ECRONICON OPEN ACCESS

EC DENTAL SCIENCE

Research Article

Bio-Active Restorative Materials Based on Chilean and Uruguyan Propolis as Alternative Pit and Fissure Sealants in Pediatric and Preventative Dentistry: *In Vitro* Investigation

V. Tamara Perchyonok* and Rafael Fellitti

Research and Development Department of VTPCHEM PTY LTD, Australia

***Corresponding Author**: Tamara Perchyonok V, Research and Development Department of VTPCHEM PTY LTD, Australia.

Received: December 30, 2016; **Published:** January 28, 2017

Abstract

Background: Resin-based pit-and-fissure sealants are often used to form a barrier on the occlusal surface of molars to treat caries lesions; however, bacteria can remain in the pit and fissures without detection, increasing the risk of secondary caries. During this time, pit and fissure sealants have been shown to be effective in reducing the risk of occlusal caries.

Aim: The aim of the investigation is to further develop and evaluate a versatile designed chitosan based bio-active materials based on Chilean and Uruguanon for use as bonding free fissure sealant/fissure protectors on permanent dentition and evaluate remineralization/demineralization capacity of the materials through pH cycling, as well as shear bond strength etch and no etch prototype and compare the property with the commercially available standard. Mechanical properties of the designed materials were investigated and they include: compressive strength, modulus of elasticity, biodegradability. Preliminary microbiological performance of the materials was carried out.

Results: Shear bond strength to enamel has increased in the presence of conventional etch and bond technique as well as non etch/bond techniques increase in the enamel treated with the modified Premise containing bio-active materials compared to the bond strength of the conventionally bonded teeth. The increase in bond strength was also observed in the groups of hydrogen peroxide exposed samples (*in vitro* system to evaluate effects of conventional in-house tooth whitening products commonly present in oral hygiene products used on the day to day basis and commonly accepted as products which adversely effecting enamel strength as well as dental material bonding capacity and therefore additional benefits of antioxidant content of the bioactive fissure sealants. The increase in bonding effectiveness is durable effect (up to 6 months).

It been demonstrated for the first time in the evaluated materials that treatment with chitosan effects demineralization of tooth enamel significantly in contrast to minor effect on the remineralization process.

All the test samples gave an average inhibition zone larger than the tetracycline control disc, thereby confirming the antibacterial activity of the different bio-active containing combinations against *Staphylococcus aureus.*

Conclusion: The materials developed, designed and tested were tested for further development of "dual function restorative flowable materials". We quantified the effects of functional designed biomaterials on the enamel bond strength to the enamel, bio-adhesion, microbiology properties as well as remineralization/demineralization and the total phosphorous loss as a model for evaluation of the designed materials as effective fissure sealant restorative materials with additional bioactive capacity. Preliminary microbiological data cytotoxicity data suggests that propolis containing flowable restorative materials have a promising future as a bioactive restorative materials.

Clinical significance: Fissure sealants are recommended to be applied soon after the tooth eruption, mainly at the level of the first permanent molars. The additional benefits of the application of bioactive fissure sealant materials lies in the build-in functionality of these materials to chemical attack in oral cavity as well as additional antibacterial action.

Keywords: *Bio-Active Material; Chitosan; Demineralization; Remineralization*

Introduction

Resin-based pit-and-fissure sealants are often used to form a barrier on the occlusal surface of molars to treat caries lesions; however, bacteria can remain in the pit and fissures without detection, increasing the risk of secondary caries. Sealants with antimicrobial properties or microbial repellent actions might be advantageous [1]. and their success largely depends on the long term retention and tight micromechanical adhesion to enamel surfaces [2].

Resin based sealants can be classified as either filled or unfilled to the filler contents. There is a great deal of controversy regarding the most appropriate type for pit and fissure sealant. Droz., *et al.* reported that filled sealant is less likely to completely fill a fissure than an unfilled sealant because a less viscous sealant would penetrate the fissure more deeply [3].

Barnes., *et al.* however, reported that the viscosity and flow properties of fissure sealants did not affect their sealing ability several studies have also shown similar penetration capability and retention in the 2 sealant types [4].

Recently, flowable composites have been marketed as pit and fissure sealants with the view that flowable composites have a higher wear resistance. One study reported that 20% of practitioners used flowable composites as sealants and 29% of the practitioners used bonding systems before applying flowable composites or compomers [5].

