International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Novel Irrigant in Regenerative Endodontics - An In-Vitro Study

Research Article

Srujana Hemmanur[1], Iffat Nasim[2*]

[1] Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.
[2] Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.

Abstract

Introduction: Regenerative endodontic procedures have gained a limelight in recent times owing to the rates of success that has been reported by various authors. The potential regeneration of the tissues of the pulp-dentin complex yields us the hope to bring back the vitality of a non vital tooth. The management of a young permanent tooth with necrotic pulp has remained a challenge. In the present study, the potential of Farnesol, a sesquiterpene alcohol by origin with a good antimicrobial activity is tested for cytotoxicity, cell migration and cell proliferation assays against SCAP cells.
Aim: Aim of the study was to evaluate if Farnesol could be used as an irrigant for necrotic immature permanent teeth.
Materials and Methods: 95% Farnesol (Sigma Aldrich, US) was bought. SCAP cells from Axol Pvt. ltd (Cambridge, UK) were derived and a final concentration of 25µM/ml, 50µM/ml and 100µM/ml of the solution was tested on SCAP cells for the analysis of cytotoxicity, cell migration and cell proliferation. For cytotoxicity evaluation Sulforhodamine Assay, for evaluation of cell migration Transwell Migration Assay and cell proliferation In-Vitro Bromodeoxyuridine Assay were performed.
Results: Analysis of the results showed statistically significant increase in cytotoxicity with the increase in the final concentration of Farnesol. Also, reduction in the cell migration and proliferation was seen to significantly decrease with the increasing concentration of the novel irrigant.
Conclusion: Farnesol at a concentration lower than 25µM/ml, can be tried as a potential irrigant for the management of the necrotic immature permanent teeth by revascularisation technique. However, clinical studies need to be done to extrapolate this finding in reality.

Keywords: Immature Teeth; Necrotic Immature Permanent Teeth (NIPT); Open Spices; Revascularisation.

Introduction

Management of a young permanent tooth with non vital pulp poses a challenge. The presence of a blunderbuss canal with a wide apical opening and thin dentinal walls pose the biggest challenge as inadequate disinfection of the canal space and difficulty in obturation due to lack of an apical stop is evident [1]. Conventionally, a necrotic immature permanent tooth is managed by either apexification using calcium hydroxide or by placing a MTA apical plug. [2]. These approaches are successful in the long run as they resolve the clinical signs and symptoms of the disease but have got no benefits in the improvisation of the root length, thickness, return of the pulp vitality and nociception etc [3].

Regenerative attempts have not been new to medicine and dentistry. Iwaya et al and Banchs-Trope have demonstrated the successful regeneration of an immature tooth with not just the resolution of disease but also the increase in the length and thickness of the root and apical closure [4, 5]. Regenerative endodontics are procedures that are designed to replace damaged tooth structures and are biologically based [6]. The protocol for the regenerative endodontic procedures have been formulated based on the findings of many clinicians. The current protocol that is being followed has been chalked out by AAE and it has been revised most recently in 2018. According to the current protocol, in the first appointment, isolation and pain management is done and disinfection using copious gentle irrigation of the canal space with a lower concentration (1.5%) and 20 ml of NaOCl with preven-

***Corresponding Author**:
Iffat Nasim,
Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.
Tel: +919940063567
E-mail: iffatnasim@saveetha.com

Received: May 03, 2021
Accepted: August 11, 2021
Published: August 18, 2021

Citation: Srujana Hemmanur, Iffat Nasim. Novel Irrigant in Regenerative Endodontics- An In-Vitro Study. *Int J Dentistry Oral Sci.* 2021;8(8):4016-4021.
doi: http://dx.doi.org/10.19070/2377-8075-21000820

tion of its extrusion is performed. A closed dressing with either calcium hydroxide or TAP or DAP is given and a temporary restorative material is used to seal the access cavity. The patient is recalled for the second visit 1-4 weeks after the first and isolation and LA without vasoconstrictor is used to anesthetize the tooth. Copious gentle irrigation with 17% EDTA is done and bleeding is induced by filing beyond the apex. An alternative such as autologous platelet concentrate can be used and MTA is used as a capping material. GIC is placed above the capping material and the access cavity is sealed off. A follow up that consists of a check for clinical symptoms and radiographic examination is done after 6,12, and 24 months. [7]

