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Effect of Calcium Hydroxide and Chlorhexidine Based Gutta-Percha Points on Gingival Fibroblasts and Epithelial Tumor Cells

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Abstract

Aims and Methods: The aim of the present study was to demonstrate the possible effect of different endodontic calcium hydroxide and chlorhexidine-based gutta-percha points, on two different human cell culture systems.

Two different calcium hydroxide (Roeko, Langenau, Germany) and one chlorhexidine (Activ Point/ Roeko, Langenau, Germany) gutta-percha points were tested with gingival fibroblasts and epithelial tumor cells over a period of six days (n = 12). Conventional gutta-percha points (VDW, Munich, Germany) and cells that were not exposed to any substances served as controls (n = 12). Study parameters included cell vitality, cell count, protein synthesis and cell proliferation.

Results: All tested materials induced cell growth specific alterations. Chlorhexidine-based gutta-percha points showed a significant lower protein synthesis with both, gingival fibroblasts (0.013 ± 0.009 mg/ml) and epithelial tumor cells (0.07 ± 0.039 mg/ml), when compared with the controls ($p > 0.05$). Protein synthesis increase of the epithelial tumor cells (0.581 ± 0.013 mg/ml, control) was observed with the conventional gutta-percha points (0.688 ± 0.078 mg/ml) and with both gutta-percha points containing different calcium hydroxide-based formulations (0.776 ± 0.115 and 0.7 ± 0.047mg/ ml).

Conclusions: Under the conditions of this study, chlorhexidine containing gutta-percha points showed the highest effect on cell growth inhibition. No significant differences were observed between the tested material and the two different cell culture types.

Key words: Gutta-percha points; calcium hydroxide; chlorhexidine; cell culture

Introduction

Calcium hydroxide is the most commonly utilized, and studied root canal medication. The first report about calcium hydroxide is attributed to Nygren (1838) for the treatment of the "fistula dentalis", whilst Codman (1851) was the first to attempt to preserve an injuried dental pulp (Fava and Saunders 1999). Hermann (1920, 1936) demonstrated the biological properties and antimicrobial effect of calcium hydroxide through his pioneering work. Since then its clinical indications have expanded, and has been recommended to control microbial growth, to enhance the healing process of periapical lesions, to arrest inflammatory root resorption, to induce hard tissue deposition, as an inter-appointment root canal dressing material as well as to promote healing of vital pulps and periapical tissues (Andreasen 1981, Caliskan et al 1998). Calcium hydroxide's alkaline pH, its ionic activity, diffusion through dentinal tubules, influence on apical microleakage and placement within the root canal are examples on how this material has been evaluated since its introduction. Its alkalizing effect (Economides et al 1999, Larsen and Horsted-Bindslev 2000, Podbielski et al 2000, Schäfer and Al Behaissi 2000) is meant to support the mechanical and chemical preparation of the root canal through its antibacterial effect (Lado et al 1986, Stuart et al 1991). An inter-appointment antimicrobial medication is also recommended in order to prevent recovery and multiplication of remanent microorganisms even after careful instrumentation and debridement of the root canal system (Byström 1985). Thus, the use of various formulations and suggestions for mixing calcium hydroxide power with other substances which have antibacterial and radiopac properties have been also recommended. Conventionally, calcium hydroxide is prepared by mixing the powder with a liquid and inserted into the root canal by means of an injection, root canal instrument, and gutta-percha or paper point. Sterile water or glycerine are recommended as vehicles to make a paste with $Ca(OH)_2$ powder (Walton and Torabinejad 1989). Glycerine may be preferred as the vehicle for the placement of calcium hydroxide into the root canal when depth of penetration and paste density (Riviere 1994) should be enhanced. In addition to placement, the removal of calcium hydroxide from the root canal without leaving any residues behind is also a time-consuming and cumbersome procedure. Thus, a potential negative influence on the adhesive properties of the root canal sealer is possible (Lambrianidis et al 1999). These shortcomings prompted the development of gutta-percha points containing calcium hydroxide or chlorhexidine, which could be easily placed in the root canal especially in the apical area. Likewise, these types of gutta-percha points can be easily removed without leaving material residuals behind. In addition to calcium hydroxide, gutta-percha points containing chlorhexidine diacetate have also been used to avoid re-infection of the root canal system. Chlorhexidine is effective against a