The same study also reported that no practitioners used bonding agents with classical sealants. If flowable composites have a comparable bonding quality with enamel without using a bonding system, they can be recommended for widened occlusal fissures with the benefit of a better abrasion resistance than conventional filled sealants.

Chitosan and modified chitosans are interesting candidates in this respect. Chitosan, a natural linear bio-polyaminosaccharide is obtained by alkaline deacetylation of chitin [6]. This material is also biocompatible and biodegradable. It is positively charged and combines with the bacterial cell wall and membrane with bacteriostatic and bactericidal results. Muzzarelli., *et al.* [7] Phosphorus chemical analysis was carried out by a spectroscopic method described elsewhere [8].

Propolis, also known as bee glue, is a natural nontoxic resinous sticky substance produced by honeybees through mixing the secretions of their hypopharyngeal glands with the digested product of resins collected from leaves, flowers of plants, trees, and certain barks, which is used as a sealant and sterilizer in honeybee nests [9]. It is dark green or brown in color, and its chemical content depends on the geographic zone from which it comes [10].

Dental use of propolis has been emphasized by several studies; in addition to decrease dentinal hypersensitivity and permeability of dentin and occlude dentinal tubules [11], it has been found to be beneficial in many aspects, including prevention of dental caries [Parolia, 2010]; reduction of oral mucositis resulted from chemotherapy [Abdulrhman, 2012;29:285–92]; oral cancer [Kuo YY, 2013]; gingival and periodontal diseases; plaque inhibition and anti- inflammatory [12] as a constituent of dentrifice to control oral microbiota [13]; as an effective transport medium for increasing periodontal ligament cell viability of avulsed teeth [14]; direct pulp capping [15]; and as an analgesic [16]. Moreover, as an antiviral, it delays growth and progression of skin changes in an early stage of infection with Herpes simplex and does not cause cytotoxic effect [16].

Assay design

A random experimental design with 5 groups (Premise, 10% PC, 10% PCC, 10% PU and 10% PUC) containing 3 pieces each was used, and all groups were submitted to the pH cycling. One group (blank) was prepared without exposed dental enamel. This was achieved by completely coating the piece with acid resistant varnish. The blank was added to the experiment in order to verify that the materials employed in the pieces did not promote release or absorption of calcium and phosphorus, therefore, having no effect in quantitative analyses of these ions. Two other groups (control) did not contain chitosan in the samples, and were cycled in solutions DE4.0 and DE4.8, respectively. Bioactive modified chitosan containing flowable composites were applied on enamel by means of a small brush and cured for 20 seconds. After a certain period (time of chitosan action), the sample was submitted to pH cycling. The results were analyzed using the software Prism. Averages were compared by the Student test in the 95% confidence level.

The demineralization and the remineralization processes were followed separately by phosphorous chemical analysis. The amount of phosphorous released (DE) or absorbed by the dental specimen was calculated cycle by cycle. The sum of 5 cycles are represented in the figure, separately for the demineralization and the remineralization process for the different group of specimens studied. The figure shows also the net phosphorous loss (net P loss=DE-RE).

Shear bond strength etch and no etch prototype

Extracted human molars were used within 2 months of storage in water containing thymol crystals. Only undamaged teeth were selected. The roots of the teeth were removed and all the occlusal enamel exposed. The teeth were embedded in 10 mm length PVC (Consjit Tubing, SA PVC, JHB, RSA) pipes with cold cure acrylic resin so that the exposed enamel is projected well above the acrylic and the dentin then thoroughly washed under tap water. Two modified flowable materials composite studs each with an internal diameter of 2.6 mm and height of 1 mm, were bonded to the enamel surface of each tooth via etching with orthophosphoric acid (37%) prior to applying and curing with modified flowable material or no etching used prior to applying the modified flowable material.

In this way, 30 tooth samples (each containing two studs) were prepared and divided at random into 5 groups of 8 each. The teeth were stored in a solution of artificial saliva. After 24 h, the shear bond strength of one stud of each tooth was tested for failure (Zwick Universal Testing Machine, Germany) by means of a knife-edged rod at a crosshead speed of 0.5 mm/min. The other stud was tested after 6 months. All data were analyzed using the non-parametric ANOVA test.