The regeneration of the tissue requires three components- stem cells, scaffolds and growth factors [8]. The absence or late delivery of any one of the factors would fail the regeneration to occur. It has been suggested to use the term revitalization instead of regeneration as the tissue formed in the root canal space is vital but the nature of which is different from the original lost tissue [9]. Thus, revascularization as a regenerative modality can shift the entire paradigm in the management of a young permanent teeth with necrotic pulp as it can not only resolve the clinical signs and symptoms but also can aim in the restoration of the tissues in the root canal space that are of great importance to the clinician and scientific world.

Previously our team has a rich experience in working on various research projects across multiple disciplines [10-24] Now the growing trend in this area motivated us to pursue this project.

Our aim was to check if a novel proposed irrigant like Farnesol with good antimicrobial properties as checked in in-vitro environment [25] can be used as a potential irrigant for the management of the necrotic immature permanent teeth by a relatively newer technique of revascularization.

Materials And Methods

Stem cells from Apical Papilla (SCAP)

The cryopreserved stem cells from apical papilla (SCAP) (AXOL bioscience Ltd., UK) was derived and plated at a density of $7x10^3$ cells per well in 96-well plates with DMEM/F12 supplemented with 10% FBS and 2 mM L-glutamine, 100 UI/ml penicillin and 100 µg/ml streptomycin. Cell growth was assessed by the Neubauer counting chamber method. Every 24 hours, the cells were harvested by trypsin EDTA treatment and then were counted with a hemocytometer. After 72 h, the culture medium was replaced with a fresh one, and unattached cells were also removed. When 80-90% confluency was reached, cells were routinely subcultured and counted using a microscope.

Chemicals

Bromodeoxyuridine, Sulforhodamine, haematoxylin, and other chemicals were purchased from Rankem laboratories (Ludhiana, India).

Cytotoxicity test by sulforhodamine assay

The microtiter plates were added with culture medium (DMEM supplemented with 10% fetal bovine serum, 100 µL/well) was added and incubated for 24 h at 37 °C in the incubator with 5% CO_2. After 24 h of incubation, cell growth was observed using a microscope. Cellular concentration used was 1.4 x 104 cells/mL. Then, the medium was discarded and Farnesol with various concentrations, negative control and DMEM without fetal bovine serum were added in each well. After 24 h of incubation, the cells were fixed with trichloroacetic acid (100 µL/well) and placed under time and dried. Then the plates were stained for 20 min at room temperature with SRB 0.4% (50 µL/well). The plates were washed with 1% acetic acid solution and dried at room temperature. The unbound dye was removed after washing, the dyestuff bound to the protein was solubilized in a basic medium to determine the optical density in a plate reader at 570 nm.

The average absorbance of the negative control (AbsNC), for each concentration of the tested substances (absT) and positive control (absPC) was calculated. The percentage of living cells was given by:

% survival = [(AbsT - AbsNC)/(AbsNC)] x 100

Transwell Migration assay

Cell migration was quantitated using 24-well Transwell inserts (6.5 mm) with polycarbonate filters (5-µm pore size). SCAP cells (0.5 × 106 in 100 µl of RPMI 1640 medium/1% human albumin) were added to the well. Transwell and 700ml of serum free MEM with or without Farnesol (25-100µM/ml) was added to the lower chambers. The plates were incubated at 37°C in 5% CO_2 for 24h and cells remaining on the top of the surface of the filter were removed with a cotton swab and the cells that had traversed from the membrane to the bottom chamber were for 10mins with 4% paraformaldehyde and stained with haematoxylin for 30 mins. To quantify the migrated cells, 10 random microscopic fields per filter at 200x magnification were selected for a cell count measurement were done in triplicates and results calculated as mean in each experiment.

