SEM Analysis of Residual Dentin Surface in Primary Teeth Using Different Chemomechanical Caries Removal Agents

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SEM Analysis of Residual Dentin Surface in Primary Teeth Using Different Chemomechanical Caries Removal Agents

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Background: *The purpose of this in vitro study was to analyze the residual dentinal surfaces following caries removal using two chemomechanical methods (Papacarie Duo and Carie Care), by scanning electron microscopy (SEM).* ***Study design:*** *Twenty extracted primary molars with active occlusal carious lesions were randomly assigned two groups depending on the CMCR agent used for the caries excavation – Group 1 – with Papacarie Duo and Group – 2 with Carie Care. After the caries excavation, the specimens were subjected to SEM analysis.* ***Results:*** *Though both the agents showed the minimal smear layer with the patent dentinal tubules, Carie care showed patent dentinal tubules with a clearly exposed peritubular and intertubular collagen network.* ***Conclusion:*** *Carie Care treated surface exhibited better surface morphology of residual dentin.*

Key words: *SEM, residual dentin, chemomechanical caries removal, Carie care, primary teeth*

INTRODUCTION

Dentin substrate is a vital hydrated composite material with structural components and properties that may be altered by physiological processes, age and diseases. Significant variations in its architecture may occur according to the depth and response to previous injuries such as carious lesions and cavity preparations[1, 2]. With the advent of the adhesive restorative materials, the concept of caries excavation with preservation of healthy dentin has gained popularity. In order to accomplish this principle, the procedures available include: air abrasion with aluminum oxide, CMCR, atraumatic restorative therapy (ART) and most recently, lasers[3]. The CMCR method stands out among the other minimal invasive options as it address the advantages like cost effectiveness, patient's compliance, avoid the use of local anesthesia, preserve the healthy dentin intact and facilitate ultimate tissue preservation[4]. The CMCR system involves selective dissolution of outermost portion (infected layer), by the application of a natural or synthetic agent, followed by atraumatic mechanical removal leaving behind the affected demineralized dentin that can be remineralized and repaired[5].

Considering the advantages of CMCR, a gel based on papain containing chloramine and toluidine blue named Papacarie Duo™ was developed in Brazil in 2003. A number of studies can be found in the literature evaluating the clinical efficacy and surface topography of residual dentin and remnant tooth structure with this material after caries removal[2, 5-8]. The data corroborated, indicated Papacarie Duo™ to be a clinically efficient material in terms of the dentin surface morphology and non-interference with the resin composite bonding[2, 9, 10].

An alternative to Papacarie Duo™, named Carie Care™ was launched in India in 2011 with an intent to be more cost effective, which is composed of papain, clove oil, and chloramine[9]. This gel is based on papaya extract, an endoprotein rich in basic amino acids in combination with essential therapeutic oils. The active ingredient has proteolytic action that would soften the pre degraded collagen of the lesion without pain or any undesirable effects on adjacent healthy tissues with added antiseptic and anti-inflammatory properties of the essential therapeutic oils[11, 12].

Due to the difference in composition, the two materials will yield a different pattern of dentin substrate. Therefore giving the relevance to the subject, the emergence of new CMCR material and the scarcity of the studies in this regard on primary teeth, the present study aimed at evaluating remaining dentinal substrate after caries removal in primary teeth using two different CMCR agents–Carie Care™ and Papacarie Duo ™ using SEM.

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MATERIALS AND METHOD

Twenty recently extracted human primary molars with an active occlusal carious lesion and internal or external root resorption indicated for extraction from the out patients visiting the Department of Pedodontics and Preventive Dentistry, K.L E Society's Institute of Dental Sciences, Bangalore were included for the study. The teeth were extracted, collected and stored as per the occupational safety and health regulations[13]. The informed consent was obtained from all participating parents or legal guardians of minors for using the extracted teeth for the research purpose. The study was approved by the institutional research ethics committee prior to the commencement. Each tooth had occlusal caries to a depth of approximately 1-2mm below the central fissure, as assessed with an exploratory probe. Immediately after the extraction, the teeth were stored in deionized water and were evaluated within seven days. All the teeth were cleaned with prophylactic instruments and were randomly divided into two basic groups according to the method of caries removal for evaluation of ultrastructure of residual dentin.

