

ORIGINAL ARTICLE

Antibacterial property of locally produced hydroxyapatite

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(Received 14 February 2007, revised manuscript accepted 7 November 2007)

KEYWORDS

Hydroxyapatite,
antibacterial properties,
Streptococcus mutans

Abstract Use of synthetic hydroxyapatite (HA) in biomedical applications is well warranted. It has shown to have an excellent biocompatibility in human tooth and bones. Additionally it has been documented to possess antibacterial potentials. The present study was conducted to assess the presence of any such potential in locally produced (HA) using *Streptococcus mutans*, a common pathogen in the oral cavity. The study was carried out using 50, 100, 150, 200, 300, 400 and 800 mg/ml concentration of HA. The antibacterial property of HA was assessed using Miles and Misra method. Our studies showed that bacterial growth inhibitions of *S. mutans* occurred from 50 mg/ml, and complete inhibition was perceived at concentrations at 200mg/ml of HA. The antibacterial property HA should be used to good advantage as a bioactive biomaterial in dental and maxillofacial applications.

Introduction

Hydroxyapatite (HA), found in rocks and sea coral, is chemically similar to mineral components of bone and hard tissues in mammals. It is one of the few materials, classified as a bioactive biomaterial that supports bone in growth and osseointegration when used in orthopedic, dental and maxillofacial applications. Synthetic hydroxyapatite with a stoichiometric composition as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ has an excellent biocompatibility with human teeth and bone, making it very attractive for biomedical applications (Fang *et al.*, 1994).

Researchers are extensively investigating the use of HA in various dental applications. Mangin *et al.* (2003) tested its sealing ability in its use as a root-end filling material. Lucas *et al.* (2003) 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. Numerous animal studies have been done to test tissue reaction of the pulp to hydroxyapatite, while being used as a pulp capping material (Maruo, 1990; Jaber *et al.*, 1991; Alliot-Licht *et al.*; 1994; Hayashi *et al.*, 1999; Li *et*

al., 1998).

Li *et al.* (1998) found the use of hydroxyapatite sol as a safe biomaterial for dental pulp treatment, as it induced an early formation of dentine-bridge and possessed some antibacterial activity. It would be best to harness the antibacterial property of HA, by using it as a base in treatment of carious cavities, to act against residual cariogenic bacteria. Similar instances of bioactive material being used in the past have been noted, zinc oxide and eugenol paste as a sealant being one of them, in lieu of its antibacterial properties. But of recent, glass-ionomer is preferred.

Kouassi *et al.* (2003) 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. Since locally produced hydroxyapatite is the family of calcium phosphate, its antibacterial effect needs to be studied.

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) also studied the antibacterial effect of porous hydroxyapatite granules. They found that cultures of *Staphylococcus epidermidis* evinced inhibition

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of growth in each specimen with hydroxyapatite granules. 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. This present study has been undertaken to explore the antibacterial properties of locally produced hydroxyapatite.

Materials and method

Preparation of hydroxyapatite (HA) powder

Locally produced HA powder was weighed in 50 mg, 100 mg, 200 mg, 300 mg, 400 mg and 800 mg. Each concentration of HA was dispensed into glass test tubes. In addition a tube with 200 mg HA served as a control. HA in tubes were sterilized by autoclaving them at 15lbs /15minutes. Following this, 2ml of Brain heart Infusion (BHI) broth was added to the test tubes containing variable quantities, 50mg, 100mg, 200mg, and 300mg, of HA. 4ml of BHI was added to 400mg and 800mg HA tubes (4ml of broth was used as 2ml of broth was not enough to suspend 400 and 200mg HA). 2ml of BHI broth was added control tube.

Test strain and growth condition

Antibacterial activity of HA was evaluated against the bacterial strain, *Streptococcus mutans* (35668) procured from Micro Biologics USA. *Streptococcus mutans* stock was grown in BHI broth at 37°C. After over night incubation, a loop-full of the inoculum was transferred to 2 ml BHI

broth and inoculated for 4 hours. After incubation, the tubes were vortexed and 25 µl of bacterial suspension was added to the tubes with 50 mg, 100mg, 200mg, 300mg of HA and 50 µl to the tubes with 400mg and 800mg of HA (as 4 ml of broth was used to suspend HA) and were incubated overnight. Estimation of bacterial growth inhibition was carried out by the 'Miles and Misra' method (Collins *et al.*, 1995). On the BHI agar plate eight sections were marked, for six concentrations of HA, and one each for the controls (HA and organism control). After overnight incubation of test organism with different concentrations of HA, 10 µl of broth from the test and control tubes were transferred onto the respective marked sections on a BHI agar plate, from a height of 2.5 cm, and allowed to air dry. The plates were subsequently incubated at 37°C for 16-18 hrs, and read for the presence or absence of bacterial growth. Inhibition of bacterial growth was assessed by enumerating the number of colonies in each of the sections using a colony counter. The complete set of assay was carried out in triplicate and outcome recorded.