In vitro Bromodeoxyuridine cell proliferation Assay

A 10 mM stock solution of BrdU was prepared by dissolving 3 mg of BrdU in 1 mL water. The 10 mM BrdU stock solution in cell culture medium was diluted to make a 10 µM BrdU labeling solution. SCAP (2500-100000 cells/well) in 100 µl medium were plated in 96-well plate and incubated with the respective test substance Farnesol (25, 50 and 100 µM/ml) for 72 hr. The prepared solution of BrdU with SCAP cells served as a negative control.

A prepared 10 µl of 10X BrdU solution per well were added. The cells were placed in an incubator for 24 hr. For suspension cells, centrifugation of the plate at 300xg for 10 min was done and then the medium was removed. 100 µl/well of the fixing solution was added to each well at room temperature for 30 min. Then after the solution was removed, 100 µl/well prepared 1X detection antibody solution was added and placed at room temperature for 1 hour. The solution was discarded and the plates were washed 3 times with 1X Wash Buffer. 100 µl of prepared 1X HRP-conjugated secondary antibody solution was added in each well , and incubated at room temperature for 30 min. Then 100 µl TMB Substrate was added and incubated for 30 min at room temperature, The 100 µl STOP Solution was added and the solu-

tion's absorbance at 450 nm was read.

Statistical Analysis

The experiments were carried out in triplicates, the results were represented as Mean ± Standard error of mean (SEM). Statistical differences were determined by one-way analysis of variance (ANOVA) and post hoc comparison test. P-values <0.05 were considered statistically significant. Data were analysed using the SPSS 22.0 package (Chicago, IL, USA).

Results

The Sulforhodamine assay, Transwell migration assay, Bromodeoxyuridine immunohistochemistry indicated towards the use of Farnesol as an effective irrigant for necrotic immature permanent tooth in the in vitro scenario. The concentration of Farnesol at 25µM/ml shows the greatest potential. The graphical representation of the results have been done in Figures 1,2 and 3 evaluating cytotoxicity, proliferation and migration of SCAP cells in the presence of Farnesol at various concentrations respectively.

Discussion

Our institution is passionate about high quality evidence based research and has excelled in various fields [26-36].

The American Association of Endodontists (AAE) identifies the prospects of regenerative endodontics. Disinfection holds a very important place in the success of the procedure. Due to the presence of thin dentinal walls, minimal instrumentation of the canal must be ensured. The focus of regenerative endodontics has shifted to proper disinfection. Aurora et al suggested that tissue healing whether regeneration or repair occurs in a sterile or highly disinfected environment as the host immune defense system fails to promote tissue- destruction by inflammatory responsive processes [37]. Trevino et al, [38] evaluated the survival of SCAP cells which are supposed to be a source of stem cells in regenerative endodontic procedures as reported by Sonoyama et al, 2008 [39]. They suggested the use of EDTA promoted greater survival of SCAP cells and also had the property to reverse effects of sodium hypochlorite which is used to debride the canal space off necrotic tissue. Hence. 17% EDTA is recommended to be used as the final irrigant so as to allow the release of growth factors from dentin [38].1.5% Sodium hypochlorite has the least effect on the survival and proliferation of SCAP cells and hence it was used in copious amounts for irrigation [40].

The use of calcium hydroxide as an intracanal medicament has been suggested as it is non discolouring, can be easily removed from the canal space and has no detrimental effect on the survival of SCAP which is not seen when TAP was used [41].

Farnesol is a natural product that structurally exists as a sesquiterpene alcohol commonly found in propolis and essential oils of citrus fruits. The antimicrobial and anti-biofilm activity of Farnesol has attracted the attention of researchers in recent times. Farnesol was seen to affect the biomass and biofilm composi-

Figure 1. Graph representing cytotoxicity of SCAP cells: This figure depicts the percentage of cytotoxicity upon treatment with Farnesol . The X axis represents the concentration of Farnesol used for testing and negative control (NC) while the Y axis represents the % of cytotoxicity. Results are represented as a Mean ± SEM of three independent experiments. It can be inferred that least cytotoxicity to SCAP cells is seen when the concentration is 25µM/ml (p value <0.05; statistically significant; One-Way ANOVA).