broad spectrum of microorganisms, including yeast and fungi (McDonell and Russell 1999). Chlorhexidine diacetate is soluble and releases cations binding to the anionic surface molecules of bacterial cell membranes, thereby disrupting the osmotic equilibrium of cells (McDonell and Russell 1999). However, the biocompatibility of these calcium hydroxide and chlorhexidine-based gutta-percha points with regard to the surrounding tissue structures also needs to be considered. Tissue compatibility is decisive in the selection of the active ingredient, since the intra-canal medication may otherwise lead to irritation of the surrounding tissues; thus, a possible interference of the post-treatment healing process could be possible. The aim of the present study was to investigate and compare the effect of gutta-percha points containing either calcium hydroxide or chlorhexidine diacetate on human gingival fibroblasts and epithelial tumor cells.

Materials and Methods

Cell Cultures

Gingival fibroblasts were obtained from biopsies of healthy individuals of both genders (age range: 20–30 years), derived from the lower molar region showing no signs of inflammation. Epithelial tumor cells were obtained from one male patient with T4 squamous cell carcinoma of the sinus piriformis. The cells were cultivated in Eagle's Basal medium (Grand Island Biological Co., Grand Island, NY) with 10% fetal calf serum (Grand Island Biological Co.), 50 IU/ml penicillin (Seromed) and 50 µg/ml streptomycin (Seromed). The cultures were kept in an environment of 95% air and 5% CO_2 and a temperature of 37 °C (Martin 1973). Following caryological characterization (Dutrillaux and Couturier 1983), normal diploid gingival fibroblasts between the 5th and 7th passage and epithelial tumor cells between the 35th and 37th passage were used.

Intra-canal Materials

Two different intra-canal dressing materials in three different gutta-percha point types size ISO 30 (n = 12) were investigated: Roeko Standard $Ca(OH)_2$ Points (58% calcium hydroxide, 42% gutta-percha and <1% pigments / Roeko, Langenau, Germany); Roeko Plus Points (52% calcium hydroxide, 48% gutta-percha and <1% pigments / Roeko, Langenau, Germany) and Roeko Activ Points (5% chlorhexidine diacetate, 30% gutta-percha, 65% ZnO, and <1% pigments / Roeko, Langenau, Germany). Conventional gutta-percha points (n = 12) size ISO 30 (70% ZnO, 30% gutta-percha and <1% pigments / VDW, Munich, Germany). Cells that were not exposed to any substance served as controls (n = 12).

Cell Vitality

Cell vitality and cell counts were determined by trypan blue staining in a Fuchs-Rosenthal counting chamber. Cell vitality was additionally evaluated based on cytosolistic esterase activity using DNA-specific fluorescent markers (calcein AM: excitation 485 nm, emission spectrum 530 nm; ethidium-homodimer-1: excitation 485 nm, emission spectrum 600 nm) and a Fluoroskan Ascent 96 microplate fluorometer (Labsystem, Helsinki, Finland).

Cell Proliferation

The proliferation rate in the two cell culture systems was determined by placing the tested materials in 96 microplate with the Almar-Blue-Test (Redox-Indicator). The materials were covered with 30 000 cells per-well containing 200 µl BME full medium, followed by addition of 10 µl Almar-Blue. The fluorescent activity was measured immediately and after 1, 2, 3, 6, 12, 48, 72 and 96 hours of culturing by means of a Fluoroskan Ascent microplate fluorometer (Labsystems, Helsinki, Finland). The cultures were incubated at 37 °C.

Protein Concentration

To assess the potential impact of the different intra-canal dressing materials on protein concentration, 30.000 gingival fibroblasts and epithelial tumor cells were suspended over the different materials in 24 multi-well (Becton Dickinson, Plymouth, England). The cells were incubated and analyzed after six days using standard culture techniques (Briseño and Willershausen 1990). The protein concentration was determined by conventional photometry test methods (Boehringer, Mannheim, Germany).

Statistical Methods

The Wilcoxon's test for dependent and independent samples was used to assess statistical differences between the different materials and cell culture systems in comparison to the controls. The results deemed statistically significant at a 0.05 level.