Group I–consisted of 10 samples prepared after caries removal using Papacarie Duo™ (Formula & Acao, Sao Paulo SP, Brazil).

Group II–consisted of 10 samples prepared using Carie Care™ (Innovation - Hub 5, Bangalore, India).

The caries removal was performed using either of the two methods according to the manufacturer's instructions. The gel was applied on to the carious lesion and left undisturbed for 30- 60 seconds. The softened carious dentin was then scraped using a blunt excavator (EXC 245, Hu–Friedy Mfg. Co., LLC, Chicago). The procedure was repeated 2–3 times on an average and caries removal was ceased when the gel attained a non-turbid appearance, which was considered caries free. The residual gel was then removed and the cavity was wiped with a moistened cotton pellet and rinsed. The complete procedure of caries excavation required a mean time of 6-8 minutes. The visual and the tactile criteria were followed for confirmation of the complete removal of active carious lesion. The visual method of assessment of caries removal was based on non-turbid appearance and unchanged light color of the CMCR agent used for caries excavation. The tactile assessment of sound dentin was gauged by the test performed with an exploratory probe[14].

Sample preparation for SEM viewing

The specimens of both groups were subjected to an ultrasonic bath for 5 minutes in sterile distilled water. Subsequently they were dried overnight in a desiccator until all residual moisture had been removed. Then the prepared samples were sputtered coated (Sputter coater, Q150T S, Quorum technologies Ltd, United Kingdom) and introduced into vacuum chamber of SEM (FE-SEM, Carl Zeiss Neon 40 Crossbeam, Germany). A series of micro-photographs were taken at a magnification of x5000 and x10,000 for viewing the surface morphology.

RESULTS

Differences were noticed between the dentin surfaces obtained after using the two different caries removal systems. The images of dentin after caries removal in Group–I are shown in Figures–1 and 2. The morphological details of remaining dentinal substrate in Group–I exhibited two different patterns of remaining dentin. A very irregular and rough surface covered by an amorphic layer indicating the presence of smear layer, obliterating the dentinal tubules and few patent dentinal tubules (Figure – 1). The dentinal tubules were patent, inorganic dentin debris were detected with little smear layer and exposed dentinal tubules. (Figure – 2)

In Group II, a substantial portion of dentin surface was free from smear layer. The dentin surface possessed intertubular microporosity. The surface morphology showed patent dentinal tubules with a clearly exposed peritubular and intertubular collagen network. The exposed superficial intertubular collagen network is obviously different from that of the normal dentin sample. The distinctly reticular collagen fiber network with a random orientation was noted (Figure – 3). The dentinal tubules were open in most locations due to a minimal smear layer. Some residual smear layer and a fibrous structure inside the tubules were revealed. Partial opening of dentinal tubules with a residual mineral deposit was demonstrated (Figure – 4). A noticeable smear layer and occluded dentinal tubules remained on the residual dentin surface were present in some areas (Figure – 5).

DISCUSSION

It has been reported that chemo-mechanically treated dentin has a higher surface energy than conventionally treated dentin. This implies that the chemo-mechanically treated dentin may have greater affinity for adhesive materials and better bonding than conventionally treated dentin[15]. Thus the present study was undertaken to assess the ultramorphology of residual dentin in primary teeth after caries excavation with two CMCR agents. The result of this study supports our hypothesis and suggests that samples prepared using Carie Care™ exhibited better surface morphology than that obtained with Papacarie Duo™.

In order to accomplish the objective of biomimetic dental reconstruction and maintain the pulpal vitality after restoration, it is important to evaluate the state of residual dentin after excavation of caries[16]. Using histologic, biochemical, biomechanical, microscopic and microbiologic techniques, researchers were able to distinguish two layers in caries lesions that were very different in nature i.e. outer carious dentin and inner carious dentin[17]. The remineralization concept involves systematic approach to caries removal end point determination especially till the transparent zone within the inner carious dentin followed by the adhesive restoration[16]. This may be attributed to the presence of sound collagen structure and vital odontoblastic process[18].