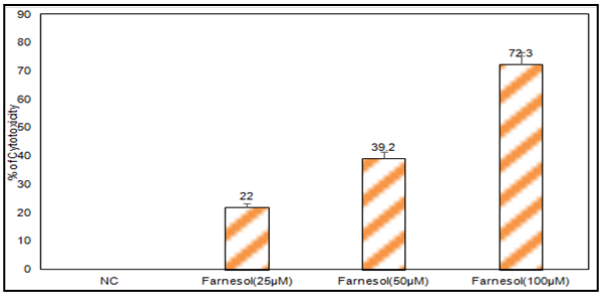


Figure 2. Graph representing the migration of SCAP cells; This figure depicts the relative migration of SCAP cells upon treatment with Farnesol . The X axis represents the concentration of Farnesol used for testing and negative control (NC) while the Y axis represents the relative migration rate of the SCAP cells. Results are represented as a Mean ± SEM of three independent experiments. It can be inferred that the relative migration rate of SCAP cells is the greatest when 25µM/ml is used as the test agent and the rate decreases with increase in the concentration of Farnesol. (p value <0.05; statistically significant; One-Way ANOVA)

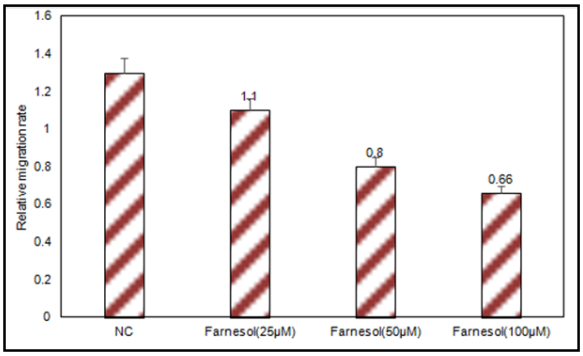
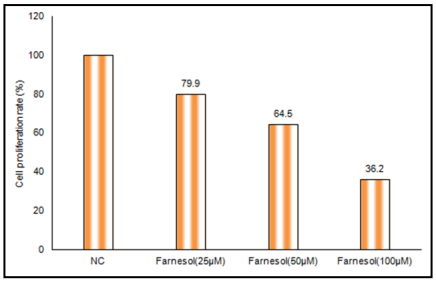


Figure 3. Graph representing the percentage of cell proliferation; This figure depicts the percentage of SCAP proliferation upon treatment with Farnesol .The X axis represents the concentration of Farnesol and negative control (NC) used for testing while the Y axis represents the % of cell proliferation of the SCAP cells. Results are represented as a Mean ± SEM of three independent experiments.It can be inferred from the graph that the SCAP cells proliferate the most when the concentration is 25µM/ml and it decreases with the increase in concentration of Farnesol.(p value <0.05; statistically significant; One-Way ANOVA).



tion of dual species biofilm. It also effectively blocks the quorum sensing amongst multi-species biofilm which helps in fighting the resistance [25, 42-44] . An in-vivo evaluation of Ti6Al4V implants treated with Farnesol has shown a decreased rate of colonisation of S.aureus and the dental implant commonly used is of the same composition [45]. Farnesol has suggested an effective induced cell cycle arrest and apoptosis of tumor cells in carcinoma like pancreatic cancer [46, 47]. Farnesol by preventing the conversion of fungi to hyphae seems to decrease the pathogenicity of vulvovaginal candidiasis [48] and oral candidiasis in mice [49]. Farnesol is also reported to have low cytotoxic effects and no genotoxicity towards fibroblasts [44].

Given the wide array of use of Farnesol, antimicrobial nature and low cytotoxicity and genotoxic effects of Farnesol, current study was designed to check if Farnesol can effectively be used as an irrigant for necrotic immature permanent teeth ,based on the results of in-vitro analysis done by Andrade et al, 2017 in which they suggested that it can be used as a potential irrigant for root canal treatment [44].

