Comparing the Effect of Hyperpure Chlorine-dioxide and Conventional Endodontic Disinfecting Agents *In Vitro* On *Enterococcus Faecalis* Intracanal Biofilm

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Introduction

The success of modern endodontic treatment depends on the effective use of files, rotary instrumentation and irrigation solutions to clean, shape and disinfect root canals. The elimination by irrigation of harmful pathogens present in the canals and dentinal tubules is especially critical to prevent post-endodontic recontamination. The type microorganism of the invetigation is *Enterococcus faecalis* bacterium due to resistance, adaptability to harsh environmental changes and the fact that it can grow in root canal walls as a biofilm.

Current irrigant therapy most commonly includes the use of NaOCl, CHX, EDTA or ozonated water. ClO_2 is a well-known and popular disinfectant. Current practical uses include surface desinfection, water treatment, food processing, veterinary care, dental waterline treatment and is found in some commercially available mouth rinses. ClO_2 has many beneficial properties for dental application. First, it kills a broad spectrum of pathogen microorganisms such as bacteria, fungi and viruses. It is not toxic for humans till 3000 ppm and is not allergenic. It has the capacity to penetrate into the skin and mucous membranes in a few tenth of millimeters depth. This feature may also be an important property in case of treating a biofilm. These beneficial properties could position ClO_2 as an ideal candidate for root canal disinfectant. This study aims to investigate the effecacy of high purity ClO_2 solution in comparison to the standard irrigants (NaOCl, CHX) in the elimination of the intracanal *E. faecalis* biofilm.

Materials and Methods

Forty single, straight-rooted human teeth previously extracted because of periodontal disease were decoronated maintaining 16mm of root lengths. Root canals were prepared up to a 25 K-file and autoclaved. The apical foramens were covered and sealed with cyanoacrylate from the outer surface. The roots were separately inoculated with 108 CFU/mL *E. faecalis* (ATCC29212) in Brain Heart Infusion in sterile eppendorf tubes and incubated at 37° C. Every 2 days the suspension was refreshed through a period of 14 days. On day 14 the effectiveness of the infection in all infected root canals were checked. Microbiological samples were collected by absorbent paper points from each canal. The paper points were placed into eppendorf tubes containing 50µL sterile physiologic saline solution, vortexed and centrifuged on 10000 RPM for 5 mins. Forty µL from the supernatant was inoculated onto Columbia agar in duplicate and incubated for 2 days. The numbers of colony-forming units (CFU) were counted.

The preinfected roots were randomly divided into four groups (10 teeth/group). The canals were further enlarged up to 40 K-file. The ClO_2 group was irrigated with 0.5 mL, 0.12 % Solumium Dental for 1 min followed by 2 mL of 20x fold diluted ClO_2 with sterile destilled water for 1 min. For comparison in the other groups the canals were irrigated with the same parameters: 2 min with 2.5 mL 5.25 % NaOCl, 2 % CHX, or physiologic saline control solution. The irrigants were washed out with 2 mL sterile distilled water in all groups. We collected samples (S1) for microbiological analysis right after the chemical procedures as described above.

The manipulated outside surface of the chemo-mechanically treated, sampled roots were then disinfected by 70 % ethyl alcohol and placed back into sterile eppendorf tubes containing physiologic saline solution and stored at 37° C. After 2 and 5 days the second and the third samples (S2, S5) were collected the same way to investigate the reinfection of the root canals.

Results

All root canals were infected by *E. faecalis* after 14 days of incubation. Only the control physiological saline treated group had a detectable number of bacteria right after the application of the chemo-mechanical procedure. No bacteria could be detected in any of the irrigant administered groups (Figure 1). All test solutions reduced reinfection significantly after 2 days compared to the massive reinfection of the control group, but there was a significant difference between NaOCl and CHX, as well as between NaOCl and ClO_2. No difference was observed between CHX and ClO_2. After the fifth day of reinfection there were significant differences among all investigated irrigants. ClO_2 was the most powerful in eliminating reinfection. Furthermore, there was a significant increase in reinfection between the 2nd and 5th day in the case of the NaOCl group, but not in the CHX or the ClO_2 treated groups. The least reinfection CFU count was found in the ClO_2 treated group both on day 2 and 5. It is interesting to note, that from the investigated eight roots, in the ClO_2 irrigated group, in five cases no reinfection was detected at all, neither after the 2nd nor after the 5th day. This was not observed in the case of the other solutions.

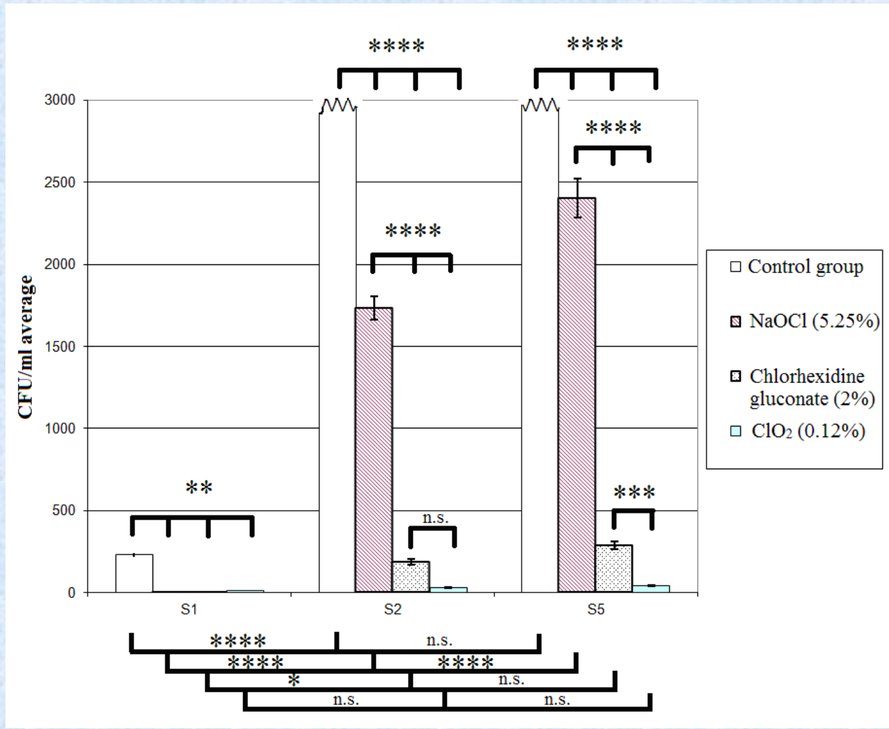

Figure 1
The antibacterial property of irrigants in artificially infected in vitro canal model
Legend: mount of *E. faecalis* after chemo-mechanical preparation (S1), or 2 (S2) and 5 (S5) days later; wavy line represents uncountable amounts, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$; n.s.=no significance

Conclusion

- High purity ClO_2 solution powerfully and quickly eliminate the experimental *E. faecalis* infection from the root canal system
- It is more effective against the reinfection than the conventionally used NaOCl or CHX desinficients
- Our findings strongly suggest applying the safe and effective high purity real ClO_2 as a root canal irrigant in the clinical practice

Support: Semmelweis University Faculty Research Application FOK/DH/3-5/2011, 2013.
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