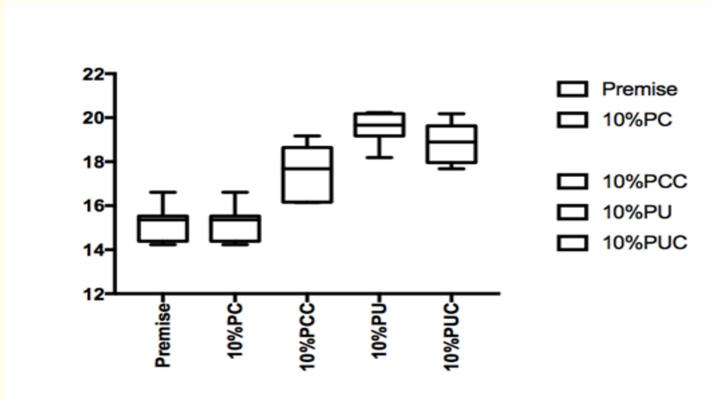
Microbiology Investigations

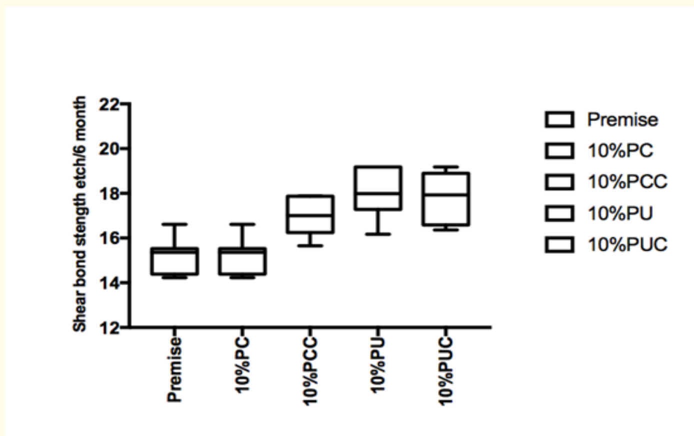
A type strain of *Staphylococcus aureus* (ATCC 12600), obtained from the American Type Culture Collection (Manassas, USA) was used as test bacterium for estimating the antibacterial activity of the hydrogels. The antibacterial activity of the prepared chitosan hydrogels were tested using the standard Kirby-Bauer agar disc diffusion method. Five to 6 mm deep Muller-Hinton agar (Oxoid, Basingstoke, UK) plates were inoculated by streaking a standardized inoculum suspension that match a 0.5 McFarland standard and containing 10^7 - 10^8 colony forming units/ml with a throat cotton swab. For each test sample 500 µg of hydrogel was applied to a 6 mm diameter paper disc. The paper discs were placed on the inoculated Muller-Hinton agar medium and incubated at 37^0C for 24 hours. The diameter of the zones of growth inhibition was measured with a caliper. Each measurement was done in triplicate and the testing of each sample was repeated 3 times. The antibacterial efficacy of the prepared gels were compared to antibiotic sensitivity discs (Mast Laboratories, Merseyside UL) containing 30 µg of tetracycline per disc.

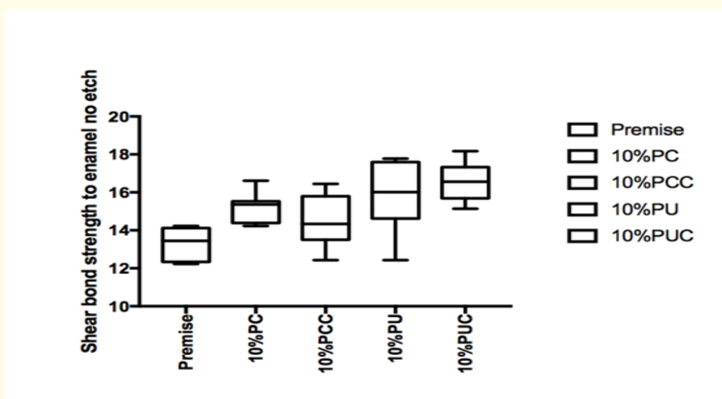
Results and Discussion

Figure 1a and Figure 1b gives the shear bond strength values (MPa) after 24 hours and after 6 month of storage of samples in artificial saliva, respectively, using conventional fissure sealant protocol discussed in the experimental section. In general there was an increase in bond strength of the enamel treated with the modified Premise containing bio-active materials compared to the bond strength of the conventionally bonded teeth.

