Assessment of decontamination of gutta-percha cone and the change of surface texture after rapid chemical disinfection

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ABSTRACT

The purposes of this study were firstly to identify the microbial species on gutta-percha (GP) cones exposed at outpatient clinics using polymerase chain reaction, and secondly to evaluate the rapid sterilization effect of two chemical disinfectants at chair side. It also evaluated the alteration of surface texture of GP cones after 5-min soaking into two chemical disinfectants. A total of 100 GP cones from two endodontic departments were randomly selected for microbial detection using PCR assay with universal primer. After inoculation on the sterilized GP cones with the same microorganism identified by PCR assay, they were soaked in two chemical disinfectants: 5% NaOCl and 2% chlorhexidine for 1, 3, 5, and 10 minutes. The sterilization effect was evaluated by turbidity and subculture. The change of surface textures using a scanning electron microscope was also examined after 5 min-soaking in two chemical disinfectants. Results showed that four bacterial species were detected in 17 GP cones, and all the species belonged to the genus *Staphylococcus*. Two chemical disinfectants were effective in sterilization with just 1 minute soaking. On the SEM picture of NaOCl-soaked GP cone, a cluster of cuboidal crystals was seen on the cone surface. Present data demonstrate that two chemical disinfectants are useful for rapid sterilization of GP cone just before obturation at chair side, while CHX-soaked GP cone has cleaner surface without crystal precipitation than that of NaOCl-treated cone. (J Kor Acad Cons Dent 31(2):133-139, 2006)

Key words : Gutta-percha cone, PCR, SEM, sterilization effect, surface texture

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Ⅰ. INTRODUCTION

Apical periodontitis is a microbial disease, and therefore, all effort must be taken during endodontic treatment to avoid root canal cross-infection by instruments or canal filling materials. Gutta-percha (GP) cones, now widely used to fill root canals, may become contaminated by pathogens during manufacturing and exposed to the air in clinics for several

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months. It is controversial whether the sterilization process is necessary because of anti-bacterial characteristic of a component of cone itself[1,2], however, the sterilization of cones prior to root canal filling has been recommended in most cases.

The chemical agents that have been used to sterilize GP cone prior to canal filling are diverse. Recently, numerous experiments have been performed to seek chemical agents that act in a shorter time and sterilize various bacteria, and it has been reported that NaOCl, glutaraldehyde, and chlorhexidine, etc. are effective[3-7]. However, in these experiments, GP cone was infected with artificially selective bacteria and the sterilization effect among different sterilizers was compared. Therefore, it is necessary to identify the microbial species on GP cones exposed at the outpatient clinics and evaluate which chemical solution is more effective on such bacteria.

Therefore, the purpose of this study was first, to identify the type of bacteria that contaminate GP cone exposed at two hospital-based dental clinics by polymerase chain reaction (PCR), and secondly to evaluate the rapid sterilization effect of two chemical disinfectants. It also analyzed the alteration of the surface texture using scanning electron microscope after rapid sterilization.

Ⅱ. MATERIALS AND METHODS

1. Identification of contaminated bacteria on gutta-percha cone by PCR

100 GP cones (Meta Biomed Co. Chung-Ju, Korea) kept exposed in two hospital-based dental clinics were collected and used in this study. From 10 immediately opened GP packs, total 30 cones were collected and contamination was assessed as control group. Each GP cone was placed in tube containing 200 ㎕ PBS buffer solution, and vortexed for 5 minutes. All GP cones were removed from the tube, 200 ㎕ PBS buffer solution was inoculated to a Brain Heart Infusion (BHI) agar plate, and cultured for 48 hours in a 37℃ incubator. The number of colonies formed from each GP cone (CFU) was counted and the contamination level of total gutta-percha cones was evaluated. The colony pattern formed on the BHI agar plate was examined and classified by microscope.

