Volume : 3 | Issue : 5 | May 2014 • ISSN No 2277 - 8179

Research Paper

Antibacterial property of hydroxyapatite compared to Glass Ionomer Cement and Amalgam

Medical Science

KEYWORDS : Hydroxyapatite, GIC, Amalgam retrograde, antibacterial properties

*** Dr M. K. Hilal**

Department of Oral Maxillofacial surgery , Faculty of dentistry , Al-jabal Al-gharbi University, Gharian, Libya. * Corresponding Author

ABSTRACT

The aim of this study was to evaluate antimicrobial activity of Hydroxyapatite (HAP) which been fully investigated previously by the author24,25 (in its biocompatibity In vitro and in vivo its chemical, physical, mechanical sealing ability properties) compared with Gutta-percha (GP), glass ionomer cement (GIC) and amalgam (Amg) used as retrograde root filling materials. The antibacterial activity of retrograde Filling material was assessed using the Agar diffusion inhibitory test. HAP and GIC, GP were compared with amalgam commonly used retrograde root filling materials. The four retrograde materials were placed on agar (MH) plate inoculated with Streptococcus anginosus (milleri) and Enterococcus faecalis, bacteria commonly found in peri-apical lesions (. At interval of 24, 48, and 72 hours). the presence and diameter of zone of inhibition were recorded with precision ruler for each material and its incubation period. The diameter of zone of inhibition increased with time for all materials, GIC had the most pronouncede antibacterial activity against bacteria, producing the largest zone of inhibition, followed by HAP.

Introduction:

The root-end filling material should improve the sealing of the existing canal filling or provide an apical sealing to an otherwise unobturated root canal, preventing the movement of bacteria and bacterial products from the root canal system to periapical tissues[1,2]. These substances should also be biocompatible, non-toxic, non-carcinogenic, easy to use, and should not be sensitive to moisture[3,4].

With the purpose of obtaining root-end sealing in endodontic surgery, many different materials have been suggested. The most commonly used ones are amalgam, glass ionomer cement, zinc oxide, eugenol cement, gutta-percha and composite resins[4]

More recent studies have shown mineral trioxide aggregate (MTA) as a promising material for root-end filling[5,6]. Root-end filling materials have been tested for leakage assessment, marginal adaptation, cytotoxicity and usage tests[1,2,5,6,7,8,9].

GIC release fluoride affects bacterial viability with respect to carbohydrate transport over the cell membrane and subsequent acid production GIC act against cariogenic property of bacteria[11].

Gutta percha has shwn to exhibit some antimicrobial activity due to zinc content recently antseptis MGP with iodoform or chlorhexidin have more antimicrobial activity[12].

Hydroxyapatite (HAP) has been studied extensively and its biocompatibility is well recognized wheter implanted in bone or soft tissue[13,14,15,] or hard tissue[16,17] Synthetic hydroxyapatite with a stoichiometric composition as Ca10 (PO4)6(OH)2 has an excellent biocompatibility with human teeth and bone,. Mangin et al. (2003)[18] tested its sealing ability in its use as a root-end filling material. Lucas et al. (2003)[19] tested the improvement of the mechanical strength of glass ionomer cement by adding HA. Their results indicated that hydroxyapatite-added glass ionomer cement has a greater potential as a reliable restorative material with improved fracture toughness, long-term bonding strength to dentin and unimpeded ability of sustained fluoride release[23].. . . Kouassi et al.(2003)20 studied the antibacterial effect of hydraulic calcium phosphate for dental applications and found that calcium bis- dihydrogenphosphate monohydrate CaO-based cement has an antibacterial effect and is potential candidate for pulp capping and cavity lining. In study by Tin-Oo et al 200721of newly produced hydroxyapatite they found that HAP possess antibacterial activity against streptococcus mutans. Studies on biological properties of IRM (Intermediate Restorative Material), with the addition of HA as a retrograde root filling material showed antibacterial activity against Streptococcus anginosus (milleri) and Enterococcus faecalis on blood agar plates with standardized pellets of HAP-modified forms (Owadally et al., 1994). Ingram et al. (1996)[22] also studied the antibacterial effect of porous hydroxyapatite granules. They found that cultures of Staphylococcus epidermidis evinced inhibition

The aim of retrograde filling is to seal the root canal system by means of an apical cavity preparation, which is then filled using a material with adequate physical, chemical and biological properties. The ideal root-end filling material must possess certain characteristics, including biocompatibility, adequate marginal sealing, ability to allow or induce bone repair, and antimicrobial activity [(21)]. As hydroxyapatite ceramic is one of the specialty Calcium Phosphate Products being used as, adjuvant to dental implants and cements (dental and orthopedic), dentrifices, maxillo-facial surgery, pulp-capping materials, repair of periodontal defects and repair of failing implants, the study of antibacterial properties of this material is an area of profound interest.

