs of the sections were obtained. The resin tag lengths were then measured. (Figure 3) The average of each photograph was calculated.



Figure 1: Tooth Sample Mounted



Figure 2: Buccolingual sectioning of tooth specimen



Figure 3: Resin Tag length of Reinforced Ultra seal XT

Figure 4: Contact Angle of Clinpro, Ultraseal XT and Reinforced Ultraseal XT

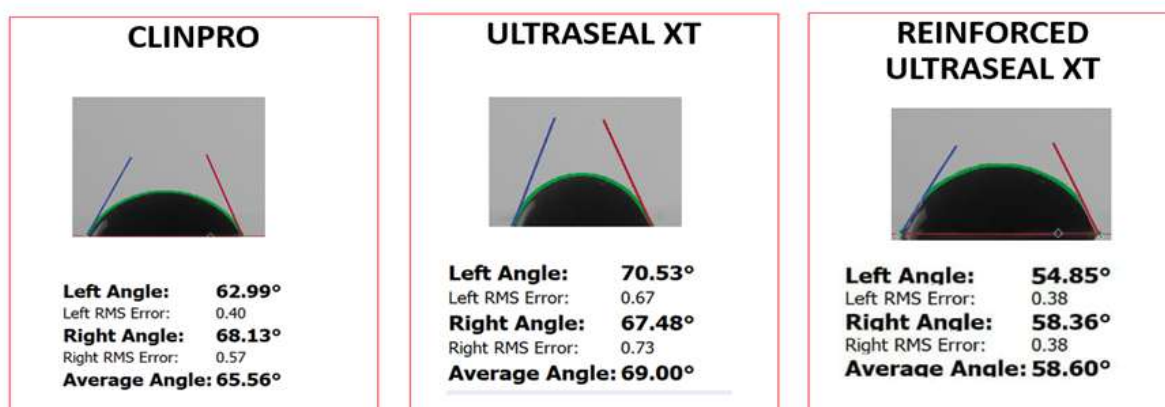




Figure 5: Ossila Goniometer

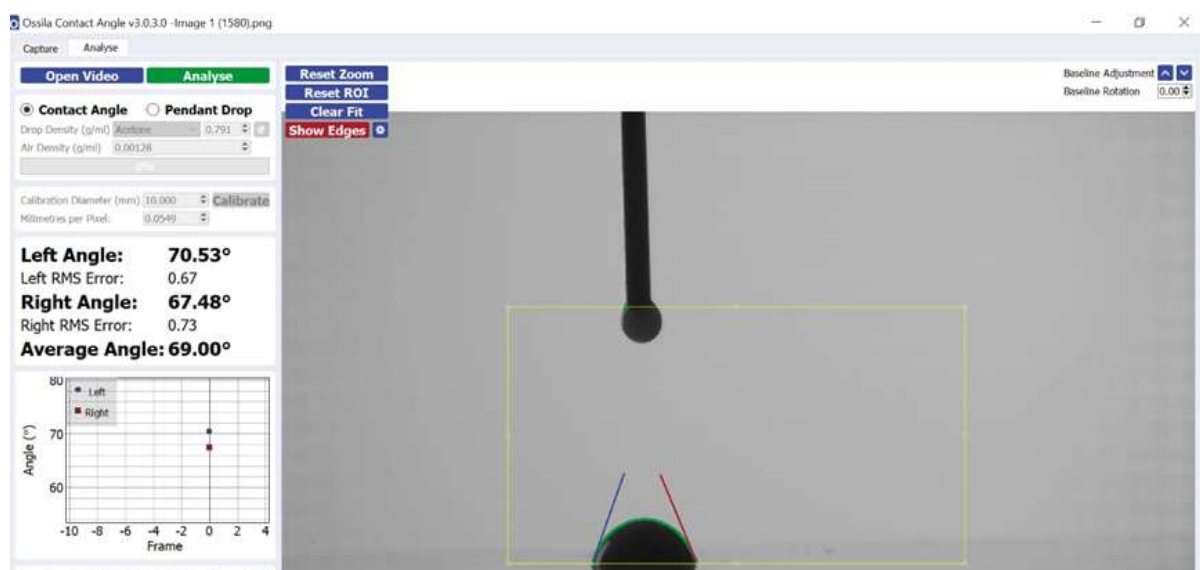


Figure 6: Contact Angle assessment in Goniometer

Measurement of Contact Angle:

Goniometer / Tensiometer is used to calculate the contact angle of the sealants to the surface, the lesser the contact angle - lesser the surface tension, which leads to more wettability of sealant.

