

Cell Survival After Exposure to a Novel Endodontic Irrigant

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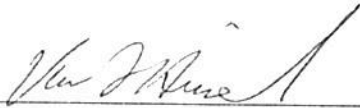
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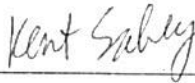
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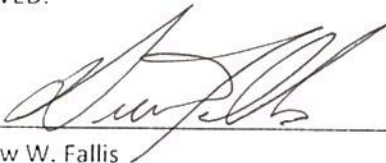


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A handwritten signature in black ink, appearing to read "G. Zilinski".

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Cell Survival After Exposure to a Novel Endodontic Irrigant

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Abstract

Introduction: Endocyn, a pH-neutral solution of hypochlorous acid and hypochlorite has been developed for use as an endodontic irrigant. The purpose of this study was to evaluate the effect of Endocyn on human periodontal ligament (PDL) fibroblasts and rat osteosarcoma cells (UMR-106) and compare this with other commonly used endodontic irrigants. **Methods:** To determine cytotoxicity, cultured PDL and commercially purchased UMR-106 cells were exposed to 1%, 5%, 10%, 20%, and 50% concentrations of Endocyn, 6% NaOCl, 17% EDTA, and 2% CHX for 10 minutes, 1 hour, and 24 hours by dissolving the irrigants into cell medium. Distilled water was used as the control. The cells were rendered fluorescent with Calcein AM to allow microscopic examination and determination of survival. Comparison of cell survival was performed by using an unpaired, 2-tailed Student t-test ($p < 0.05$). **Results:** Compared to distilled water, Endocyn showed no increased cytotoxicity to PDL cells at 10 minutes for all concentrations tested. The 20% and 50% concentrations of Endocyn resulted in a 10% decrease in PDL cells at 1 hour, while the 50% concentration resulted in a 20% decrease in PDL cells at 24 hours. At all concentrations, Endocyn showed no increased cytotoxicity to UMR-106 cells at 10 minutes and 24 hours and a slight increase (10%) in cytotoxicity at 1 hour. All other experimental irrigants demonstrated significant cytotoxicity to both PDL and UMR-106 cells at all time intervals. **Conclusion:** Endocyn was less cytotoxic to PDL and UMR-106 cells compared to other commonly used endodontic irrigants.

Key Words

Endocyn, Irrigation, Cell Survival

Introduction

It is well established that microorganisms are the principle cause of apical periodontitis (1,2,3). Since the goal of endodontic therapy is the prevention and treatment of apical periodontitis, the paramount goal of endodontic treatment is the elimination of microorganisms. A variety of different endodontic irrigants can be used to accomplish this goal. Sodium hypochlorite (NaOCl) has proven to be an effective irrigant because of its ability to effectively disrupt microbial biofilms (4) and dissolve necrotic tissue (5). A significant disadvantage of using sodium hypochlorite is its toxicity to periodontal ligament cells (6), stem cells of the apical papilla (7) and the significant pain and morbidity involved when it is extruded beyond the confines of the tooth (8). Chlorhexidine (CHX) is another frequently used endodontic irrigant that has shown efficacy in the elimination of microorganisms but has also proven to negatively affect periodontal ligament cells (6) and decrease the migration capacity and viability of human gingival fibroblasts (9). It is quite clear that prevention of irrigant extrusion is critically important and many methods of prevention have been suggested, for example, limiting needle penetration depth, keeping delivery tip loose in the canal, and using negative pressure systems such as the EndoVac® device (10). However, in cases with large apical foramina or perforations, prevention of irrigant extrusion can be very difficult. Additionally, because regenerative endodontic procedures rely primarily on chemical debridement, medicaments must not only demonstrate antimicrobial abilities, but also promote survival and proliferation of the patient's stem cells (11). The perfect endodontic irrigant would eliminate microorganisms but have insignificant or no toxicity to healthy host cells.

A new irrigant, Endocyn™, has been developed for endodontic use. It is a pH-neutral solution of hypochlorous acid and hypochlorite created by a patented electrochemical treatment of purified water and NaCl. According to the manufacturer, this proprietary process has created

a superoxidized water solution with superior product stability (12). Microcyn™, which is chemically similar to Endocyn, has been shown in hospital settings to be an effective disinfectant with a wide antimicrobial range (13). It has been successfully used as a treatment for infected diabetic foot ulcers with minimal adverse effects (14). When compared to hydrogen peroxide, Microcyn showed significantly less cytotoxicity to human dermal fibroblast cultures (15). To date, there have been no published reports regarding the effects of Endocyn on human periodontal ligament (PDL) fibroblasts and rat osteosarcoma cells (UMR-106). Therefore the purpose of this study was to evaluate the effect of Endocyn on PDL fibroblast and UMR-106 cell survival and compare this with other commonly used endodontic irrigants.