In the Papacárie Duo™ group, two patterns of residual dentin were observed. The first pattern revealed an amorphic layer indicative of smear layer and obliterated dentinal tubules, in accordance to previous studies[2, 19, 20]. The second presented a regular surface with patent dentinal tubules and minimal smear layer was discerned[2]. Whereas in case of Carie Care™ micrographs, it was observed that the dentinal tubules were open with some residual smear layer and fibrous structure inside the tubules. Another observation was the

Figure – 1 Scanning Electron Micrograph of dentin surface in Group I (x5000, 3µm) revealed amorphic layer indicative of smear layer and obliterated dentinal tubules

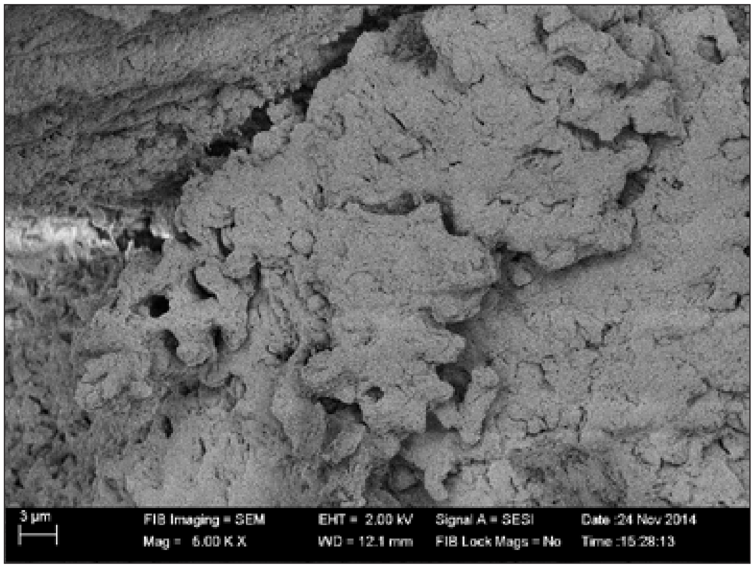


Figure – 2 Scanning Electron Micrograph of dentin surface in Group I (x10,000, 2µm) indicating patent dentinal tubules

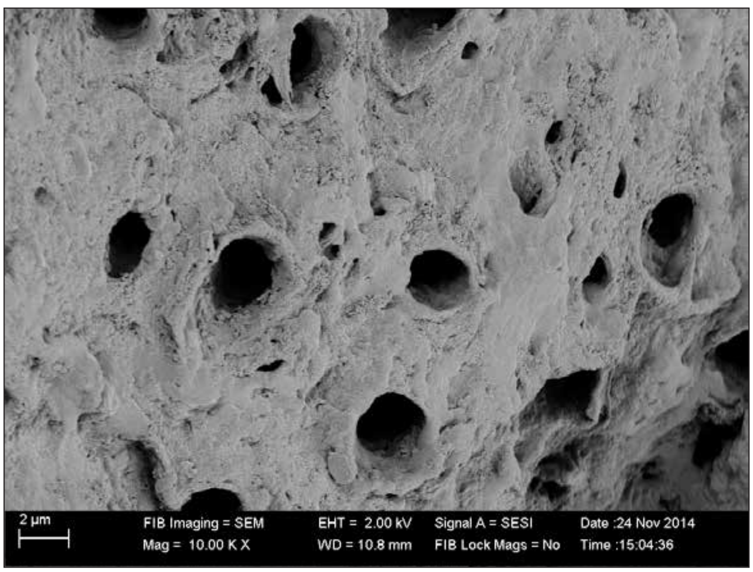


Figure – 3 Scanning Electron Micrograph of dentin surface in Group II (x10,000, 1µm) revealed intertubular microporosity with open dentinal tubules and minimal smear layer

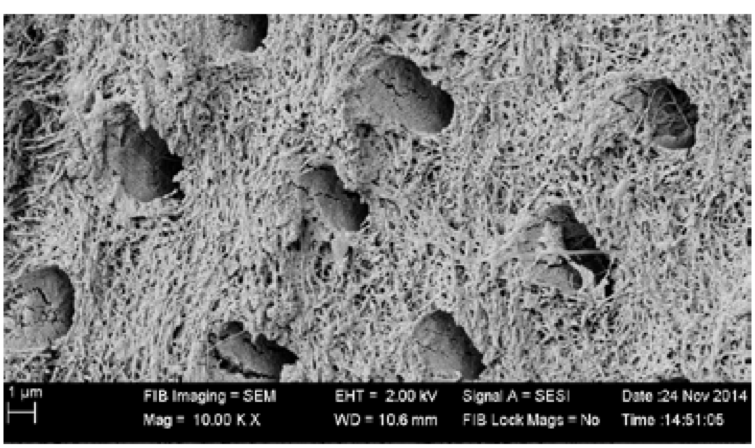


Figure – 4 Scanning Electron Micrograph of dentin surface in Group II (x10,000, 1µm) demonstrated fibrous structure inside the tubules and cross banded collagen fibrils