An increase in the shear bond strength was also previously reported [17] for chitosan containing hydrogels. Interestingly the increase in bond strength was also observed in the groups of hydrogen peroxide exposed samples suggesting that there additional benefits associated with bioactive flowable composites system are in need of further investigations [18].


22
20
18
16
14
12
Premise
10%PC
10%PCC
10%PU
10%PUC
Premise
10%PC
10%PCC
10%PU
10%PUC


Shear bond stength etch/6 month
22
20
18
16
14
12
Premise
10%PC
10%PCC
10%PU
10%PUC
Premise
10%PC
10%PCC
10%PU
10%PUC


Shear bond strength to enamel no etch
20
18
16
14
12
10
Premise
10%PC
10%PCC
10%PU
10%PUC
Premise
10%PC
10%PCC
10%PU
10%PUC

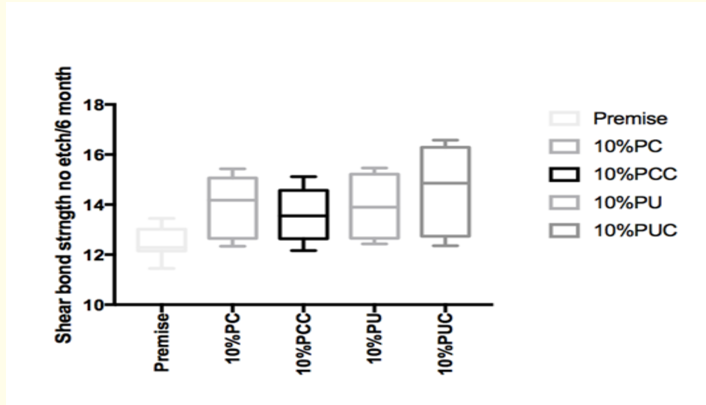


Figure 1: *a, b, c and d Shear bond strength in mPa. a. enamel bonding with etching after 24 hours debonding. b. enamel bonding with etching after 6 month debonding. c. shear bond strength to enamel bonding with no etching after 24 hours debonding. d. shear bond strength to enamel bonding with no etching after 6 month debonding.*

The results of this study suggests that the optimum results for the strengthening of enamel can be achieved throughout the immediate treatment with bioactive: chitosan with the increase of enamel bond strength. Also, impressively an almost immediately after the corresponding modified flowable material treatment and proceeding with bonding procedures is recommended with the significant increase in bond strength. The additional benefit of using chitosan: antioxidant system as a bonding/pre-bonding to enamel and dentin system lies in its ability to show favorable immediate results in terms of bonding effectiveness as well as the durability of resin-enamel bonds for a prolonged time (up to 6 months) [17].

Remineralization/de-mineralization results and total P loss: Effects of chitosan on de-remineralization of dental enamel

The influence of chitosan can also clearly be seen in Figure 2. This figure shows the release and uptake of phosphorous after 5 cycles of de-remineralization, according to the chemical analysis of the solutions. It is seen that release of phosphorus into the demineralizing solution (i.e., loss of phosphorus from the samples) showed larger amplitude (from 610.2 mg to 101.3 mg) than the uptake of phosphorus by the samples from the remineralizing solution (from 125.2 mg to 66.1 mg). Therefore, the treatment with chitosan seems to act more on the demineralization of tooth enamel with little effect on the remineralization process. Regarding the net phosphorus loss (net P loss), it can be seen that net demineralization occurs in all cases. However, the net amount of phosphorous released by the control group samples was significantly higher than those groups treated with chitosan. The net P loss for the control group was 485 mg of P, whereas the groups containing chitosan had a net P loss in the range of 30 - 182 mg.

The important aspect of any newly designed/ developed restorative material is cytotoxicity as Grobler., *et al*. [18] investigated the cytotoxic effect of bioactives such as Chilian or Uruguyan propolis.