Materials and Methods

Human PDL fibroblasts were obtained from patients with healthy gingiva and no periodontal disease who underwent extraction of impacted third molars at the Department of Oral Surgery at the Louisiana State University School of Dentistry, New Orleans, LA. All tissues were obtained from subjects after informed written consent as prescribed in an approved institutional review board protocol. Cells were maintained in minimum essential medium alpha (MEM α) containing 10% fetal bovine serum (FBS) at 37°C and 5% CO₂. Cell lines of rat osteosarcoma cells (UMR-106) were obtained from American Type Culture Collection (ATCC-CRL-1661) and were maintained in Dubelco's Modified Eagles Media (DMEM) containing 10% fetal calf serum (FCS) and 200 U/mL penicillin and 200 mg/mL streptomycin.

All cells were isolated by trypsinization and plated onto 48-well plates and allowed to adhere for 24 hours in MEM α containing 10% FBS. The MEM α was then replaced with fresh MEM α containing endodontic irrigant obtained by diluting 6% NaOCl, distilled water, 2%CHX,

17% EDTA, and Endocyn™ separately in MEM α to final concentrations of 1%, 5%, 10%, 20%, and 50%. The negative control was the PDL fibroblast and UMR cells in MEM α .

Following exposure to the solutions for 10 minutes, 1 hour, and 24 hours, the cells were rinsed twice with phosphate buffered saline (PBS) and allowed to recover for 24 hours at 37°C. Cell viability was assessed by treating the cells with Calcein AM (Invitrogen) for 1 hour to render live cells fluorescent. Following this, the cells were rinsed twice with PBS and cell viability was determined using a Biotek Synergy 2 plate reader (Biotek Instruments, Inc., Winooski, VT), calibrated to measure fluorescence intensity with filters appropriate for ~480nm excitation and ~520nm emission. Eight replicates of each concentration were performed for each test. Statistical analysis of mean cell survival between the control and each treated group was analyzed using an unpaired, 2-tailed Student t-test ($P < 0.05$).

Results

As anticipated, NaOCl and CHX were toxic to both cell types (Figure 1), with a loss of 80% viability after exposure for only 10 minutes (Figure 1A and B), and virtually no cell survival after 1 hour of exposure (Figure 1C and D) at concentrations as low as 1%. EDTA solutions of 1% and 5% displayed intermediate toxicity to these cells when exposed for up to 1 hour (Figure 1C and D), but at 24 hours, cell viability was again reduced to nearly 0% (Figure 1E and F). In contrast, distilled water did not alter cell viability up to concentrations of 50%. Endocyn displayed no significant toxicity for these cells up to 50% ($P < 0.05$). Thus, Endocyn was no more toxic to these cells than was distilled water.

Discussion

In this study, Endocyn demonstrated significantly less cytotoxicity to PDL and UMR-106 cells compared to traditionally used endodontic irrigants. Results for EDTA should be interpreted with caution, because EDTA functions as a chelating agent and cellular binding to the experimental wells occurs via a calcium dependent binding mechanism. EDTA may have caused dislodgement of the cells, allowing them to be rinsed away before plate reading, giving a false impression of cytotoxicity.

Ideal properties for endodontic irrigants include antimicrobial activity, the ability to dissolve necrotic tissue, to aid in the debridement of the canal system, and be nontoxic to the periradicular tissues (16). Additionally because of heightened interest in regenerative endodontic procedures, irrigants selected for that specific treatment modality must also not appreciably affect the proliferation of the patient's own stem cells (11). The active ingredient in Endocyn is hypochlorous acid which has significant bactericidal activity due to its ability to penetrate bacterial cell membranes resulting in protein degradation (17). If Endocyn demonstrates the ability to eliminate microorganisms and disrupt microbial biofilms, it may become an additional choice for irrigation during root canal therapy. Perhaps the most intriguing findings of this investigation was that Endocyn proved to be relatively nontoxic to PDL and UMR-106 cells. Because of its relative non-toxicity, it may become a valuable irrigant in cases where extrusion of irrigants is more likely as in perforations or open apex cases. It could prove to be a useful irrigant in undergraduate dental education, where aspiring clinicians have limited experience with endodontic irrigation. Another interesting consideration would be the effect of Endocyn on stem cells of the apical papilla (SCAP). If Endocyn were found to have minimal toxicity for SCAP, or perhaps promote their proliferative capacity, it may prove to be a useful irrigant during regenerative endodontic procedures.