The Sulforhodamine assay was developed by Skehan and colleagues in 1990 for anticancer drug screening [50]. It is a colorimetric technique, with results as sensitive as obtained from other cytotoxicity assays like the MTT. This assay is based on the ability of a protein dye (sulforhodamine B) to bind electrostatically to the basic protein amino acid residues of acetic acid fixed cells [51]. The cytotoxic effect of Farnesol on SCAP cells can be seen to be increasing with an increase in the concentration. The least cytotoxic effect was seen when Farnesol at a concentration of 25µM/ml was used as the test product (Figure 1).

Transwell migration assay provides an analysis to check for the ability of a particular group of cells to sense the presence of a chemoattractant and to migrate towards it through a physical barrier that is present [52]. In this study, the migration of SCAP in the presence of Farnesol was checked as without the migration of the stem cells to the canal space, the regeneration cannot occur. The number of migrated cells was quantified by counting them underneath a microscope or by taking pictures and evaluating them. The chemotaxis and directional cell migration of any group of cells in an in-vitro setup can be checked through this assay. In the present study, greatest amounts of cell migration was appreciated when Farnesol in the concentration of 25µM/ml was used as the test product. The relationship between the cell migration and concentration of farnesol can thus be considered as inversely proportional, with the least migration in higher concentration of the product (Figure 2).

Bromodeoxyuridine immunohistochemistry in the 1980s was used to examine the proliferation and migration of cells of CNS [53]. BrdU competes with thymidine and gets incorporated into the nuclear DNA. The presence of BrdU marks DNA synthesis and this DNA when fixed, incubated and treated with anti-BrdU monoclonal antibodies produce a colorimetric reaction when the secondary antibody gets exposed to an added substrate. This can be visualised under a microscope [54]. In the present study, maximum proliferation of SCAP was seen when Farnesol was used in the concentration of 25µM/ml (Fig.3). As the concentration of Farnesol increased, the rate of proliferation of SCAP cells was seen to decrease.

With the current study, a concentration of 25µM/ml of Farnesol may be tried to be used as a potential irrigant for regenerative endodontics as it not only provides good antimicrobial efficacy and the cyotoxicity, cell migration and proliferation of SCAP is seen under in-vitro conditions.

Although Regenerative endodontic procedures have provided clinical success, the tissue formed in the pulp canal space is of varying nature. Hence, the regeneration of the tissues still remain bleak and it is the repair that is being induced. More advanced bioengineering approaches may in future lead to the achieving of predictable regeneration of vital and natural pulp-like tissue.

Conclusion

Farnesol 95% (Sigma Aldrich, US) at a concentration of about 25µM/ml, can be used as a potential irrigant for the management of the necrotic immature permanent teeth by revascularization technique. However, clinical studies need to be done to extrapolate this finding in reality.

References

[1]. Trope M. Treatment of the immature tooth with a non–vital pulp and apical periodontitis. Dent Clin North Am. 2010 Apr 1;54(2):313-24.
[2]. Chueh LH, Ho YC, Kuo TC, Lai WH, Chen YH, Chiang CP. Regenerative endodontic treatment for necrotic immature permanent teeth. J Endod. 2009 Feb 1;35(2):160-4.
[3]. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with a new biomaterial. J Endod. 2011 Apr;37(4):562-7.Pubmed