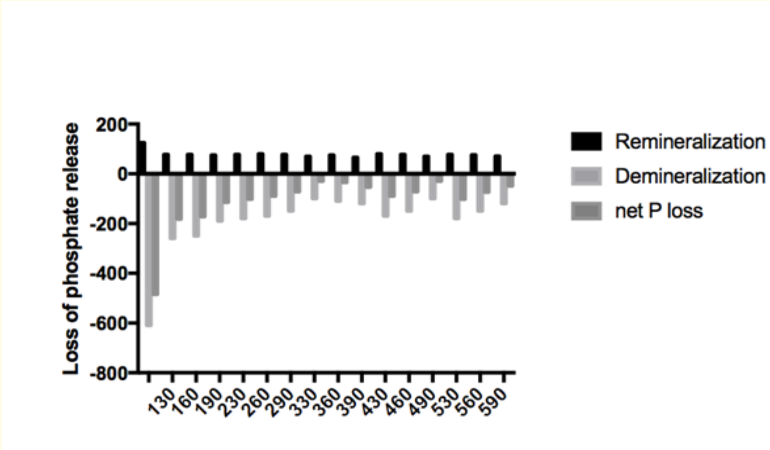


Figure 2: *Cumulative phosphorous content of demineralization (expressed as negative number) and remineralization solution and their difference (net P loss) after 5 cycles for each group of samples investigate.*

Bioadhesion and bioactive flowable materials

Higher adhesiveness of the gels is desired to maintain an intimate contact with skin or tooth structure and results are summarized in Table 1. Modified bioactive flowable composites showed the highest adhesive force and the work of adhesion this can be expected because of the well known intrinsic bioadhesive properties of chitosan as well as propolis (Uruguyan and Chielian) [19]. The adequate water absorption capacity together with the cationic nature which promotes binding to the negative surface of skin or enamel structure can also interpret this results (Table 1).

Bio-active flowable restorative materials	Adhesive Force (N) ± SD (Enamel)	Work of Adhesion (Ncm) ±SD (Enamel)
Premise	1.12 ± 0.35	3.45 ± 0.30
10%PC	1.21 ± 0.47	3.92 ± 0.46
10%PCC	1.13 ± 0.40	3.44 ± 0.29
10%PU	1.09 ± 0.24	3.45 ± 0.34
10%PUC	1.18 ± 0.40	3.89 ± 0.44

Table 1*: Bioadhesion testing in vitro.*

The presented values are an average (n = 5)

Chitosan hydrogels showed the highest adhesive force and the work of adhesion this can be expected because of the well known intrinsic bioadhesive properties of chitosan. The detailed investigation of the newly produced bio-materials with particular attention being paid towards understanding of the exact nature of interaction between bio-actives such as propolis, chitosan and enamel structure are currently on the way in our laboratory.

Microbiology and bio-active restorative materials: *in vitro* investigation

All the test samples gave an average inhibition zone larger than the tetracycline control disc, thereby confirming the antibacterial activity of the different bio-active containing combinations against *Staphylococcus aureus* (Figure 3). Using the Student's T-test ($p < 0.01$), there was a significant difference between the rest of the samples when compared to each other and the positive control.

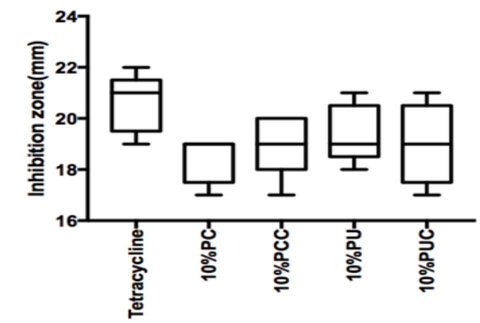


Figure 3: *Inhibition Zone against Staphylococcus aureus (Tetracycline 30 μg).*

The presence of *S. aureus* in the dental caries development and progression and therefore development the bio-active flowable composites can contribute to development of protective fissure sealant materials and work is currently on the way in our laboratory to develop this materials further [19-42].

Conclusion

The materials developed, designed and tested were tested for further development of "dual function restorative flowable materials". We quantified the effects of functional designed biomaterials on the enamel bond strength to the enamel, bio-adhesion, microbiology properties as well as remineralization/demineralization and the total phosphorous loss as a model for evaluation of the designed materials as effective fissure sealant restorative materials with additional bioactive capacity. Preliminary microbiological data cytotoxicity data suggests that propolis containing flowable restorative materials have a promising future as a bioactive restorative materials.