Results

All tested intra-canal dressing materials induced specific alterations in cell growth. Direct exposure to the tested materials resulted in cell morphology changes and in the formation of more or less marked inhibition zones (Figs. 1, 2). All gutta-percha points, whether conventional or containing calcium hydroxide or chlorhexidine, led to an inhibition of gingival fibroblast growth after 24 and 96 hours. The calcium hydroxide-based Plus points were the only material to be associated with a significant increase of proliferation at 96 hours (Fig. 3). Direct exposure of epithelial tumor cell cultures to the various tested materials led to morphological cell irregularities and influenced the proliferation patterns characterized by more or less marked inhibition zones. Compared to the controls, cell proliferation was reduced after 6 hours with all tested materials. At 24 hours, these reductions had reached statistically significant differences for the conventional gutta-percha points, the Plus points, and the Activ Points. At 96 hours only, the proliferation of epithelial tumor cells exposed to Activ Points was significantly inhibited when compared with the controls (Fig. 4).

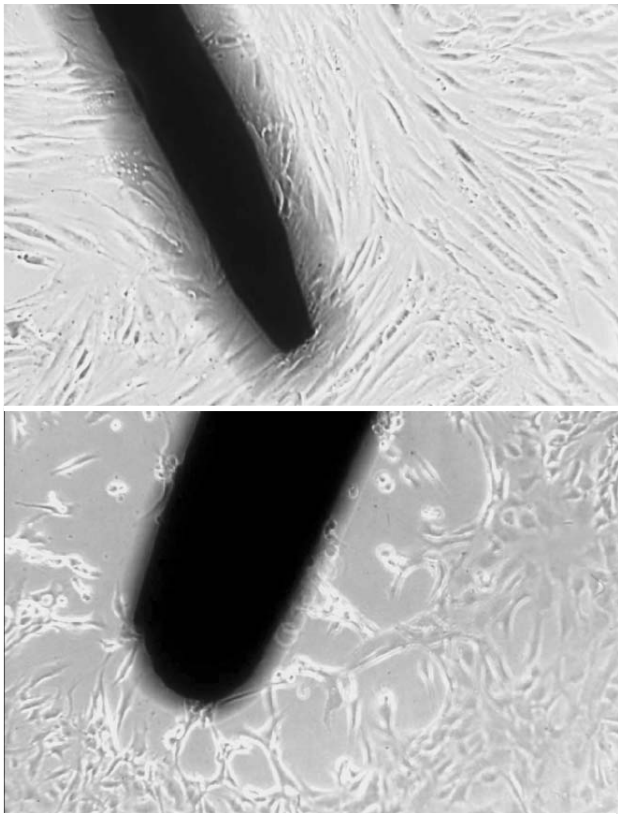

Fig. 1 a, b. Gingival fibroblasts exposed to a) conventional gutta-percha point and b) Activ Point (x 125). An inhibition zone around the testing material (b) can be observed.

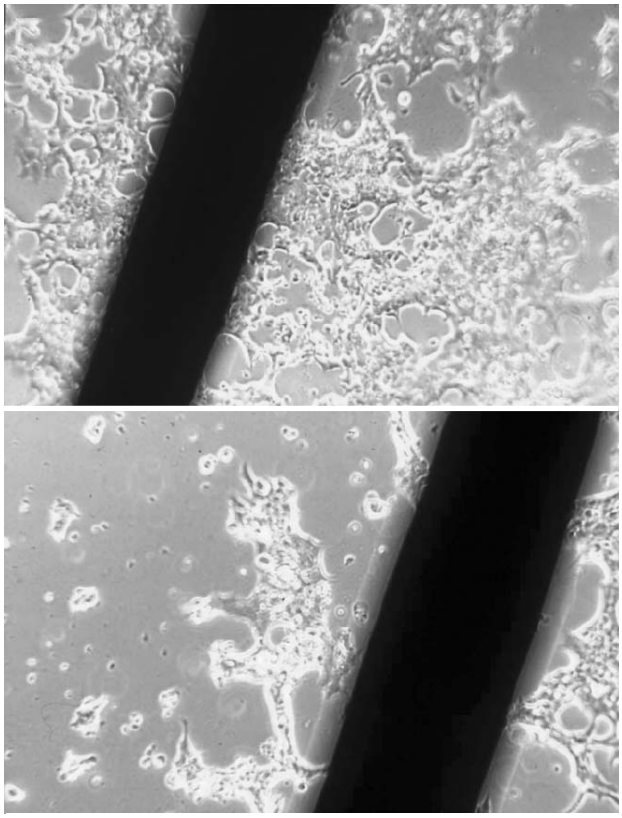

Fig. 2 a, b. Epithelial tumor cells exposed to a) conventional gutta-percha point and b) Activ Point (x125). A direct contact between the testing material and cells can be observed in a) and an inhibition zone in the vicinity of the Activ point (b).