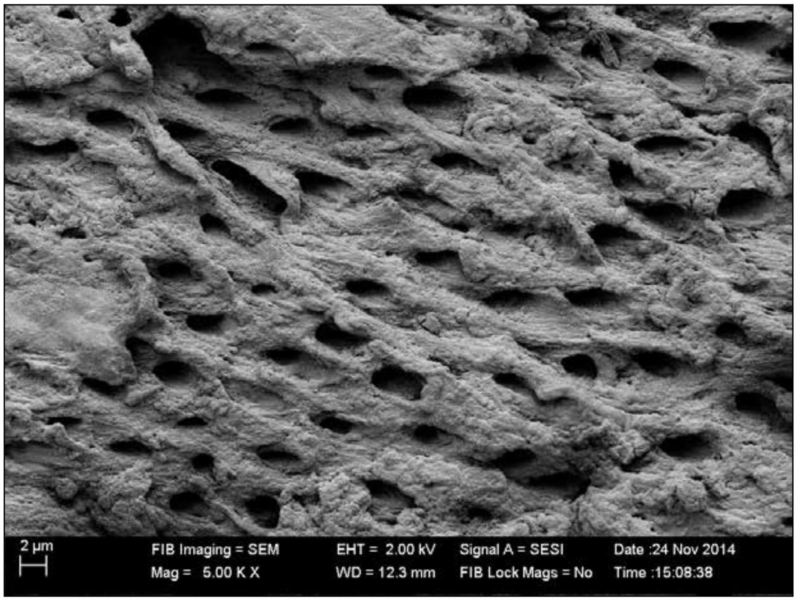
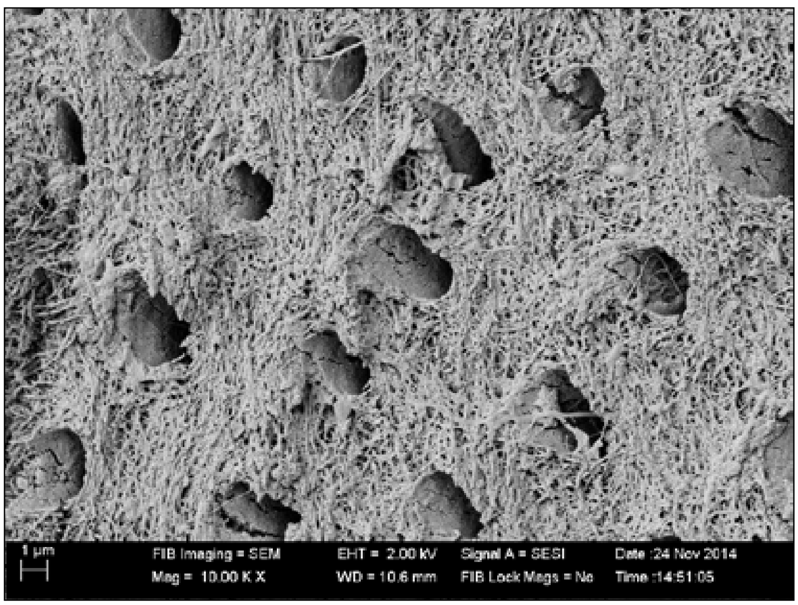


Figure – 5 Scanning Electron Micrograph of dentin surface in Group II (x5000, 2µm) with occluded dentinal tubules



presence of some fibrous structures in the dentinal tubules and the circular orientation of the peritubular collagen network suggestive of residual mineral deposit[21].