Acknowledgements

VTPCHEM PTY LTD would like to thank Paz Ovalle and Laboratorio Rotterdam for generous supply of sample propolis.

Bibliography

1. Simonsen RJ. "Pit and fissure sealant: A review of the literature". *Pediatric Dentistry* 24.5 (2002): 393-414.

2. Duangthip D and Lussi A. "Effects of fissure cleaning methods, drying agents, and fissure morphology on microleakage and penetration ability of sealants in vitro". *Pediatric Dentistry* 25.6 (2003): 527-533.

3. Droz D., *et al*. "Penetration and microleakage of dental sealants in artificial fissures". *Journal of Dentistry for Children* 71.1 (2004): 41-44.

4. Barnes DM., *et al*. "Flow characteristics and sealing ability of fissure seal-ants". *Operative Dentistry* 25.4 (2000): 306-310.

5. Duangthip D and Lussi A. "Variables contributing to the quality of fissure sealants used by general dental practitioners". *Operative Dentistry* 28.6 (2003): 756-764.

6. Vishu Kumar AB., *et al*. "Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and pronase, and their bactericidal action against Bacillus cereus and Escherichia coli". *Biochemical Journal* 391.2 (2005): 167-175.

7. Muzzarelli R., *et al*. "Antimicrobial properties of N-carboxybutyl chitosan". *Antimicrobial Agents and Chemotherapy* 34.10 (1990): 2019-2023.
8. Murphy J and Riley JP. "A modified single solution method for the determination of phosphate in natural mater". *Analytica Chimica Acta* 27 (1962): 31-36.
9. Coutinho A. "Honeybee propolis extract in periodontal treatment: A clinical and microbiological study of propolis in periodontal treatment". *Indian Journal of Dental Research* 23.2 (2012): 294.
10. Skaba D., *et al*. "Influence of the toothpaste with brazilian ethanol extract propolis on the oral cavity health". *Evidence-Based Complementary and Alternative Medicine* (2013).
11. Mahmoud AS., *et al*. "The effect of propolis on dentinal hypersensitivity and level of satisfaction among patients from a university hospital Riyadh, Saudi Arabia". *Indian Journal of Dental Research* 10.4 (1999): 130-137.
12. Wieckiewicz W., *et al*. "Does propolis help to maintain oral health?" *Evidence-Based Complementary and Alternative Medicine* (2013).
13. Ghisalbert EL. "Propolis: A review". *Bee World* 60.2 (1979): 59-84.
14. Sanghavi T., *et al*. "Evaluation and comparison of efficacy of three different storage media, coconut water, propolis, and oral rehydration solution, in maintaining the viability of periodontal ligament cells". *Journal of Conservative Dentistry* 16.1 (2013): 71-74.
15. Bretz WA., *et al*. "Preliminary report on the effects of propolis on wound healing in the dental pulp". *Zeitschrift für Naturforschung C* 53.11-12 (1998): 1045-1048.
16. Scheller S., *et al*. "Biological properties and clinical application of propolis. IX. Experimental observation on the influence of ethanol extract of propolis (EEP) on dental pulp regeneration". *Arzneimittelforschung* 28.2 (1978): 289-291.
17. Perchyonok V Tamara., *et al*. "Bioactive-Functionalized Interpenetrating Network Hydrogel (BIOF-IPN): A Novel Biomaterial Transforming the Mechanism of Bio-Repair, Bio-Adhesion and Therapeutic Capability – An In Vitro Study". *Journal of Interdisciplinary Medicine and Dental Science* 3 (2015): 166.
18. Perchyonok V Tamara., *et al*. "Evaluation of tetracycline containing chitosan hydrogels as potential dual action bio-active restorative materials capable of wound healing: in vitro approach". *Biointerface Research in Applied Chemistry* 4.5 (2014): 843-849.
19. Abbas G Fleming., *et al*. "Cuspal movement and microleakage in premolar teeth restored with a packable composite cured in bulk or increments". *Journal of Dentistry* 31.6 (2003): 437-444.
20. Aguiar F., *et al*. "Effect of light curing modes and light curing time on the microhardness of a hybrid composite resin". *Journal of Contemporary Dental Practice* 8.6 (2007): 1-8.
21. Alomari QD., *et al*. "Effect of liners on cusp deflection and gap formation in composite restorations". *Operative Dentistry* 26.4 (2001): 406-411.
22. Alrahlah A., *et al*. "Post-cure depth of cure of bulk fill dental resin-composites". *Dental Materials* 30.2 (2014): 149-154.
23. Bae K., *et al*. "Effect of water-soluble reduced chitosan on Streptococcus mutans, plaque regrowth and biofilm vitality". *Clinical Oral Investigations* 10.2 (2006): 102-107.

