Antimicrobial and sealant properties of nanohydroxyapatite as endodontic sealer

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Abstract

BACKGROUND: Depending on different pH values, nanohydroxyapatite materials have different purities, whether root canal sealers formed by which exert effects on antimicrobial resistance, root canal closure and apical histocompatibility has no reports.

OBJECTIVE: To compare the antimicrobial and sealant properties of nanohydroxyapatite materials with different pH, nanohydroxyapatite-composite and traditional root canal sealants.

METHODS: We detected the antimicrobial action of nanohydroxyapatite with pH 8, 9, 10, Vitapex and AH-Plus root canal sealants with and without addition of ornidazole against three microbial strains namely *Enterococcus faecalis*, *Candida albicans* and *Staphylococcus aureus* using agar diffusion method. And we also analyzed the endodontic microleakage of six root canal sealants by determining the apical reservoir glucose concentration using Glucose Oxidase Method.

RESULTS AND CONCLUSION: Pure nanohydroxyapatite with different pH did not show antimicrobial properties. Addition of ornidazole to nanohydroxyapatite showed greater inhibitory action against *Enterococcus faecalis*, lesser in *Staphylococcus aureus*, followed by *Candida albicans*. Vitapex root canal sealer had inhibitory effects only against *Staphylococcus aureus*. AH-Plus, itself, had antimicrobial activity against all the three strains, but the antimicrobial activity decreased after addition of ornidazole. Nanohydroxyapatite, as a root canal sealant, was superior to zinc oxide eugenol and Vitapex, but inferior to AH-Plus. Addition of ornidazole to nanohydroxyapatite for a short period showed no impact on sealant properties of the material.

Subject headings: biocompatible materials; nanoparticles; durapatite; ointments

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INTRODUCTION

One of the major goals of endodontic treatment is to eliminate microorganisms from the root canal system. This is achieved through mechanical cleaning and shaping, supplemented by antibacterial irrigants, adequate filling of the empty space, and the possible use of antimicrobial dressings[1]. However, treatment might reduce, but not necessarily eliminate them completely. A possible treatment modality is the use of root canal sealers with antimicrobial properties to improve the outcome of endodontic treatment. Commonly used root canal sealants like Calcium hydroxide, AH-Plus, Vitapex, etc, has their own advantages and disadvantages[2].

Hydroxyapatite can be found in teeth and bones within the human body, which is biocompatible and has a potential to promote the healing of bone in endodontic therapy[3]. Although currently more researches are being done on nano hydroxyapatite (nHA) as an endodontic sealer, the material purity varies with change in pH. The antimicrobial effect and histocompatibility of nHA as THE endodontic sealant are yet not to be reported.

Thus, the objective of this study was to analyze the antimicrobial properties of nHA composites, against different microorganisms based on different pH values and also to find out the sealant ability of the material, so as to provide a theoretical basis for its clinical application.

MATERIALS AND METHODS

Design

A comparative observation.

Materials

Electronic mixers, incubator, ultra-quiet bench, BHI Agar, Sabouraud Medium, glucose peroxidase kit, nHA with pH 8, 9, 10, AH-Plus root canal sealer, Vitapex root canal sealer, zinc oxide eugenol, ornidazole and glycerin. Microbial strains: *Enterococcus Faecalis* (ATCC 29212), *Candida Albicans* (ATCC 10231), *Staphylococcus Aureus* (ATCC 25923).

Methods

Antimicrobial test

Experiment groups: (1) nHA with pH 8, 9, 10, Vitapex and AH-Plus, totally five groups. (2) Ornidazole was added to the above mentioned five groups at a mass ratio of 7:3 to make another five groups.

Strain recovery and activation: After individual microbial strain recovery and activation, using Agar diffusion method, we prepared different strains corresponding to the plate medium. Three holes of 0.7 mm in diameter were prepared on each plate and bacterial mediums were uniformly coated on the flat surface of the agar plate. 100 mg of root canal sealants were added to the prepared holes and the plates were inverted and incubated at 37 ℃ for 24 hours to prepare 1×10^5 CFU/mL bacterial liquid for observations and recording.