Results

Bacterial growth was seen only in drop areas of broth from 50 mg and 100 mg HA tubes. No bacterial growth was found in the drops areas from 200 mg tubes onwards (Figure 1). On counting the colonies, a reducing trend in bacterial count was seen in concentrations from 50 mg HA upwards, when compared with the organism control. Similarly, bacterial count was reduced in 100 mg HA when compared to 50 mg. Bacterial counts ranged between 20 to 25 in 50 mg HA and 8 to 10 in HA with 100 mg (Figure 2).

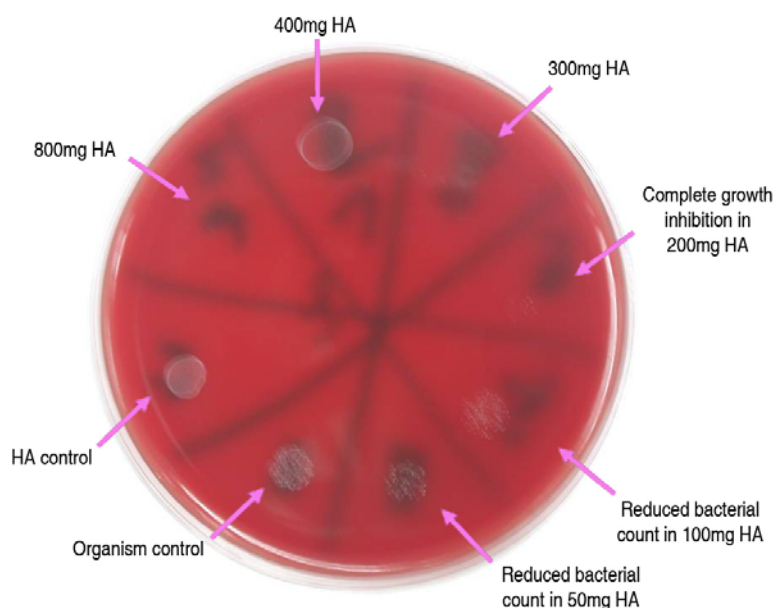


Figure 1 On the BHI agar plate, bacteria colony count was not found in drop areas taken from 200mg tube onwards