24. Ceballos L., *et al*. "Curing effectiveness of resin composites at different exposure times using LED and halogen units". *Medicina Oral Patologia Oral y Cirugia Bucal* 14.1 (2009): E51-E56.

25. De Las Casas., *et al*. "Residual Stresses and Cracking in Dental Restorations due to Resin Contraction Considering In-Depth Young's Modulus Variation". *Applied Biological Engineering - Principles and Practice* (2012).

26. El-Nawawy M., *et al*. "Depth of Cure and Microhardness of Nanofilled, Packable and Hybrid Dental Composite Resins". *American Journal of Biomedical Engineering* 2.6 (2012): 241-250.

27. García AH., *et al*. "Composite resins. A review of the materials and clinical indications". *Medicina Oral Patologia Oral y Cirugia Bucal* 11.2 (2006): E215-E220.

28. Leprince J., *et al*. "Investigating filler morphology and mechanical properties of new low-shrinkage resin composite types". *Journal of Oral Rehabilitation* 37.5 (2010): 364-376.

29. Lindberg A., *et al*. "Effect of power density of curing unit, exposure duration, and light guide distance on composite depth of cure". *Clinical Oral Investigations* 9.2 (2005): 71-76.

30. Meredith N and Setchell DJ. "In vitro measurement of cuspalstrain and displacement in composite restored teeth". *Journal of Dentistry* 25.3-4 (1997): 331-337.

31. Mulder R., *et al*. "Volumetric change of flowable composite resins due to polymerization as measured with an electronic mercury dilatometer". *Oral Biology and Dentistry* 1 (2013): 1-5.

32. Poskus LT., *et al*. "Influence of placement techniques on Vickers and Knoop hardness of class II composite resin restorations". *Dental Materials* 20.8 (2004): 726-732.

33. Price RB Labrie., *et al*. "Effect of Distance on Irradiance and Beam Homogeneity from 4 Light-Emitting Diode Curing Units". *Journal of the Canadian Dental Association* 77 (2011): b9.

34. Ramp LC., *et al*. "Hardness and wear resistance of two resin composites cured with equivalent radiant exposure from a low irradiance LED and QTH light-curing units". *American Journal of Dentistry* 19.1 (2006): 31-36.

35. Rastelli ANS., *et al*. "Effect of different light-curing techniques on hardness of a microhybrid dental composite resin". *Brazilian Dental Science* 17.1 (2014): 45-53.

36. Rueggeberg F A., *et al*. "Lifetime intensity profiles of 11 light-curing units". *Journal of Dental Research* 75 (1996): 380.

37. Salerno M., *et al*. "Surface morphology and mechanical properties of new-generation flowable resin composites for dental restoration". *Dental Materials* 27.12 (2011): 1221-1228.

38. T Arnaud., *et al*. "Chitosan effect on dental enamel de-remineralization: An in vitro evaluation". *Journal of Dentistry* 38.11 (2010): 848-852.

39. Tagliferro EPS., *et al*. "Combined effects of carbon dioxide laser and fluoride on demineralized primary enamel: an in vitro study". *Caries Research* 41.1 (2007): 74-76.

40. Watts DC., *et al*. "Characteristics of visible-light-activated composite systems". *British Dental Journal* 156.6 (1984): 209-215.

41. Yaman BC., *et al*. "The effects of halogen and light-emitting diode light curing on the depth of cure and surface microhardness of composite resins". *Journal of Conservative Dentistry* 14.2 (2011): 136-139.

42. Zorzin J., *et al*. "Bulk-fill resin composites: Polymerization properties and extended light curing". *Dental Materials* 31.3 (2015): 293-301.

Volume 7 Issue 5 January 2017