Measuring zone diameter and result determination[4-5]: After the plates had been overnight incubated, there should be a noticeable "clearing zone" around each of the materials used. The diameter of each inhibition zone was measured and recorded in millimeters (mm). The criteria for recording the microbial inhibition zone test results are as follows: ≥ 0.5 mm as inhibitory effect and < 0.5 mm as no inhibition which is recorded as 0.

Microleakage test

Grouping: This *in vitro* experiment consisted of 60 extracted single rooted, single-canalled teeth where cleaning and shaping of the canals were performed to file size #40. Five experimental groups were: nHA pH=8, nHA pH=9, nHA pH=10+ornidazole (mass ratio 7:3), AH-Plus and Vitapex sealants and one control group: zinc oxide eugenol sealant.

Root canal filling: Lateral condensation technique was used with gutta percha and the individual group sealant to obturate the canals. The length of the gutta percha points from the canal orifice to the root apex was 10 mm and the excess was removed using #4 Gates Glidden drill. Later, vertical pressure was performed for compression of gutta percha in the canals. Thereafter, three layers of nail polish were applied to the tooth surface, except for the apical 2 mm and the canal orifice, which remained exposed, in such a way that the solution could pass through the canal. Teeth were stored in a 37 ℃, 100% humidity incubator for a week to allow for setting of the sealers. The coronal part of the teeth was fixed and sealed on to the larger opening of an Eppnedorf tube, where two-thirds of the tooth is seen outside the tube opening. The upper part of tube was connected to a transparent infusion tube, through which 0.2% N_aN_3 and 1 mL/mol glucose solution was passed. The solution should be passed from the length of the infusion tube to the canal orifice where gutta percha was seen, which was around 15 cm. The solution level in the infusion tube was maintained constantly throughout the experiment. The end of the Eppnedorf tube with the tooth was fixed and sealed on to small bottles. A #12 needle was inserted on the bottle cap for atmospheric contact. The complete setup was placed at 37 ℃ saturated humid atmospheres.

10 μL of the samples was collected from the glass bottles at the intervals of 2, 4, 7, 10, 15, 20, 25 and 30 days respectively. 10 μL of distilled water was added to all the glass bottles after completing each day sample collections. With glucose oxidase kit, the automatic biochemical analyzing instrument was used to record the absorbance value at the wavelength of 490 nm and the corresponding glucose concentration was then calculated.

RESULTS

Inhibition zone for five root canal sealants with and without addition of ornidazole against three microbial strains (**Figure 1**)

AH-Plus sealant showed a defined antimicrobial effect against all three strains. Vitapex sealer had antibacterial effects only with *Staphylococcus aureus*. No inhibitory zone, thereby no antimicrobial effect, was seen for nHA sealants with different pH values (**Table 1**). All three microbial strains showed varying degrees of inhibition zones to all five root canal sealants after addition of ornidazole, with *Enterococcus faecalis* showing the larger inhibition zone diameter followed by *Staphylococcus aureus*, and *Candida albicans* (P < 0.05). Among the three microbes, *Candida albicans* showed the maximum inhibition zone diameter in Vitapex+ornidazole group (P < 0.05; **Table 1**).

Table 1 Inhibition zone diameters of three microbial strains for five types of Sealants with or without ornidazole ($\bar{x}\pm s$, mm)

Group	Without ornidazole			After addition of ornidazole		
	Enterococcus faecalis	*Staphylococcus aureus*	*Candida albicans*	*Enterococcus faecalis*	*Staphylococcus aureus*	*Candida albicans*
pH=8 nHA	0	0	0	20.22±0.47	16.53±0.24	4.27±0.25
pH=9 nHA	0	0	0	18.73±0.27	16.27±0.23	5.56±0.43
pH=10 nHA	0	0	0	19.57±0.45	18.23±0.32	11.89±0.39
Vitapex	0	2.16±0.70	0	13.61±0.17	16.14±0.10	21.14±0.11
AH-Plus	10.44±0.96	6.52±0.39	5.07±0.39	17.39±0.11	10.39±0.17	3.17±0.52

Notes: AH-Plus sealant showed a defined antimicrobial effect against all three strains. Vitapex sealer had antibacterial effects only with *Staphylococcus aureus*. No inhibitory zone, thereby no antimicrobial effect, was seen for nano-hydroxyapatite (nHA) sealants with different pH values. All three microbial strains showed varying degrees of inhibition zones to all five root canal sealants after addition of ornidazole, with *Enterococcus faecalis* showing the larger inhibition zone diameter followed by *Staphylococcus aureus*, and *Candida albicans* (P < 0.05). Among the three microbes, *Candida albicans* showed the maximum inhibition zone diameter in Vitapex+ornidazole group(P < 0.05).