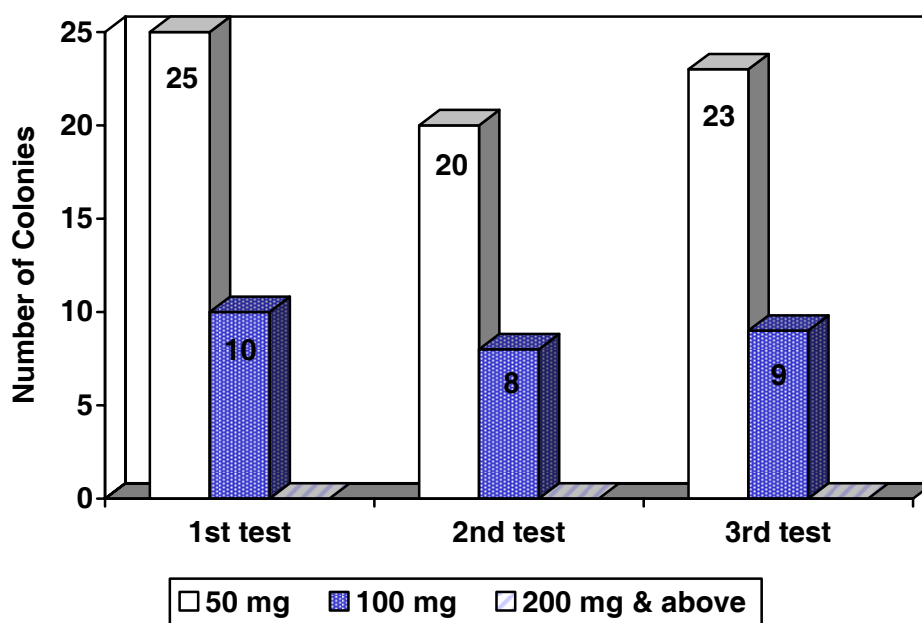


Figure 2 Reducing trend of colonies in increased HA concentration

Discussion

The antibacterial effect of hydroxyapatite has been widely studied by different researchers using various methodologies and different sets of organisms. HA was also incorporated with other materials and assessed for antibacterial potentials. In one such study of antimicrobial effect of human gingival biocompatibility of HA sol-gel coatings was carried out. In this, HA coating containing 100 ppm Ag ions suppressed the growth of *S. mutans* (Chung *et al.*, 2006). Likewise, when HA was added in Intermediate Restorative Material (IRM) as a retrograde root filling material (Owadally *et al.* 1994) it was found that *Streptococcus anginosus (milleri)* and *Enterococcus faecalis* growth inhibition were increased with time with IRM HA-modified forms. In this present study, the antibacterial effect of HA was tested on its inhibition potentials. As HA is not readily dissolved in distilled water or saline, the assessment of microbial growth inhibition using the agar-diffusion test was not a suitable option. Therefore, bacteria were grown in the tubes containing different quantities of HA, and studied using the broth dilution technique. This has an added advantage that, the antibacterial property of HA against the different organism tested can be quantitated.

Tubes containing 200mg of HA or more showed complete inhibition of *S. mutans*, as no bacterial growth was found. This finding has shown that HA possesses antibacterial properties. This study reiterates the fact of HA possesses antibacterial activity as evinced by Ingram *et al.* (1996) and Owadally *et al.* (1994).

In management of dental caries, the infected and necrotic tissue is removed from the cavity to restore the lost tooth structure. Even though after the evacuation the dentin appears without marked discoloration, there can still be a possibility of microbes left in open tubules (Thylstrup and Fejerskov, 1994). As this study has evinced antibacterial activity, against *S. mutans* a cariogenic organism, by HA, it validates its use as a potential candidate for dental applications such as pulp capping or as a base liner material. Further studies are required to determine and assess the spectrum of antibacterial property possessed by HA, by studying its activity against other bacteria, and also the nature of its antibacterial activity, is it bacteriostatic or bactericidal.

Acknowledgements

This research work was supported by USM short term research grant 304/PPSG/613162.

References

- Alliot-Licht B, Jean A and Gregoire M (1994). Comparative effect of calcium hydroxide and hydroxyapatite on the cellular activity of human pulp fibroblasts in vitro. *Arch Oral Biol*, **39**(6): 481-489.
- Chung RJ, Hsieh MF, Huang CW, Perng LH, Wen HW and Chin TS (2006). Antimicrobial effects and human gingival biocompatibility of hydroxyapatite sol-gel coatings. *J Biomed Mater Res B Appl Biomater*, **76**(1): 169-178.
- Collins CH, Lyne PM and Grange JM (1995). Culture media. In: Collins CH, Lyne PM, Grange JM (eds.), *Collins and Lyne's Microbiological Methods*, 7th Edn. Oxford: Butterworth-Heinemann Ltd., pp. 60-93.

- Fang Y, Agrawal DK and Roy DM (1994). Thermal stability of synthetic hydroxyapatite. In: Brown PW, Constantz B (eds.), *Hydroxyapatite and Related Materials*. London: CRC Press, pp. 269-282.
- Hayashi Y, Imai M, Yanagiguchi K, Vilorio IL and Ikeda T (1999). Hydroxyapatite applied as direct pulp capping medicine substitutes for osteodentin. *J Endo*, **25**(4): 225-229.
- Ingram AE, Robinson J and Rohrich RJ (1996). The antibacterial effect of porous hydroxyapatite granules. *Plast Reconstr Surg*, **98**(6): 1119.
- Jaber L, Mascres C and Donohue WB (1991). Electron microscope characteristics of dentin repair after hydroxyapatite direct pulp capping in rats. *J Oral Pathol Med*, **20**(10): 502-508.
- Kouassi M, Michales 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.
- Li T, Akao M and Takagi M (1998). Tissue reaction of hydroxyapatite sol to rat molar pulp. *J Mater Sci Mater Med*, **9**(11): 631-642.
- 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.
- 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.
- Maruo K (1990). Histopathologic study on the application of synthetic hydroxyapatite and alpha-tricalcium phosphate for vital pulpotomy. *Gifu Shika Gakkai Zasshi*, **17**(1): 223-245.
- 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.
- Thylstrup A and Fejerskov O (1994). Clinical and pathological features of dental caries. In: Thylstrup A, Fejerskov O (eds.), *Textbook of Clinical Cariology* (2nd edn.). Copenhagen: Munksgaard, pp. 111-115.