PMID: 21419310.
[4]. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol. 2001 Feb;17(4):185-7.
[5]. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol?. J Endod. 2004 Apr 1;30(4):196-200.
[6]. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod. 2007 Apr;33(4):377-90.Pubmed PMID: 17368324.
[7]. Curriculum/Syllabus for Advanced Programs in Endodontics on Revascularization and Regenerative Endodontics. 2017. Available from: https://www.aae.org/specialty/wp-content/uploads/sites/2/2017/06/currentregenerativeendodonticconsiderations.pdf
[8]. Langer R, Vacanti JP. Tissue engineering. Science. 1993 May 14;260(5110):920-6.
[9]. Lenzi R, Trope M. Revitalization procedures in two traumatized incisors with different biological outcomes. J Endod. 2012 Mar;38(3):411-4.Pubmed PMID: 22341086.
[10]. Govindaraju L, Gurunathan D. Effectiveness of Chewable Tooth Brush in Children-A Prospective Clinical Study. J Clin Diagn Res. 2017 Mar;11(3):ZC31-ZC34.Pubmed PMID: 28511505.
[11]. Christabel A, Anantanarayanan P, Subash P, Soh CL, Ramanathan M, Muthusekhar MR, et al. Comparison of pterygomaxillary dysjunction with tuberosity separation in isolated Le Fort I osteotomies: a prospective, multi-centre, triple-blind, randomized controlled trial. Int J Oral Maxillofac Surg. 2016 Feb;45(2):180-5.Pubmed PMID: 26338075.
[12]. Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery--a systematic review. Int J Oral Maxillofac Surg. 2013 Aug;42(8):974-80.Pubmed PMID: 23702370.
[13]. Mehta M, Deeksha, Tewari D, Gupta G, Awasthi R, Singh H, et al. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206-215.Pubmed PMID: 31136735.
[14]. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115-121.Pubmed PMID: 30451321.
[15]. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol. 2014 Jan 1;13(1):44-58.
[16]. Sneha S. Knowledge and awareness regarding antibiotic prophylaxis for infective endocarditis among undergraduate dental students. Asian J Pharm Clin Res. 2016 Oct 1:154-9.
[17]. Christabel SL, Linda Christabel S. Prevalence of type of frenal attachment and morphology of frenum in children, Chennai, Tamil Nadu. World J Dent. 2015 Oct;6(4):203-7.
[18]. Kumar S, Rahman R. Knowledge, awareness, and practices regarding biomedical waste management among undergraduate dental students. Asian J Pharm Clin Res. 2017;10(8):341.
[19]. Sridharan G, Ramani P, Patankar S. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Cancer Res Ther. 2017 Jul 1;13(3):556-61.
[20]. Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S. Herbs as an antioxidant arsenal for periodontal diseases. J Intercult Ethnopharmacol. 2016 Jan 27;5(1):92-6.Pubmed PMID: 27069730.
[21]. hamaraiselvan M, Elavarasu S, Thangakumaran S, Gadagi JS, Arthie T. Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession. J Indian Soc Periodontol. 2015 Jan;19(1):66-71.
[22]. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, et al. Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. PLoS One. 2016 Jun 9;11(6):e0156582.Pubmed PMID: 27280700.
[23]. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019 May;29(4):276-290.Pubmed PMID: 30461321.
[24]. Ramakrishnan M, Shukri M. Fluoride, Fluoridated Toothpaste Efficacy And Its Safety In Children-Review. Int. J. Pharm. Sci. Res. 2018 Oct 1;10(04):109-14.
[25]. Chávez-Andrade GM, Tanomaru-Filho M, Basso Bernardi MI, de Toledo Leonardo R, Faria G, Guerreiro-Tanomaru JM. Antimicrobial and biofilm anti-adhesion activities of silver nanoparticles and farnesol against endodontic microorganisms for possible application in root canal treatment. Arch Oral Biol. 2019 Nov;107:104481.Pubmed PMID: 31336203.
[26]. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441-1448.Pubmed PMID: 31257588.
[27]. Pc J, Marimuthu T, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res. 2018 Apr 6;20(4):531-4.
[28]. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241-1248.Pubmed PMID: 30044495.
[29]. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJ. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig. 2019 Sep;23(9):3543-50.
[30]. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019 Apr;48(4):299-306.
[31]. zhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115-121.Pubmed PMID: 30451321.
[32]. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. Clin Oral Investig. 2020 Sep;24(9):1-6.Pubmed PMID: 31955271.
[33]. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285-286.Pubmed PMID: 32416620.
[34]. R H, Ramani P, Ramanathan A, R JM, S G, Ramasubramanian A, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020 Sep;130(3):306-312.Pubmed PMID: 32773350.
[35]. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. Prog Orthod. 2020 Oct 12;21(1):38.Pubmed PMID: 33043408.
[36]. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol. 2018 Oct;94:93-98.Pubmed PMID: 30015217.
[37]. Aurora AB, Olson EN. Immune modulation of stem cells and regeneration. Cell stem cell. 2014 Jul 3;15(1):14-25.
[38]. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, et al. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod. 2011 Aug;37(8):1109-15.Pubmed PMID: 21763903.
[39]. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod. 2008 Jun 1;34(6):645-51.
[40]. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod. 2014 Jan;40(1):51-5.Pubmed PMID: 24331991.
[41]. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod. 2012 Oct;38(10):1372-5.Pubmed PMID: 22980180.
[42]. Alves FR, Silva MG, Rôças IN, Siqueira JF Jr. Biofilm biomass disruption by natural substances with potential for endodontic use. Braz Oral Res. 2013 Jan-Feb;27(1):20-5.Pubmed PMID: 23306623.
[43]. Alves FR, Neves MA, Silva MG, Rôças IN, Siqueira JF Jr. Antibiofilm and antibacterial activities of farnesol and xylitol as potential endodontic irrigants. Braz Dent J. 2013;24(3):224-9.Pubmed PMID: 23969910.
[44]. Chávez-Andrade GM, Tanomaru-Filho M, Rodrigues EM, Gomes-Cornélio AL, Faria G, Bernardi MIB, et al. Cytotoxicity, genotoxicity and antibacterial activity of poly(vinyl alcohol)-coated silver nanoparticles and farnesol as irrigating solutions. Arch Oral Biol. 2017 Dec;84:89-93.Pubmed PMID: 28965044.
[45]. Constantino JA, Delgado-Rastrollo M, Pacha-Olivenza MA, Pérez-Giraldo C, Quiles M, González-Martín ML, et al. In vivo bactericidal efficacy of farnesol on Ti6Al4V implants. Rev Esp Cir Ortop Traumatol. 2016 Jul-Aug;60(4):260-6.Pubmed PMID: 27239017.
[46]. Joo JH, Jetten AM. Molecular mechanisms involved in farnesol-induced apoptosis. Cancer Lett. 2010 Jan 28;287(2):123-35.Pubmed PMID: 19520495.
[47]. Burke YD, Stark MJ, Roach SL, Sen SE, Crowell PL. Inhibition of pancre-