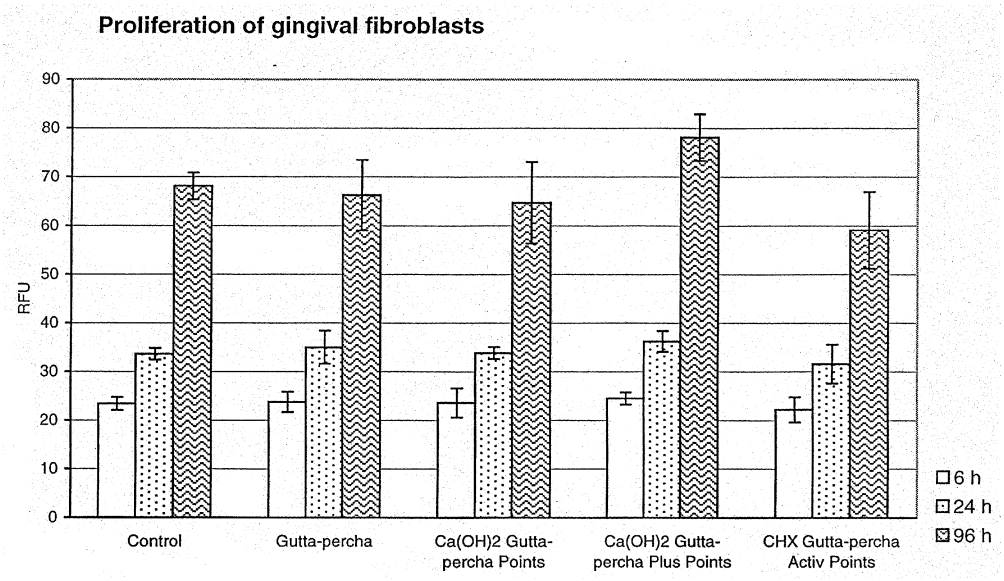

Fig. 3. Effect of gutta-percha containing intracanal dressing material on the proliferation rate (RFU) of gingival fibroblasts at different observation times (n = 12).

The protein concentration of gingival fibroblasts in the control group was 0.1 mg/ml (±0.008) at the beginning and reached 0.115 mg/ml (± 0.014) at the 6th day of culturing. Similar protein concentration values at the 6th day were also found when the gingival fibroblasts were exposed to conventional gutta-percha and to calcium hydroxide-based Plus points, while the calcium hydroxide-based conventional points resulted in a statistically higher increase of protein concentration. The chlorhexidine-based Activ Points was the only material to yield a significant decrease in fibroblast protein concentration when compared with the controls (Fig. 5). Likewise, the epithelial tumor cells exposed to Activ Points produced significantly less protein than the controls, whereas the other gutta-percha points showed higher protein concentration values than the controls