Alghough bacteria superficially adhering to root canal dentin might be more easily killed than those protected in the depths of dentinal tubules , bacteria inside dentinal tubules might also be affected by antibacterial activity of retrograde filling material and sealer such as calcium hydroxide, HAP, GIC, MTA and Kalizinol[21].

Root-end resection may be the treatment of choice for teeth in which adequate nonsurgical retreatment had failed to elemnate existing periapical pathosis, thus following the resection, efforts should be made to seal the root-end and prevent microleakage, improving the surgical endodontic procedure by selecting proper root end fiiling material to elemenate the etiological factor and attempt to prevent microleakage and promote periradicular (bone) tissue healing. Some materials used in RCT may elict foreign body reaction in the perapical tissue such as talc-contaminated gutta-percha, the celluse component of paper points, cotton wool and food material of vegetable origin .[20,23].

The purpose of this study was to evaluate the antmicrobial activity of Hydroxyapatite (which fully investigated in our previous studies regarding thermal stability, mechanical, physical chemical, and ((biocompatibity In vitro and In vivo)) with bone tissue)[24] againest Streptococcus miller and Entrococcus faecalis compared with the antibacterial activity of Glass ionomer amalgam and guttapercha commonly used

Materials and Methods

In this study, we had four experimental groups as follows: group 1, Hydroxyapatite ;group 2, Glass ionomer cement; group 3, Amalgam; group 4, Gutta Percha.Amalgam (SDI Ltd Australia) Glass ionomer cement GIC(AHL Generic UK) 3. Hydroxyapatite (HAP) (Plasma biotal UK) 4. Gutta-percha (calium hydroxide sealapex)(Dencare Ltd UK)

The study was conducted on double-layered plates, in which the base layer was made of 10 mL of sterilized Muller-Hinton agar (MH) poured into 2×10 cm sterilized Petri plates. Four uniform cavities or wells (4-mm diameter, one for each test material) were punched at equidistant points in agar by means of a sterile copper coil after 24 h. The cavities were filled by materials immediately after being perpared according to the manufacturer's instructions standerized pellets as 4mm diameter.

Antibacterial activities of the selected materials were evaluated against the Streptococcus anginosus (milleri) and Enterococcus faecalis, commonly found in Per iapical lesions and a mixture of these bacteria was evaluated by the agar diffusion method. The strains were obtained from the Department of Microbiology, Faculty of Medicin TRIPOLI, University. After activation from stock culture, microorganisms were maintained in MH broth until use. Overnight cultures of the microorganisms were done. All the microbial strains were grown at 37°C for 24 h in MH broth and then seeded into 15 mL of the MH agar, to produce a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 10^8 colony forming units ml^{-1}. For the mixture group, the second layer contained equal concentrations of each microorganism. The seeded agar was added over the plates immediately after the insertion of freshly prepared pellets of test materials. The plates were kept at room temperature for 2 h for pre-diffusion of the materials and then incubated at 37°C for 72 h. under anaerobic condition and zone of growth inhibition were measured at 24, 48, and 72 hours. The inhibition zones around the wells were then measured with a precision millimeter ruler with accuracy of 0.5 mm. Data were subjected to the Kruskal-Wallis and Dunn test at a 5% significant level. The mean and standerd diviation of diameter of growth of inhibition zone of the test materials were statistically analysed using Analysis of variance (ANOVA) and the inhibition zones associated with each material and each duration were compared by Tukey's honest significant difference(HSD) pairwise comparison. All statistical analyses were performed with the SPSS statistical software package, and all results were evaluated at the 5% significance level.

Results:
The mean diameters of zones of inhibition caused by the four root-end materials are presented in Table 1. The 4 trials yielded consistent results.