Sealant tube is placed on the holder present in the goniometer, one drop of sealant material is made to fall on the glass slide, Goniometer captures and calculates the contact angle which is formed. Same procedure is repeated for all the three types sealants. (Figure 4,5,& 6)

Statistical Analysis:

For statistical analysis, SPSS software version 23.0 was used. One way ANOVA was used to compare the surface tension and resin tag between the three

groups and for post hoc comparisons Tukey's test was used.

3. Results

Results revealed that with regard to surface tension of the sealants, Reinforced Ultraseal had a statistically significant lower surface tension than Clinpro and UltraSeal (table 1). Post hoc tukey's revealed that reinforced UltraSeal had a statistically significant difference of 4.782 and 9.868 lesser surface tension than Clinpro and UltraSeal respectively (table 2).

With the resin tag formation, Reinforced Ultraseal had a statistically significant higher resin tag than clinpro and ultraseal (table 3). Post hoc tukey's revealed that reinforced ultraseal had a statistically

significant difference of 0.82 and 0.14 more resin tag formation than clinpro and ultraseal respectively (table 4).

Table1: Surface Tension - One-Way Anova Test

	N	Mean \pm Std. Deviation	95% Confidence Interval for Mean		F value	P value
			Lower Bound	Upper Bound		
Clinpro	10	64.17 \pm 2.20	61.44	66.91	21.604	0.000
Ultraseal	10	69.26 \pm 1.63	67.23	71.30		
Reinforced UltraSeal	10	59.39 \pm 1.71	57.27	61.52		
Total	30	64.27 \pm 4.51	61.78	66.78		

Table 2: Multiple Comparisons – Tukey's Post Hoc

Groups (I)	Groups	Mean Difference (I-J)	Sig.	95% Confidence Interval		Groups (I)
				Lower Bound	Upper Bound	
Clinpro	Ultraseal	-5.086*	.003	-8.24	-1.93	

	Reinforced UltraSeal	4.782*	.004	1.63	7.93
Ultrasal	Clinpro	5.086*	.003	1.93	8.24
	Reinforced UltraSeal	9.868*	.000	6.72	13.02
Reinforced UltraSeal	Clinpro	-4.782*	.004	-7.93	-1.63
	Ultrasal	-9.868*	.000	-13.02	-6.72

Table 3: Resin Tag - One-Way Anova Test

		Mean \pm Std. Deviation	95% Confidence Interval for Mean		F Value	P Value
			Lower Bound	Upper Bound		
Clinpro	10	5.86 \pm 0.46	4.81	6.89	57.29	0.086
Ultrasal	10	6.54 \pm 1.1	5.75	7.31		

Reinforced UltraSeal	10	6.68±0.4	5.86	7.50		
Total	30	6.36 ± 0.65	5.47	7.20		

Table 4: Multiple Comparisons – Tukey's Post Hoc

(I) Groups		Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Clinpro	Ultraseal	-0.68	0.08	-0.94	-0.42
	Reinforced Ultraseal	-0.82	0.1	-0.95	-0.69
Ultraseal	Clinpro	0.68	0.08	0.42	0.94
	Reinforced Ultraseal	-0.14	0.07	-0.2	-0.08

Reinforced Ultraseal	Clinpro	0.82	0.1	0.69	0.95
	Ultraseal	0.14	0.07	0.08	0.2

4. Discussion:

For many years, the occlusal surface, particularly the pits and fissures of posterior teeth, has been known for its high caries susceptibility. ⁽²¹⁾ Because of the unique morphology of the pits and fissures, it is unquestionably more fragile. ⁽²²⁾ Occlusal pits and fissures come in a variety of shapes, but they're usually narrow and tortuous, and they're thought to be a good place to keep bacterial and food remains. This is due to the fact that the morphology makes mechanical debridement impossible. ⁽²³⁾