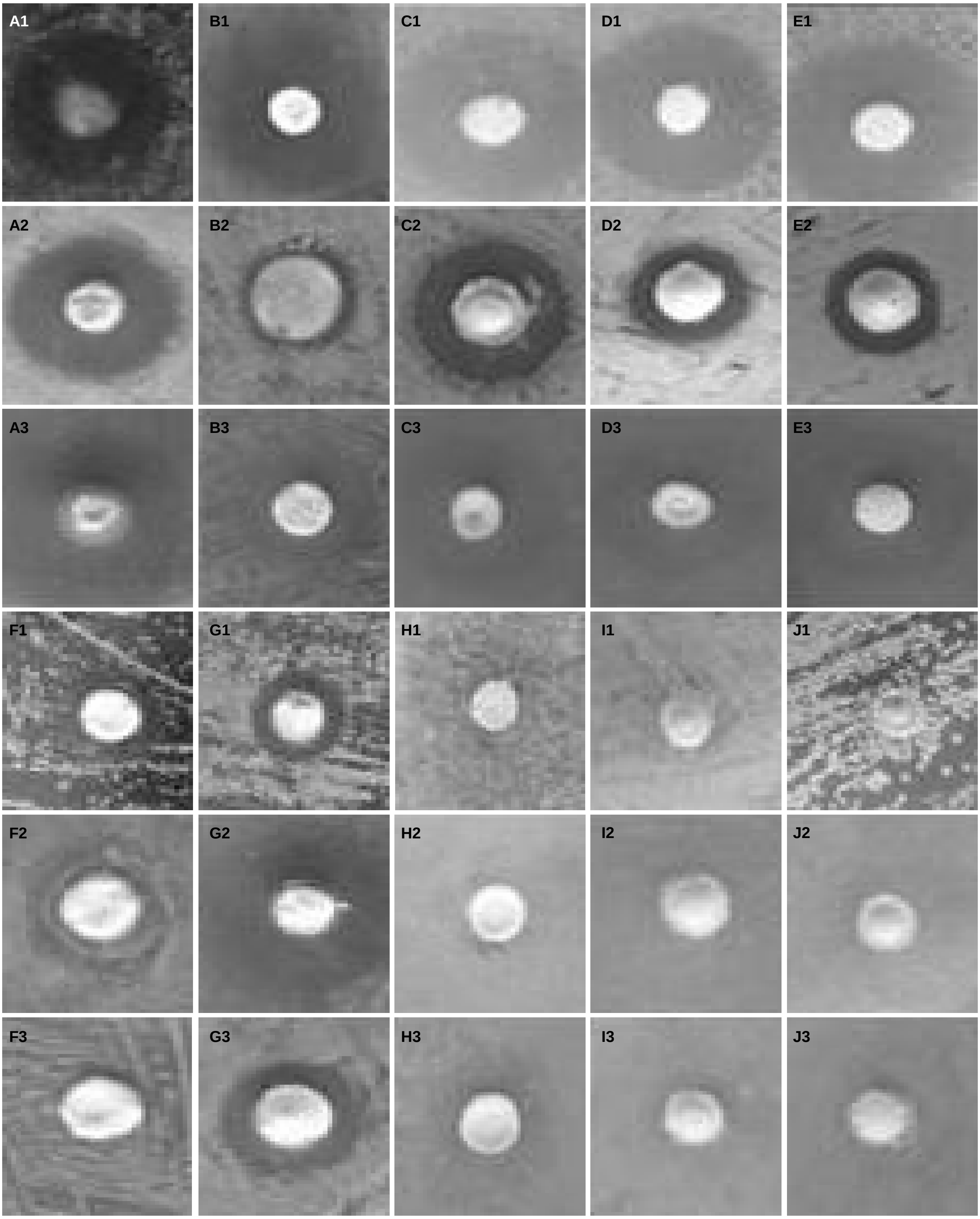

Figure 1 Inhibition zone for different root canal sealants with and without addition of ornidazole against *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*