All four root-end materials caused zones of inhibition. However, the Amalgam had little effect on the tested micro-organism. The Glass ionomer cement and Hydroxyapatite exhibited the largest inhibition zones. The Amalgam and Gutta-percha showed mild antimicrobial activity against micro-organism.

The results after 48 and 72 hours showed that the effectiveness of the root–end materials increased slightly with time.

Duration of incubation and mean diameter (SD) of inhibition zone (mm)a

Table 1: Mean diameter of zones of growth inhibition with four root-end materials

Materials	72hours	48 hours	24 hours
GIC	(30,31,31,32) 31 (1.5)	(29,30,30,31) 30(1.7)	(27,28,28,29) 28(1.25)
HAP	(25,25,24,22) 24(2.16)	(20,21,23,24) 22(2.16)	(19,20,22,23) 21(2.21)
Amalgam	(20,21,23,24) 22(2.06)	(18,19,21,22) 20(1.70)	(17,18,19,22) 19(1.41)
Gutta-percha	(17,21,22,23) 20(0.81)	(18.19,19,20) 19(0.95)	(16,18,18,20) 18(1.63)

Each value is the mean of four wells SD = stander diviation

Discussion
The agar diffusion method used in the present study is one of the most commonly employed techniques for evaluation of antimicrobial activity[26,27]. The prediffusion period, which consists of maintaining the innoculated culture medium at room temperature for 2 hours, is an important step for demonstrating the antimicrobial activity of HAP, Amalgam, GIC and Gutta-percha. Our method was in accordance with those obtained by Leonardo et al.[21,29], who employed similar methodology. Dental materials/cements may show different degrees of diffusion in agar.

The data corresponding to the antimicrobial activity of each material were analyzed statistically using the, as advised by Siqueira et al.[28] The microorganisms utilized in this study are predominant in persistent or refractory periapical lesions in teeth subjected to periapical surgery[31,31].

Our results revealed that all of the materials tested possessed antimicrobial activity, substantiated by the formation of growth inhibition zones in all strains evaluated. The antimicrobial activity of GIC release fluoride which may it interfere with bacteria methabolism and colonization was evaluated by Torabinejad et al.[23], who demonstrated its effectiveness against some facultative bacteria.

Gutta percha has shown to exhibit some antimicrobial activity due to zinc content recently antseptis [38] Hydroxyapatite-added to GIC has potential as a reliable retrograde filling material with impreove fracture toughness, bonding to dentine and ability of fluoride release. Fouride can inhibite bacteria's ability to create acids and reduce its survive in dental plaque, hydroxyapatite and fluoroapatite is created during remineralization process Fuoroapatie (fluoride release from GIC added to HAP) rendering the tooth structure less suscebtible to decay. Fluoride reduce cariogenic potential of bacteria by inhibiting their metabolism[21]MGP with iodoform or chlorhexidin have more antimicrobial activity[12] .Several studies investigated fluoride levels released from amalgam[34,36,37]

In this study the antibacterial activity of GIC, HAP, amalgam against Entrococcus faecalis and Streptococcus millers may related to fluoride release which play role in bacteria inhibition by interference with its biosynthetic metabolism and reduce its colonoization, Other component release simultaneously release from some retrograde materials (aluminium, zinc, monomers and catalysts) may involove in antibacterial activity of the root-end materials[35],

Amalgam has been the most commonly used material for retro grade filling, as it is easy to manipulate, available in all dental offices, radio-opaque, well tolerated by peri apical tissues, seals with acceptability due slight expansion on complete setting and non-resorbable. Its disadvantages include, it shows an initial leakage due to contraction at the time of primary setting[7,8,] non sterile, slow setting, amalgam 'cloud', amalgam 'tattooing', reaction of mercury to tissues and corrosion. Different amalgams (including Zn free or high Copper amalgams) are available but vary in properties regarding corrosion and cytotoxicity.[9]

Gutta-percha is malleable to a certain degree and thus adaptable to irregular cavity walls and also inert, inexpensive, does not corrode but heat sealed gutta-percha has showed significant marginal defects, pull-aways, and heat blistering.[10]