In the present study the fibrous structure inside the tubules were appreciated in the specimens treated with the Carie Care™ representative of mineral deposit. The presence of mineral in the tubules is a well-known phenomenon that leads to formation of acid resistant transparent dentin, due to occlusion of the tubules which acts as a barrier to penetration of various irritants [1, 22, 23]. Moreover the presence of crossbanded collagen in the inner dentin is believed to be essential in the remineralization process since apatite crystals can attach to these collagen molecules [1, 24, 25]. Similar findings of mineral crystals along with cross banded collagen in the peritubular and the intertubular dentin was reported in the specimens treated with Carie Care which confirms the removal of the caries infected dentin and approaching the transparent zone of the caries affected dentin approaching the transparent zone of caries[23].

According to the literature, peritubular dentin does not induce significant changes in overall mechanical properties of the dentin, and dentin properties are largely dependent on the properties of the intertubular dentin. Similarly, it is likely that the intratubular mineral deposits do not contribute significantly to the mechanical properties of the dentin[23, 26, 27]. Moreover, in some instances, mineralization appears to increase substantially in caries affected dentin, and such an increase can be accounted for only if the intertubular dentin becomes hypermineralized [23]. Similar results have also been reported for primary carious dentin [28].

The presence of open dentinal tubules in chemo-mechanical caries removal with various CMCR agents is attributed to the initial high pH of the gel and the mechanical preparation technique[29, 30]. This may be due to the presence of chloramine, a component of the CMCR agents which resulted in the opening of dentinal tubules in the outer layer of carious dentin. Chloramine may interact with both collagen and other proteins, facilitating dissociation of the collagen fibre structure [30]. It decomposed degenerated collagen, which caused selective softening of the dentin consistently removing the smear layer and exposing the dentinal tubules of carious dentin [15]. The essential therapeutic oils which is one of the component of the Carie Care is rich in phenolic compounds namely eugenol and eugenol derivatives which are precursors of flavones, isoflavones and flavonoids. They have antioxidant, anti-inflammatory, DNA-protective, analgesic and antimicrobial properties [31]. It will provide the further protection to the residual dentin.

CONCLUSION

Scanning electron microscopy analysis revealed a difference in ultramorphology of residual dentin in primary teeth treated with the two CMCR agents. The specimens treated with Carie Care™ demonstrated the presence of the cross banding of peritubular and intertubular dentin representative of transparent zone of the caries affected dentin. Our finding supports the concept of obtaining sound dentin and therefore should provide a better bonding surface morphology to adhesive restorative material.