atic cancer growth by the dietary isoprenoids farnesol and geraniol. Lipids. 1997 Feb;32(2):151-6.Pubmed PMID: 9075204.

[48]. Fernandes Costa A, Evangelista Araujo D, Santos Cabral M, Teles Brito I, Borges de Menezes Leite L, Pereira M, et al. Development, characterization, and in vitro-in vivo evaluation of polymeric nanoparticles containing miconazole and farnesol for treatment of vulvovaginal candidiasis. Med Mycol. 2019 Jan 1;57(1):52-62.Pubmed PMID: 29361177.

[49]. Hisajima T, Maruyama N, Tanabe Y, Ishibashi H, Yamada T, Makimura K, et al. Protective effects of farnesol against oral candidiasis in mice. Microbiol Immunol. 2008 Jul;52(7):327-33.Pubmed PMID: 18667031.

[50]. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990 Jul 4;82(13):1107-12.

[51]. Voigt W. Sulforhodamine B assay and chemosensitivity. Chemosensitivity. 2005 : 39-48.

[52]. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. J Vis Exp. 2014;(88).

[53]. Miller MW, Nowakowski RS. Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. Brain Res. 1988 Aug 2;457(1):44-52.Pubmed PMID: 3167568.

[54]. Crane AM, Bhattacharya SK. The use of bromodeoxyuridine incorporation assays to assess corneal stem cell proliferation. Methods Mol Biol. 2013;1014:65-70.Pubmed PMID: 23690005.