HAP and GIC had similar antimicrobial activity, suggesting that the addition of a HAP agent to GIC does not hinder its antimicrobial action.[30] In vivo GIC leads to larger decrease in count of streptococcus mutans and lactobacilli than amalgam inaccordance with our study[(169,170)]. In contrast Wearheijm et al[(171)] found no difference in number of oral strepotococci and lactobacilli. Likewise when Hap was added in IRM as retrograde root filling material (Owadally et al 1994) it was found that streptococcus anginosus (miller) and Entrococcus faecalis growth inhibition were increased with time which is in aacordance with our study The finding of this study has shown that HAP possesses antibacterial properties This studies reiterates as evinced by Ingram et al 1996[39], Owadally 1994[40] and Tin-OoMM et al 2007[21]

Conclusion:

GIC and HAP had the most pronounced anctibacteial activity compared with the other materials Guttapercha and Amalgam. The antibacterial activity of the materials was ranked in the following order GIC>HAPl>guttapercha> amalgam HAP bioactive promote priapical tissue healing and possessed antibacterial activity so it can be used as retrograde root filling material but further clinical studies are required to assess its performance clinically

Acknowledgements

Thanks goes to Who assesst in this work Prof.P. Hauton*, Prof. I. Brook**,Prof. R. VanNoort***

Depatment Biomaterial and tissue engeneering*, OMFS**, Adulth dental health*** School of clinical dentistry University of sheffeild, UK