REFERENCES

1. Marshall GW, Marshall SJ, Kinney JH, & Balooch M. The dentin substrate: structure and properties related to bonding. Journal of Dent 25(6): 441-458, 1997.
2. Corrêa FNP, Rodrigues Filho LE, Rodrigues Delgado CRM. Evaluation of residual dentin after conventional and chemomechanical caries removal using SEM. J Clin Pediatr Dent. 32(2): 115-120, 2007.
3. Beeley JA, Yip HK and Stevenson AG. Chemochemical caries removal: a review of the techniques and latest developments. Br Dent J 188(8): 427-430, 2000.
4. Ramamoorthi S, Nivedhitha MSB, Vanajassun PP. Effect of two different chemomechanical caries removal agents on dentin microhardness: An in vitro study. Journal of Conservative Dentistry 16(5): 429-433, 2013.
5. Corrêa FN, Rocha RDE, Rodrigues Filho LE, Muench A, Rodrigues Delgado CRM. Chemical versus conventional caries removal techniques in primary teeth: A microhardness study. J Clin Pediatr Dent. 31(3): 187-192, 2007.
6. Kotb RM, Abdella AA, El Kateb MA, Ahmed AM. Clinical evaluation of Papacarie in primary teeth. J Clin Pediatr Dent 34(2): 117-23, 2009.
7. Matsumoto SFB, Motta LJ, Alfaya TA, Guedes CC, Fernandes KPS, Bussadori SK, Assessment of chemomechanical removal of carious lesions using Papacarie Duo ™: Randomized longitudinal clinical trial. Indian Journal of Dental Research, 24(4): 488-492, 2013.
8. Motta LJ, Martins MD, Porta KP, Bussadori SK. Aesthetic restoration of deciduous anterior teeth after removal of carious tissue with Papacarie. Indian J Dental Res. 20(1): 117-120, 2009.
9. Lopes MC, Mascarini RC, Garcia da Silva BMC, Florio FM, Basting RT. Papain-based Gel for Caries Removal on Dentin Shear Bond Strength. Journal of Dentistry for Children 74(2): 93-97, 2007.
10. Bussadori SK, Castro LC, Galvao AC. Papain gel: a new chemomechanical caries removal agent. J Clin Pediatr Dent 30(2): 115–119, 2005.
11. Venkataraghavan K, Kush A, Lakshminarayana C, Diwakar L, Ravikumar P, Patil S, Karthik S. Chemomechanical Caries Removal: A Review & Study of an Indigen-ously Developed Agent (Carie Care (TM) Gel) In Children. J Int Oral Health 5(4):84-90, 2013.
12. Thakur R, Patil S, Kush A. Chemo-mechanical Caries removal technology – Dentistry at ease. Indian Dentist 9(3): 22-27, 2014.
13. CDC. Guidelines for infection control in dental health-care settings .mmwor2003; 52(RR17):1-66, 2003.
14. Sakoolnamarka R, Burrow MF, Tyas MJ. Morphological study of demineralized dentin after caries removal using two different methods. Aust Dent J 47(2): 116–122, 2002.
15. Hosoya Y, Kawashita Y, Marshall GW Jr, Goto G. Influence of Carisolv for resin adhesion to sound human primary dentin and young permanent dentin. J Dent. 29(3): 163-171, 2001.
16. Alleman DS, Magne P. A systematic approach to deep caries removal end points: The peripheral seal concept in adhesive dentistry. Quintessence Int 43:197-208, 2012.
17. Magne P, Oganesyan T. CT scan based finite elemental analysis of premolar cuspal defelction following operative procedures. Int J of Periodontics Restorative Dent 29: 361-546, 2009.
18. Fusayama T. Intratubular crystal deposition and remineralization of carious dentin. J Biol Buccale **19**: 255-262, 1991.
19. Arora R, Goswami M, Chaudhary S, Chaitra TR, Kishore A, Rallan M. Comparative evaluation of effects of chemomechanical and conventional caries removal on dentinal morphology and its bonding characteristics – A SEM study. Eur Arch of Ped Dent 13(4): 179-183, 2012.
20. Cardoso PEC, Moura SK, Miranda Júnior WGM, Santos JFF, Tavares AU. Carisolv as an alternative method for caries removal. Revista da Postgraduacao 11(2): 109–113, 2004.
21. Sakoolnamarka R, Burrow MF, Tyas MJ. Morphological study of demineralized dentin after caries removal using two different methods. Aust Dent J 47(2): 116–122, 2002.
22. Marshall, GW, Habelitz S, Gallagher R, Balooch M, Balooch G, & Marshall SJ Nanomechanical properties of hydrated carious human dentin. Journal of Dental Research 80(8): 1768-1771, 2001.
23. Ogawa K, Yamashita Y, Ichijo T, & Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. Journal of Dental Research 62(1):7-10, 1983.
24. Wefel JS. Root caries histopathology and chemistry. Am J of Dent **7**: 261-265, 1994.
25. Perdigao J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater 26: e24-37, 2010.
26. Kinney JH, Balooch M, Marshall SJ, Marshall GW Jr, & Weihs TP. Hardness and young's modulus of human peritubular and intertubular dentin. Archives of Oral Biology 41(1): 9-13, 1996.
27. Kinney JH, Balooch M, Marshall GW, & Marshall SJ. A micromechanics model of the elastic properties of human dentin. Archives of oral Biology 44(10): 813-822, 1999.
28. Hosoya Y, Marshall SJ, Watanabe LG, & Marshall GW. Microhardness of carious deciduous dentin. Operative Dentistry 25(2): 81-89, 2000.
29. Tonami KI, Araki K, Mataki S, Kurosaki N. Effect of chloramines and sodium hypochlorite on carious dentin. J Med Dent Sci 50: 139-146, 2003.
30. Teodorovici MP, Sandu AV, Pancu G, Stoleriu S, & Andrian S. A comparative study on the topography of the dentinary surface resulting after the removal of pathological tissues by various techniques. Odontology 14(4): 278-282, 2010.
31. Grespan R, Paludo M, Lemos HDP, Barbosa CP, Bersani-Amado CA, Dalalio MMDO, & Cuman RKN. Anti-arthritic effect of eugenol on collagen-induced arthritis experimental model. Biological and Pharmaceutical Bulletin 35(10): 1818-1820, 2012.