Polymerase chain reaction (PCR) amplication was performed in a thermal cycler (Perkin-Elmer Inc. Boston, USA) using colony selected from BHI agar plate as a template. Primer, dNTP (Bioneer, Daejeon, Korea), Taq-polymerase (Bioneer, Daejeon, Korea), PCR buffer, and distilled water were added to a PCR tube together. At that time, the universal 16S rRNA primer (TpU1: 5′-AGAGTTTGATCMTGGCTCAG-3′, RTU3: 5′-GWATTACCGCGGCKGCTG-3′, Bioneer, Daejeon, Korea) was used as a primer for PCR amplication. The PCR conditions used in this study were as follows: the initial denaturation was at 95℃ for 5 minutes. Thirty amplication cycles were then performed: the denaturation reaction at 95℃ for 1 minute, the annealing at 56℃ for 1 minute, and the extension reaction at 72℃. After the electrophoresis and confirming of PCR products, they were purified using PCR purification system (Bioneer, Daejeon, Korea). Automatic nucleic acid sequencer analyzed the purified DNA. Using the nucleic acid sequence of bacteria thus obtained, the name of bacteria was identified using the BLAST program of nucleotide database (NCBI).

2. Evaluation of sterilization effect of two chemical disinfectants

The bacteria identified above were inoculated to BHI liquid medium and cultured at 37℃ for 24 hours. A GP cone sterilized with EO gas was transferred to the BHI medium that bacteria were cultured, and remained in contact with the bacteria for 2 hours to promote surface contamination. The contaminated GP cone was transferred to a petri dish matted with two layers of filter paper and dried for 24 hours at room temperature. A contaminated GP cone was immersed in each chemical solution for 1 minute, 3 minutes, 5 minutes, and 10 minutes, and dried. As chemical disinfectants, 5.25 % NaOCl and 2 % chlorhexidine were used.

After each decontamination method, all the GP cones were transferred to sterile trial tube contain-

ing sterile BHI and incubated at 37℃ for 7 days. Growth, as indicated by the turbidity and the subculture of the examined BHI liquid medium, was then recorded. The subculture was performed with the following method: the observed liquid medium 150 ㎕ was inoculated to a Brain Heart Infusion (BHI) agar plate, and cultured for 24 hours in a 37 ℃ incubator. The sterilization was confirmed by colony forming state. The positive control group was the GP cone immersed in the BHI liquid medium that bacteria were cultured, and dried without sterilization, and cultured 24 hours in fresh BHI culture medium. The negative control group was a GP cone sterilized with EO gas without bacterial contamination and cultured in BHI liquid medium as is.

3. SEM observation of the surface texture of GP cone after 5 min soaking in chemical disinfectants

GP cones were immersed in 2 types of chemical disinfectants (5.25 % NaOCl, 2 % chlorhexidine) for 5 minutes. Their surfaces were compared with that of a fresh GP cone by a scanning electron microscope (X500, SEM-820, Tokyo, Japan).

Ⅲ. RESULT

1. PCR identification of contaminated bacterial species on GP cone surface

The immediately opened control GP cones showed negative cultures in all cases. Among total 100 GP cones opened in clinics, bacteria were detected on the surface of 17 gutta-percha cones. The numbers of GP cone showing total 1-10 bacteria colonies were 10, the cones showing 11-100 bacterial colonies were 5, and the cones detected over 101 colonies were 2.

In addition, examining the pattern or color of the colonies formed on BHI agar plate, 1 or 2 types of bacteria were detected on each GP cone. From these colonies, 24 PCR products were purified, and finally, 22 bacteria were identified. Most of them were *Staphylococcus spp*. In particular, *Staphylococcus epidermidis* was the most prevalent one. In addition, *Staphylococcus caprae*, *Staphylococcus capitis*, and *Staphylococcus xylosus* were also detected.

2. The sterilization effect of chemical solutions

Two chemical disinfectants showed sterilization effect after only 1 minute immersion, which was confirmed not only by the measurement of the turbidity but also by the subculture of the liquid culture medium. Detailed results are shown in Table 1.