The physical occlusion of pits and fissures is thought to be responsible for sealants' cariostatic characteristics. This stops new bacteria from colonising the pits and fissures, as well as the supply of fermentable carbohydrates to any bacteria that remain in the pits and fissures. ⁽²⁴⁾⁽²⁵⁾⁽²⁶⁾⁽²⁷⁾ A viscosity that allows penetration into deep and narrow cracks, enough working time, rapid cure, good and prolonged adhesion to enamel, low sorption and solubility, wear resistance, minimal tissue irritation, and cariostatic action are all requirements for an effective sealant. For a better viscosity, the sealant should possess low surface tension and low contact angle to the tooth structure. ⁽²⁸⁾

The viscosity measurement and resin tag formation are crucial aspects that must be addressed for a sealant to be effective and held for a longer period of time. ⁽²⁹⁾

This study evaluated the resin tag length and contact angle of three pit and fissure sealants, Ultraseal XT, Clinpro, Ultraseal Xt reinforced with silver nanoparticles. Scanning Electron Microscopy analysis allowed the assessment and measurement of resin tag length, its penetration depth. No comparative studies have been done comparing the resin tag formation and surface tension. Molars extracted for therapeutic purpose, which were free of caries, developmental defects, fractures and discolouration were included in the study.

The tooth specimens were treated to a thermocycling technique to simulate the temperature encountered intraorally. Thermocycling is a common method in dental research, especially for testing adhesive materials' performance. Its goal is to thermally stress the adhesive bond at the tooth–restoration interface by exposing restored teeth to severe temperatures similar to those found intraorally. ⁽³⁰⁾

Alterations in the caries morphology might influence the caries progression. Previous studies have proved that the resin tag length of Ultraseal XT Hydro is better than the Clinpro 3M ESPE sealant. In this study, the resin tag length of Silver nanoparticle reinforced Ultraseal XT Hydro showed better results compared to the other two groups, but it was not statistically significant.

The estimation of surface tension has been done for the first time in this study; it also demonstrated that the surface tension of reinforced Ultraseal Xt Hydro is lower compared to the Clinpro and plain Ultraseal XT Hydro group. This implies that lower the contact angle, lower the surface tension and higher the wettability. Lower the surface tension, lower will be the viscosity and many studies are present in the literature with evidence. Viscosity is the resistance exhibited by the liquid/sealant to flow. ⁽³¹⁾ Internal frictional forces within the liquid controls the resistance of the liquid. Higher the viscosity of the liquid, slower the liquid flows. Factors influencing the penetration of the sealant are viscosity, resin tag length, surface tension, etc., ⁽³²⁾

The longer the resintag formed, the more the retention of the sealant, this has been proved in many studies. In the present study, the mean length of resin tags obtained ranged from 5.86 to 6.68 μm for all groups. Similar in vitro studies conducted by Prabhakar et al. ⁽³³⁾ showed mean resin tag length in the range of 5–10 μm . Surface tension ranged from 57.27 to 71.30 degree.

5. Conclusion:

Based on the findings of the present study, it can be concluded that the wettability of silver reinforced UltraSeal is better than the comparator Clinpro and plain UltraSeal and also the resin tag length is more in silver reinforced UltraSeal, thus adding silver enhances the micromechanical retentive properties and wettability of the sealant.