Notes: A: Vitapex+ornidazole, B: AH-Plus+Ornidazole, C: pH=10 nano-hydroxyapatite+ornidazole, D: pH=9 nano-hydroxyapatite+ornidazole, E: pH=8 nano-hydroxyapatite+ornidazole, F: Vitapex, G: AH-Plus, H: pH=10 nano-hydroxyapatite, I: pH=9 nano-hydroxyapatite, J: pH=8 nano-hydroxyapatite; 1–3 refer to *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*. The bone marrow mesenchymal stem cells are identified by immunofluorescent staining method, and selected by the adhesive method and cultured, and positive staining is observed in the cells and is localized in a perinuclear pattern.

Microleakage test results (**Table 2**)

As shown in Figure 2, in comparison with the control group (zinc oxide eugenol), three nHA groups showed less microleakage *in vitro*, with statistical significance (P < 0.05). Change in the pH of nHA had no significant effect on microleakage (P > 0.05). Significant difference in microleakage was seen in Vitapex group compared with AH-Plus and nHA groups (P < 0.05). nHA and the other root canal sealants, Vitapex and AH-Plus, were much better than zinc oxide eugenol. The least to highest order of microleakage is as follows: AH-Plus, nHA, Vitapex and zinc oxide eugenol sealant. Addition of Ornidazole to nHA for a 30-day period had no significant effects on microleakage.

DISCUSSION

It has been recognized for decades that the ideal end result of root canal therapy would be a closure of the apical foramen with reparative cementum. The goals for stability of successful endodontic therapy are total obliteration of the canal and perfect sealing of the apical foramen at the dentino-cemental junction and accessory canals at locations other than the root apex with an inert, dimensionally stable and biologically compatible material[5-6].

According to other researchers, endodontic sealers are used to eliminate the interface between the gutta-percha and the dentinal walls. Thus, the quality of the filling depends largely on the sealing capacity offered by sealers[7-8].

Various studies has shown that nHA has better biocompatibility and is non cytotoxic. The structure and composition of nHA is similar to natural bone and the particle size of 1–100 nm makes it a better biocompatible material[9-12]. The change in pH affects the structural characterization and purity levels of nHA. The purity level increases with increasing pH where a maximum purity level of nHA is attained at 10.5. The purity of nHA, under acidic conditions, is relatively based on lower amount of calcium in $CaHPO_4$ and that in alkaline conditions , the purity is based on the higher amounts of calcium and phosphorus in $Ca_3(PO_4)_2$ and $Ca_{10}(PO_4)_6(OH)_2$. In this study, the three pH values of nHA under alkaline conditions were tested.

The leakage values are higher for zinc oxide eugenol than nHA. Lower leakage values of nHA as sealer in the present study, may be because of better sealing abilities. The recent studies have shown that commonly used zinc oxide eugenol sealer has continued material shrinkage and poor long term seal. Studies have also shown that Vitapex sealer, of which active ingredients are calcium hydroxide and iodoform, also shows poor sealant properties with long-term postoperative microleakage[13-14]. From this study, AH-Plus sealer has the least microleakage, followed by nHA, Vitapex and finally ZnOE, and addition of ornidazole to nHA has no significant effects on microleakage, which is consistent with previous results[15-17]. Due to short-time duration (30 days), this experiment does not adequately reflect on the long-term sealing ability of nHA as a root canal sealant. To use nHA as a root canal sealant, it has to be considered on to which drugs have to be added as a composite with nHA, so as to produce the required antimicrobial effects. It can be concluded from this study that the nHA based endodontic material can be used as a root canal sealant when it used in combination with a drug that could provide the required antibacterial effect. This study also confirms that nHA has better histocompatibility, root canal sealant properties and is a promising future endodontic sealer. Before reaching a definitive conclusion nHA requires further extensive researches both clinically and *in vitro*.