3. SEM Observation of the surface texture of GP cone after 5-min soaking

In comparison with GP cone that was not sterilized, the surface of GP cone immersed for 5 minutes in 2 % chlorhexidine was slightly wrinkled with the

Table 1. Sterilization effect of two chemical agents from *Staphylococcus-inoculated* GP cones.

Sterilization Time (Min)	Experimental solutions for sterilization of GP cones							
	5.25% NaOCl		2% CHX		Positive control		Negative control	
	T*	C§	T	C	T	C	T	C
1	-	-	-	-	+	+	-	-
3	-	-	-	-	+	+	-	-
5	-	-	-	-	+	+	-	-
10	-	-	-	-	+	+	-	-

T*: Turbidity, C§: Culture

Positive control: contaminated cone without chemical soaking

Negative control: No artificially contaminated cone

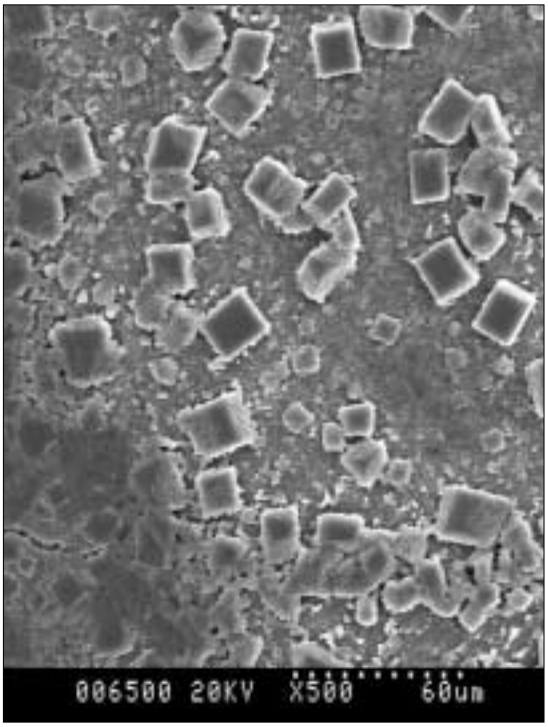

Figure 1. SEM images of the surface of gutta-percha cone after 5-min sterilization with 5.25% NaOCl. A cluster of cuboidal crystals on the surface of GP cone (Magnification × 500).

pattern of shrinkage along the long axis of cone, and the adhesion or release of other materials on the surface was not detected. However, on GP cone immersed in 5.25 % NaOCl, the precipitate with cuboidal crystal was attached to the overall surface, and it was found to be particularly abundant in the defect area or folded area of cone (Fig. 1).

Ⅳ. DISCUSSION

Molecular genetic methods, particularly the polymerase chain reaction (PCR), have been widely used for microbial identification purposes. PCR assays are very sensitive and enable a reliable identification of microbial species or strains that are difficult or even impossible to culture[10]. In this study, the method of assay should detect the smallest possible number of microorganism. The high sensitivity of the PCR method makes it ideal for this purpose.

Our present study clearly showed that GP cones exposed in clinics can be contaminated by specific organisms, particularly *Staphylococcus spp.* *Staphylococcus epidermidis* detected most frequently in this study, is a normal flora residing in the skin and the mucosa of respiratory system in normal individuals. Nevertheless, in immune suppressed patients or in the case at a site different from the distribution of normal flora such as implanted medical devices, or when its number is increased suddenly by other reasons, it may cause severe infection as opportunistic microorganism[11].

In a previous study[12], GP cones in over-filled areas were covered with a biofilm structure and a colony of cocci was observed in the cracks of the biofilm structure. These findings suggested that cocci were located in deeper layers of the biofilm structure and these cocci might play an important role in initiating biofilm formation. In addition, the biofilm which form on the extra-radicular area of gutta-percha cone are related to refractory periapi-