6. Reference:

- Usha C, R S. Dental caries - A complete changeover (Part I). J Conserv Dent. 2009 Apr;12(2):46-54.
- Liu W,Xiong L,Li J,Guo C,Fan W,Huang S, The anticaries effects of pit and fissure sealant in the first permanent molars of school-age children from Guangzhou: a population-based cohort study. BMC oral health. 2019 Jul 16
- Griffin SO, Oong E, Kohn W, et al. The effectiveness of sealants in managing caries lesions. J Dent Res 2008;87:169-74.
- Aldhuwayhi, Sami, Sreekanth Kumar Mallineni, Srinivasulu Sakhamuri, Amar Ashok Thakare, Sahana Mallineni, Rishitha Sajja, Mallika Sethi, Venkatesh Nettam, and Azher Mohiuddin Mohammad. 2021. "Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey." Risk Management and Healthcare Policy14 (July): 2851–61.
- Dua, Kamal, Ridhima Wadhwa, Gautam Singhvi, Vamshikrishna Rapalli, Shakti Dhar Shukla, Madhur D. Shastri, Gaurav Gupta, et al. 2019. "The Potential of siRNA Based Drug Delivery in Respiratory Disorders: Recent Advances and Progress." Dr Development Research 80 (6): 714–30.
- Gan, Hongyun, Yaqing Zhang, Qingyun Zhou, Lierui Zheng, Xiaofeng Xie, Vishnu Priya Veeraraghavan, and Surapaneni Krishna Mohan. 2019. "Zingerone Induced Caspase-Dependent Apoptosis in MCF-7 Cells and Prevents 7,12-Dimethylbenz(a)anthracene-Induced Mammary Carcinogenesis in Experimental Rats." Journal of Biochemical and Molecular Toxicology 33 (10): e22387.
- Ahovuo-Saloranta A, Forss H, Walsh T, Nordblad A, Mäkelä M, Worthington HV. Pit and fissure sealants for preventing dental decay in permanent teeth. Cochrane Database Syst Rev. 2017 Jul 31;7(7):CD001830. doi: 10.1002/14651858.CD001830.pub5. PMID: 28759120; PMCID: PMC6483295.
- Simonsen, RJ. Pit and fissure sealants. In: Clinical applications of the acid etch technique, 1st ed. Chicago, IL: Quintessence publishing Co, Inc, 1978; pp. 19–42.
- Ratnaditya, Akurathi & Kumar, Mallela & Sai Sankar, A J & Zahirunnisa, Mohammad & Kopuri, Raj. (2015). Clinical Evaluation of Retention in Hydrophobic and Hydrophilic Pit and Fissure Sealants-A Two Year Follow-Up Study. Journal of Young Pharmacists. 7. 171-179. 10.5530/jyp.2015.3.6.
- Naaman R., El-Housseiny A.A., Alamoudi N. The use of pit and fissure sealants-a literature review. Dent. J. 2017;5:34. doi: 10.3390/dj5040034.
- Jayaraj, Gifrina, Pratibha Ramani, Herald J. Sherlin, Priya Premkumar, and N. Anuja. 2015. "Inter-Observer Agreement in Grading Oral Epithelial Dysplasia – A Systematic Review." Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. <https://doi.org/10.1016/j.ajoms.2014.01.006>.
- Li, Zhenjiang, Vishnu Priya Veeraraghavan, Surapaneni Krishna Mohan, Srinivasa Rao Bolla, Hariprasath Lakshmanan, Subramanian Kumaran, Wilson Aruni, et al. 2020. "Apoptotic Induction and Anti-Metastatic Activity of Eugenol Encapsulated Chitosan Nanopolymer on Rat Glioma C6 Cells via Alleviating the MMP Signaling Pathway." Journal of Photochemistry and Photobiology B: Biology.
- Griffin SO, Oong E, Kohn W, et al. The effectiveness of sealants in managing caries lesions. J Dent Res 2008;87:169-74.
- Khogli AE, Cauwels R, Vercruysse C, Verbeeck R, Martens L. Microleakage and penetration of a hydrophilic sealant and a conventional resin-based sealant as a function of preparation techniques: a laboratory study. Int J Paediatr Dent. 2013;23:13–22.
- Barrere F., van der Valk C., Meijer G., Dalmeijer R., de Groot K., Layrolle P. Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats. J. Biomed. Mater. Res. Part B Appl. Biomater. 2003;67:655–665.
- E. Ahmadian, S. Shahi, J. Yazdani, S. Maleki Dizaj, and S. Sharifi, "Local treatment of the dental caries using nanomaterials," Biomedicine & Pharmacotherapy, vol. 108, pp. 443–447, 2018.
- R. Emmanuel, S. Palanisamy, S.-M. Chen et al., "Antimicrobial efficacy of green synthesized drug blended silver nanoparticles against dental caries and periodontal disease causing microorganisms," Materials Science and Engineering: C, vol. 56, pp. 374–379, 2015.
- Markov, Alexander, Lakshmi Thangavelu, Surendar Aravindhan, Angelina Olegovna