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Table 2 Microleakage for six root canal sealers ($\bar{x}\pm s$, mmol/L)

Time (day)	pH=8 nHA	pH=9 nHA	pH=10 nHA+ornidazole	ZnOE	AH-Plus	Vitapex
1	0.06±0.05	0.06±0.06	0.07±0.06	0.09±0.08	0.06±0.07	0.07±0.06
2	0.11±0.10	0.12±0.11	0.12±0.10	0.19±0.14	0.11±0.10	0.12±0.11
4	0.20±0.15	0.21±0.16	0.20±0.16	0.33±0.29	0.19±0.15	0.22±0.16
7	0.42±0.36	0.41±0.35	0.43±0.37	0.59±0.46	0.39±0.32	0.43±0.38
10	0.53±0.40	0.52±0.42	0.53±0.43	0.75±0.52	0.48±0.41	0.55±0.43
15	0.94±0.46	0.96±0.45	0.93±0.44	1.48±0.67	0.92±0.44	0.98±0.50
20	16.8±6.13	16.7±6.19	16.9±6.22	25.39±7.42	15.86±6.06	17.79±6.27
25	21.5±7.36	22.0±7.29	21.7±7.25	34.58±8.92	19.89±7.15	23.37±7.66
30	33.6±8.37	33.1±8.69	34.0±8.53	45.39±9.88	31.76±8.13	37.56±8.57

Notes: The least to highest order of microleakage is as follows: AH-Plus, nano-hydroxyapatite (nHA), Vitapex and zinc oxide eugenol sealant (ZnOE). Addition of ornidazole to nHA has no significant effects on microleakage.

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纳米羟基磷灰石复合根充糊剂的抑菌性及微渗漏

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文章亮点：

实验创新性地在碱性范围内选了 3 个 pH 值的纳米羟基磷灰石进行实验，以了解不同 pH 值纳米羟基磷灰石复合根充糊剂是否对细菌产生抑制作用，同时加入奥硝唑，观察对根管封闭性产生的影响，为临床上纳米羟基磷灰石糊剂的应用提供理论基础。

关键词：
生物材料；口腔生物材料；纳米羟基磷灰石；根充糊剂；抑菌性；葡萄糖氧化酶比色测定法；微渗漏

主题词：
生物相容性材料；纳米粒；硬羟基磷灰石；软膏

摘要

背景：不同 pH 值形成纳米羟基磷灰石材料的纳米纯度不同，所形成的根充糊剂是否对抑菌性、根管封闭及根尖组织相容性产生影响尚无报道。

目的：比较不同 pH 值纳米羟基磷灰石根管充填材料、纳米羟基磷灰石复合材料和传统根管充填材料的抑菌性及与根管的密合性。

方法：采用抑菌环实验，研究 pH 值为 8.0，9.0，10.0 的纳米羟基磷灰石复合根充糊剂，Vitapex 糊剂和 AH-Plus 糊剂及对以上各组根充糊剂添加奥硝唑以后的 5 组实验样本(共 10 组)对粪肠球菌、白色念球菌、金黄色葡萄球菌的抑菌性能；此外建立微渗漏模型，通过葡萄糖氧化酶比色测定法测量 pH 值为 8.0，9.0 的纳米羟基磷灰石复合根充糊剂，pH 值为 10 的纳米羟基磷灰石复合根充糊剂+奥硝唑，氧化锌丁香油，Vitapex 糊剂和 AH-Plus 糊剂与根管的密合性。

结果与结论：不同 pH 值的纳米羟基磷灰石本身无抑菌性，加入奥硝唑后有抑菌性，对粪肠球菌抑制性最高，其次是金黄色葡萄球菌，最后是白色念珠株菌。Vitapex 仅对金黄色葡萄球菌有抑制作用。AH-Plus 本身有抑菌性，加入奥硝唑后抑菌性降低。不同 pH 值纳米羟基磷灰石糊剂的根管封闭性优于氧化锌丁香油糊剂、Vitapex 糊剂，但次于 AH-Plus 糊剂。将奥硝唑加入纳米羟基磷灰石糊剂对根管封闭性短期无影响。

作者贡献：全体作者共同进行实验设计、实施及评估，资料收集为王建平，王建平成文、审校并对文章负责。

利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：实验未涉及与伦理冲突的内容。

学术术语：根管充填-是根管治疗术的最后一个环节，其目的是封闭根管系统避免细菌、组织液进入造成根管的再感染，同时还要借助根管充填材料的消毒作用继续消除根管内残余的感染，促进根尖周组织病变的愈合防止复发。

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