REFERENCE

1. Dorn SO, Gartner AH. Retrograde filling materials: a retro- spective success-failure study of amalgam, EBA, and IRM. J Endod 1990;16:391-3. | 2. Fischer EJ, Arens DE, Miller CH. Bacterial leakage of min- eral trioxide aggregate as compared with zinc-free amal- gam, intermediate restorative material and Super-EBA as a root-end filling material. J Endod 1998;24:176-9. | 3. Fogel HM, Peikoff MD. Microleakage of root-end filling ma- terials. J Endod 2001;27:456-8. | 4. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am 1992;36:357-78. | 5. Jou Y, Pertl C. Is there a best retrograde filling material? Dent Clin North Am 1997;41:555-61. | 6. Peters CI, Peters OA. Occlusal loading of EBA and MTA root-end fillings in a computer-controlled masticator: a scanning electron microscopic study. Int Endod J 2002;35:22-9. | 7. Regan JD, Gutmann JL, Witherspoon DE. Compari- son of Diaket and MTA when used as root-end filling ma- terials to support regeneration of the periradicular tissues. Int Endod J 2002;35:840-7. | 8. Siqueira Júnior JF, Rôças IN, Abad E, Castro AJR, Ga- hyva SM, Favieri A. Ability of three root-end filling materials to prevent bacterial leakage. J Endod 2001;27:673-5. | 9. Subay RK, Subay A. In vitro sealing ability of dentin bonding agents and cavity varnish with amalgam as ret- rofills. J Endod 1999;25:157-60. | 10. Torabinejad M, Pitt Ford TR. Root end filling materials:a review. Endod Dent Traumatol 1996;12:161-78. | 11. Anti bacterial intervention GIC MI Compendium, 2nd Edition 2006 | 12. A. Podbieiski: Growth inhibition activity of Guttapercha containing root canal medications J. Anim. Sci. 2001. Apri. : 79($) 934-41 | 13.M. OGISO, H. KANEDA, J. ARASAKI and T. TABATA, in ''Biomaterials 1980'',G.D.WINTER, .F.GIBBONS and H.PLENCK (Eds), (John Wiley & Sons, Ltd., London, 1982) p. 59 J. A. JANSEN, J. R. de WIJN, J. M. L. WOLTERS-LUTGERHORST and P. | 14. J. van MULLEM, J. Dent. Res.64 (1985) 891. C. A. van BLITTERSWIJK, S. C. HESSELING, J. J. GROTE, H. 15 1KOERTEN and K. de GROOT, J. Biomed. Mater. Res. 24 (1990) 433.M. JARCHO, J. F. KAY, I. KENNETH, K. I. GUMAER, R. H. DOREMUS | 16. and H. P. DROBECK, J. Bioeng. 1 (1977) 79. | 17. H. W. DENISSEN, K. de GROOT, P. C. H. MAKKES, A. van den HOOFF and P. J. KLOPPER, J. Biomed. Mater. Res.14 (1980) 713. | 18. Mangin C, Yesilsoy C, Nissan R and Stevens R (2003). The comparative sealing ability of hydroxyapatite cement, mineral trioxide aggregate, and super ethoxybenzoic acid as root-end filling materials. J Endo, 29(4): 261-264. | 19. Lucas ME, Arita K and Nishio M (2003). Toughness, bonding and fluoride-release properties of hydroxyapatite-added glass ionomer cement. Biomaterials, 24(21): 3787-3794. | 20. Kouassi M, Michailes P, Lacoste-Armynao A and Boudelle P (2003). Antibacterial effect of hydraulic calcium phosphate cement for dental applications.Journal of Endodontics, 29(2): 100-103. | 21. Tin-Oo MM, Gopalakrishnan, V. Antibacterial property of locally produced Hydroxyapatite: Archives of Orofacial Sciences (2007) 2: 41-44 | 23. Torabinejad et al Fluoride release and recharge property of HAP-GIC 9th word congress on preventive dentistry Sep. 8-10 2009) | 24. Hilal. M. K. Development of a high strength bioactive bone substitute: PhD thesis, 1996 Sheffield University, UK and JDR 1995 Abstr. 609 | 25. Hilal, M.K. In vitro Evaluation of the sealing ability of Hydroxyapatite compared with Glass ionomer cement, Amalgam and Gutta-perch: 2009 Libyan Dental confernce | 26. Georgopoulou M, Kontakiotis E, Nakou M (1993) In vitro evaluation of the effectiveness of calcium hydroxide and paramonochlorophenol on anaerobic bacteria from the root canal. Endod Dent Traumatol 9, 249-253 | 27. Lui JN, Sae-Lim V, Song KP, Chen NN (2004) In vitro antimicrobial effect of chlorhexidine- | impregnated gutta percha points on Enterococcus faecalis. Int Endod J 37, 105-113 | 28. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR Lima KC, Lopes HP (2000) Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 26, 274-277 | 23. Begue WJ, Kline RM (1972) The use of tetrazolium salts in bioautographic procedures. J Chromatogr 64, 182-184 | 29. Begue WJ, Kline RM (1972) The use of tetrazolium salts in bioautographic procedures. J Chromatogr 64, 182-184 | 30. Leonardo MR, Silva LAB, Tanomaru-Filho M, Bonifácio KC, Ito IY (2001) In vitro evaluation of | the antimicrobial activity of a castor oil-based irrigant. J Endod 27, 717-719 | 31. Molander A, Reit C, Dahlen G, Kvist T (1998) Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 31, 1-7 | 32. Sundqvist G, Figdor D, Persson S, Sjogren U (1998) Microbiologic analysis of teeth with failed | endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral | Pathol Oral Radiol Endod 85, 86-93 27. | 33. Silva LAB, Leonardo MR, da Silva RS, Assed S, Guimarães LF (1997) Calcium hydroxide root canal sealers: evaluation of pH, calcium ion concentration and conductivity. Int Endod J 30, 205-209 | 34. Forsten L. Short and long –term fluoride release profiles of restorative glass ionomer formulations Den. Mater. 1996: 12: 88-95 | 35. Hayacibara MF, Ambrozano, GM Simultaneous release of fluoride and aluminium from dental materials Oper. Dent. 2004: 29: 16-22 | 36. Skartveit L. Tveit AB: Fluoride release from a fluoride containing amalgam in vivo : Scand J. Dent. Res.: 1985: 93: 448-52 | 37. Garcia-Goddoy F, Olsen BT: Fluoride release from amalgam restoration lined with silver-reinforced glass ionomer cement: Amr. J. Dent., 1990:3: 94-6 | 38. Imazato S. Antibacterial properties of resin compositions and dentin bonding systems. Dent. Mater. 2003: 19, 449-57 | 39. Ingram AE, Robinson J and Rohrich RJ (1996). The antibacterial effect of porous hydroxyapatite granules. Plast Reconstr Surg, 98(6): 1119. | 40. Owadally ID, Chong BS, Pitt Ford TR and Wilson RF (1994). Biological properties of IRM with the addition of hydroxyapatite as a retrograde root filling material. Endod Dent Traumatol, 10(5): 228-232. | 41. Torabinejad M, Watson TF, Pitt Ford TR (1993) Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 19, 591-595 | 42. Abedi HR, Ingle JI (1995) Mineral trioxide aggregate: a review of a new cement. J Calif Dent Assoc 23, 36-39 |