cal pathosis, and gutta-percha cones might play a role in the initiation of biofilm infection in cases of excessive root filling with periapical lesions[12,13]. Therefore, considering the importance of the aseptic root canal and the contamination of the GP cone in clinics, it may be necessary to decontaminate the gutta-percha cones by means of a chemical disinfectant before canal filling at chair side. The time needed for disinfection of GP cone is various according to the types and concentrations of chemical disinfectants or the selective microbial species. It has been reported previously that 1 minute short sterilization with 5.25 % NaOCl eradicated spores as well as bacteria. Because of its superior antibacterial effect, the sterilization of the GP cone with 5.25% NaOCl prior to canal filling has been recommended for a long time[4-7]. However, Short *et al.* have shown that the cluster of cuboidal crystals on the surface of NaOCl-soaked GP cone was detected at various levels and mentioned that at the time of canal filling, it might affect the apical sealing [14]. Our present study demonstrated cuboidal crystals on the surface of NaOCl-soaked GP cone under high magnification. Therefore, it is essential to find other chemical disinfectants, which are more stable and clinically useful, to sterilize the contaminated GP cone.

Our results showed that two chemical disinfectants were efficient for rapid sterilization of GP cones by immersion only for 1 minute, which was confirmed by the turbidity measurement and subcultures. We contribute this result to the contamination of GP cone by *Staphylococcus*, which is a different species from the spore-forming bacteria, fungus with a thick lipid layer, and small virus lacking the envelope that need more powerful sterilization agents. Therefore, GP cone could be easily sterilized in two chemical disinfectants in the present study. Time is more critical when it is necessary to disinfect extra accessory cones during lateral or vertical compaction techniques for multi-rooted tooth, because it needs 5 to 10 min for cone disinfection. Therefore, in this study we evaluated the changes of surface textures of gutta-percha cones only after 5-min soaking in chemical solutions.

In conclusion, our present study demonstrates that two chemical solutions are useful for rapid sterilization of gutta-percha cone before canal filling at chair side. Further research is, however, needed to investigate the changes of physical properties such like elongation rate or tensile strength of GP cone after chemical disinfection.

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국문초록

화학소독제 처리 후 가타파차 콘의 멸균 효과 및 표면 성상의 변화 평가

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본 연구의 목적은 첫째 임상에서 진료실에 노출된 가타파차 콘 표면의 오염 균종을 중합효소연쇄반응법 (polymerase chain reaction, PCR)을 이용해 동정하고, 둘째 이들 세균으로 오염시킨 가타파차 콘에 대해 2종의 소독제의 rapid sterilization 효과를 비교하였다. 또한 이들 소독제에 5분간 처리된 가타파차 콘 표면 성상의 변화를 주사전자현미경으로 관찰하였다. 진료실에 수 개월간 노출된 가타파차 콘 100개를 수거하여 배양지에 넣어 배양 후 universial primer를 사용한 PCR assay 를 통해 오염 균종을 동정하였다. 실험실 상에서 이 균종을 다시 배양하여 소독된 가타파차 콘에 접종하고 1주일간 배양한 후 2종의 소독제(5% NaOCl, 2% Chlorhexidine)에 1, 3, 5, 10 분간 담근 후 각 소독제의 종류와 적용시간에 따른 멸균 효과를 turbidity test 와 subculture 를 이용하여 평가하였다. 또한 각 소독제에 5분간 처리된 가타파차 콘 표면 성상의 변화를 주사전자현미경으로 관찰하였다. 중합효소연쇄반응법의 분석결과 17개의 가타파차 콘이 오염된 것으로 나타났고 대부분이 *Staphylococcus* 계통이었으며, 2종의 소독제 모두 이들 균종에 대해 1분 내에 멸균 효과를 나타냈다. 주사전자현미경상 NaOCl로 소독된 가타파차 콘 표면에는 cuboidal crystal 의 침전물이 전반적으로 관찰되었다. 본 연구 결과 2종의 소독제 모두 근관충전 전 가타파차 콘의 rapid sterilization 을 위해 유용하였으나 클로헥시딘으로 처리된 가타파차 콘이 크리스탈 침전물이 없는 좀더 깨끗한 표면을 갖는 것으로 나타났다.

주요어: 중합효소연쇄반응법, 표면성상; 주사전자현미경 (SEM), 멸균효과; 가타파차 콘