Conclusion

Endocyn™ demonstrated less cytotoxicity to host cells than other commonly used endodontic irrigants. Further testing regarding its ability to eliminate microorganisms, disrupt biofilms, dissolve tissue, and its effect on stem cells should be performed prior to its routine use as part of the endodontic irrigation arsenal.

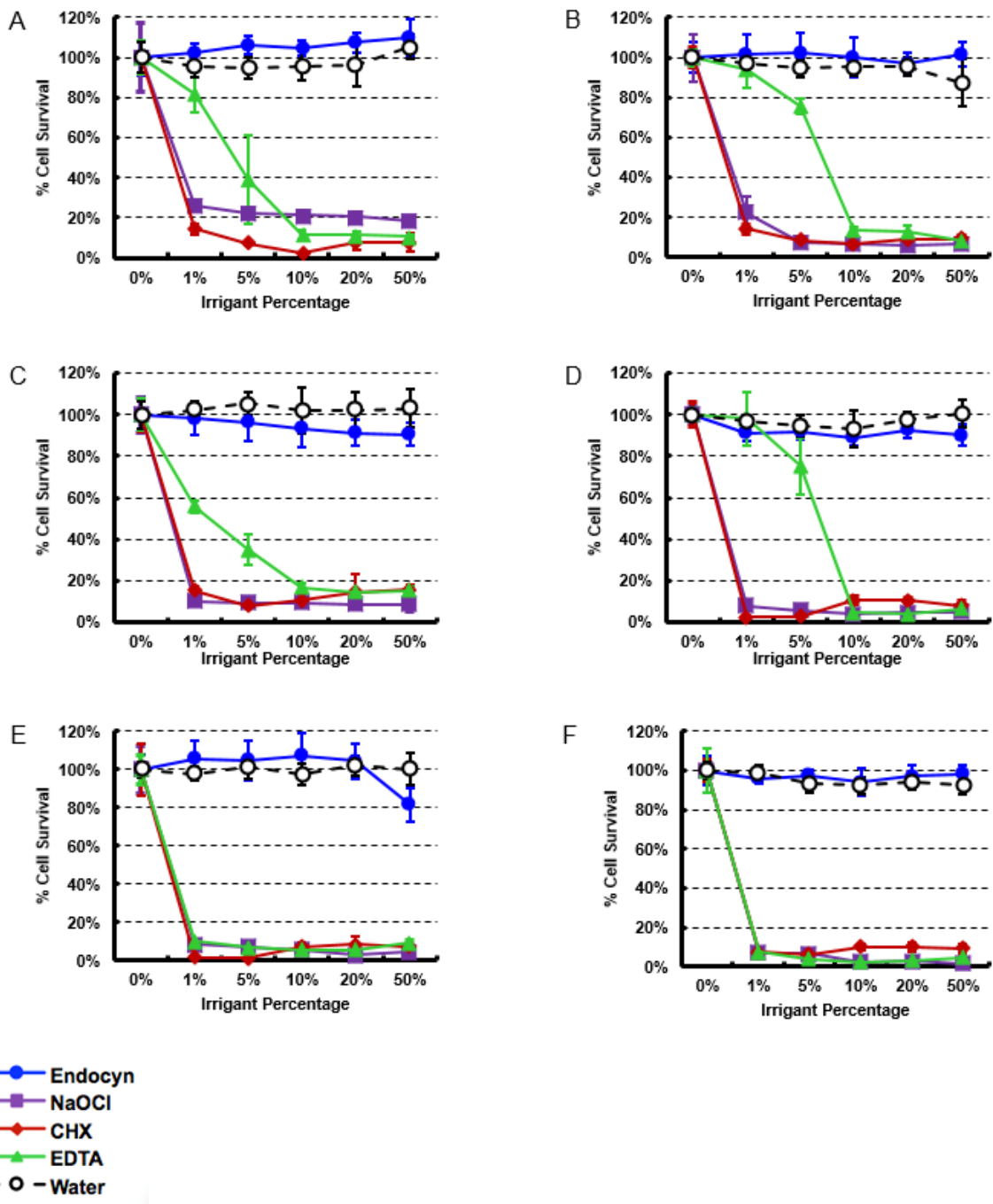


Figure 1. A, C, E: Mean PDL cell survival at 10 minutes, 1 hour, and 24 hours, respectively. B, D, F: Mean UMR-106 cell survival at 10 minutes, 1 hour, and 24 hours, respectively.

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Figure Legend

Figure 1. A, C, E: Mean PDL cell survival at 10 minutes, 1 hour, and 24 hours, respectively. B, D, F: Mean UMR-106 cell survival at 10 minutes, 1 hour, and 24 hours, respectively.