- Zekiy, Mostafa Jarahian, Max Stanley Chartrand, Yashwant Pathak, Farooq Marofi, Somayeh Shamlou, and Ali Hassanzadeh. 2021. "Mesenchymal Stem/stromal Cells as a Valuable Source for the Treatment of Immune-Mediated Disorders." *Stem Cell Research & Therapy* 12 (1): 192.
- Mohan, Meenakshi, and Nithya Jagannathan. 2014. "Oral Field Cancerization: An Update on Current Concepts." *Oncology Reviews* 8 (1): 244.
- Prabakar J, John J, Arumugham IM, Kumar RP, Sakthi DS. Comparative Evaluation of the Viscosity and Length of Resin Tags of Conventional and Hydrophilic Pit and Fissure Sealants on Permanent Molars: An In vitro Study. *Contemp Clin Dent*. 2018 Jul-Sep;9(3):388-394.
- Abou El-Yazeed M, Abou-Zeid W, Zaazou M. Effect of different enamel pretreatment techniques for pit and fissure sealing in primary and permanent teeth. *Aust J Basic Appl Sci*. 1991;7:895-9.
- Sanders BJ, Feigal RJ, Avery DR. Pit and fissure sealants and preventive resin restorations. In: McDonald RE, Avery DR, Dean JA, editors. *Dentistry for Child and Adolescent*. 8th ed. New Delhi: Elsevier; 2005. p. 355.
- Droz D, Schiele MJ, Panighi MM. Penetration and microleakage of dental sealants in artificial fissures. *J Dent Child (Chic)* 2004;71:41-4.
- Montero MJ, Douglass JM, Mathieu GM. Prevalence of dental caries and enamel defects in connecticut head start children. *Pediatr Dent*. 2003;25:235-9.
- Neelakantan, Prasanna, Deeksha Grotra, and Subash Sharma. 2013. "Retreatability of 2 Mineral Trioxide Aggregate-Based Root Canal Sealers: A Cone-Beam Computed Tomography Analysis." *Journal of Endodontia* 39 (7): 893-96.
- Paramasivam, Arumugam, Jayaseelan Vijayashree Priyadharsini, Subramanian Raghunandhakumar, and Perumal Elumalai. 2020. "A Novel COVID-19 and Its Effects on Cardiovascular Disease." *Hypertension Research: Official Journal of the Japanese Society of Hypertension*.
- Sheriff, K. Ahmed Hilal, K. Ahmed Hilal Sheriff, and Archana Santhanam. 2018. "Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College." *Research Journal of Pharmacy and Technology*. <https://doi.org/10.5958/0974-360x.2018.00101.4>.
- Irinoda Y, Matsumura Y, Kito H, Nakano T, Toyama T, Nakagaki H, et al. Effect of sealant viscosity on the penetration of resin into etched human enamel. *Oper Dent*. 2000;25:274-82.
- Garcia-Godoy F, Gwinnett AJ. Penetration of acid solution and gel in occlusal fissures. *J Am Dent Assoc*. 1987;114:809-10.
- Wahab FK, Shaini FJ, Morgano SM. The effect of thermocycling on microleakage of several commercially available composite class V restorations in vitro . *J Prosthet Dent*. 2003;90:168-74.
- Burrow JF, Burrow MF, Makinson OF. Pits and fissures: Relative space contribution in fissures from sealants, prophylaxis pastes and organic remnants. *Aust Dent J*. 2003;48:175-9.
- Breakspere RJ, Wilton A. Factors affecting the structure of a fissure sealant at the enamel/sealant interface. *Aust Dent J*. 1977;22:199-202.
- Vickram, A. S., Rao, K. A., Archana, K., Jayaraman, G., Kumar S, V., & Sridharan, T. B. (2015). Effects of various semen extenders on semen parameters for the purpose of human male fertility preservation. *Cryoletters*, 36(3), 182-186.
- Prabhakar AR, Murthy SA, Sugandhan S. Comparative evaluation of the length of resin tags, viscosity and microleakage of pit and fissure sealants – An in vitro scanning electron microscope study. *Contemp Clin Dent*. 2011;2:324-30.