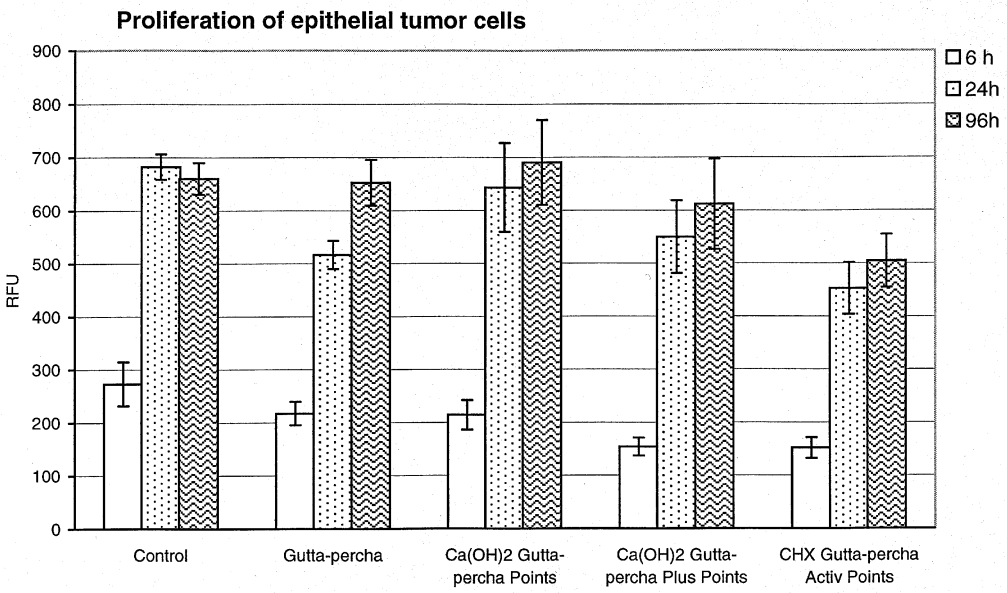

Fig. 4. Effect of gutta-percha containing intracanal dressing material on the proliferation rate (RFU) of epithelial tumor cells at different observation times (n = 12).

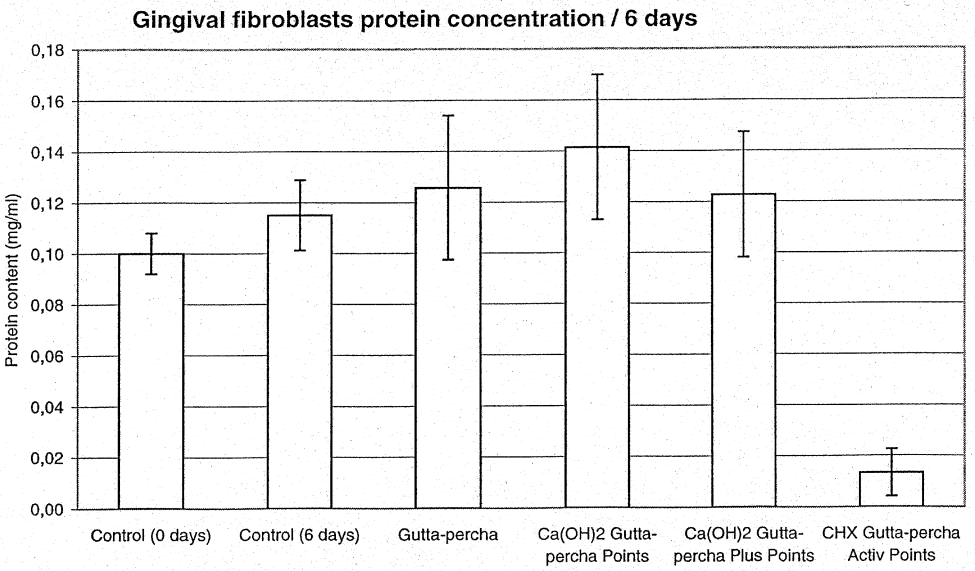

Fig. 5. Protein concentration (mg/ml) of gingival fibroblasts (n = 12) after six days of incubation.

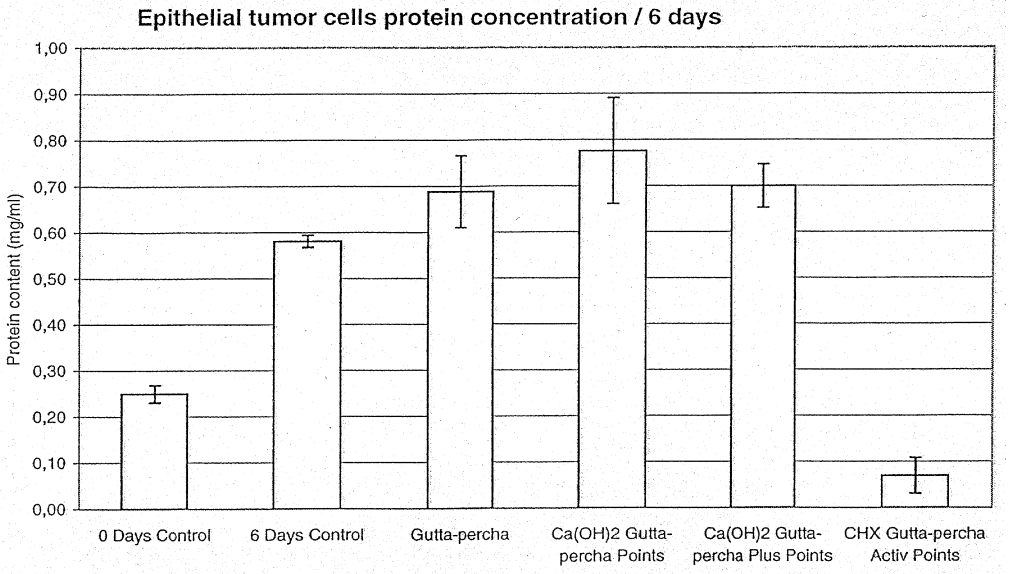

Fig. 6. Protein concentration (mg/ml) of epithelial tumor cells (n = 12) after six days of incubation.

(Fig. 6). Significantly higher protein concentration was observed only with regular calcium hydroxide based gutta-percha points.

DISCUSSION

The main use indications of calcium hydroxide are the stimulation of re-mineralization, its antibacterial properties and the dissolution of necrotic tissue (Gomes 2003, Byström 1985, Sjögren 1991, Foreman 1990). These properties are mostly correlated to its high pH and necrotizing capacity. Some authors described the destruction of epithelium present in periradicular lesions; thus, promoting connective tissue invagination and healing (Sahli 1990, Bruyne 2000). Yet, the effect of such materials in the periapical tissue has to be considered.

After root canal instrumentation different contaminated fluids such as extracellular fluid, exudates, pus or even saliva may seep into the canal. It is known that some bacteria can survive at pH 9; therefore, the application of an intra-canal dressing material that releases a considerable amount of hydroxyl and maintains a high pH in the root canal space is advantageous (Byström 1985). The application of an intra-canal dressing material will enhance the reduction of the number of microorganisms and can penetrate in areas not reached by the instruments or irrigating solutions during root canal preparation (Barbosa 1997). It has been demonstrated that conventional gutta-percha due to zinc-oxide content inhibits the growth and vitality of certain bacteria known to be present in infected root canals (Moorer and Genet 1982, Moorer and Genet 1982, Weiger et al 1993). Likewise, calcium hydroxide and chlorhexidine are also known to inhibit bacterial growth (Barbosa et al 1997, Heling et al 1992, Tchaou et al 1996). A microbiological study (Podbielski et al 2000) has shown that some microorganisms are more effectively reduced by calcium hydroxide, while others are more susceptible to chlorhexidine. Fuss et al (1996) used a freshly prepared calcium hydroxide solution whose bactericidal potential was slightly higher to that of premixed calcium hydroxide pastes. However, the bacterial advantages of calciumhydroxide and chlorhexidine could have a toxic effect in the periapical tissue. Cell culture studies can provide information on the behaviour of different materials against healthy tissues. Because the periapical tissues can be exposed to intra-canal dressing materials, the possible toxic effect of commercial available calcium hydroxide and chlorhexidine-based gutta-percha points also needs to be evaluated.

Different studies have proven the effect of calcium hydroxide (Nakamura et al 1986, Rappaport et al 1964). In a previous publication (Willershausen et al 2000) the biological compatibility of various root canal filling materials was investigated with similar research parameters. It was established that conventional gutta-percha points had a non-significant effect on the prostaglandin release of gingival fibroblasts. These findings are supported by the comparison of cell proliferation results between conventional gutta-percha points and the unexposed cell cultures. Our findings also indicate that calcium hydroxide-based gutta-percha points have only a slight inhibitory effect on the cell proliferation of gingival fibroblasts and epithelial tumor cells. The only tested material that significantly inhibited the growth of gingival fibroblasts was the chlorhexidine-based Activ Points. The protein synthesis patterns were also significantly different from the controls. During root canal treatment, it is important to create an environment in the access cavity in order to prevent the entrance of microorganisms into the root canal system. The clinical advantages or disadvantages of the use of Activ Points need to be considered. The present study shows that the culture systems employed are sensitive and provide information on the possible effects of intra-canal dressing materials on the periapical tissues. Thus, these results can facilitate employment decisions between various active ingredients of gutta-percha points and contribute to the success of root canal treatment.

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