The Comparison of the Effect of Endodontic Irrigation on Cell Adherence to Root Canal Dentin

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Abstract

The purpose of this study was to compare the effect of 10 different endodontic irrigation and chelating treatments on dental pulp stem cell (DPSC) attachment to root canal surfaces. Thirty-eight extracted human non-diseased single-canal teeth were cleaned and shaped using ProTaper and ProFile rotary instrumentation (Tulsa Dentsply, Tulsa, OK). The irrigation treatments investigated were 6% sodium hypochlorite, 2% chlorhexidine gluconate, Aquatine Endodontic Cleanser, and Morinda citrifolia juice. The irrigation treatments were used in conjunction with EDTA or MTAD. The instrumented teeth were immediately placed in cell culture with confluent DPSCs for 1 week. The number of attached DPSCs appeared to be correlated with the cytotoxicity of the root canal irrigating solution (analysis of variance, $p < 0.0001$). The presence or absence of the smear layer had little influence on DPSC activity (chi-square, $p > 0.05$). The results suggest that biocompatible irrigants are needed to promote DPSC attachment to root canal dentin, which is essential to accomplish some regenerative endodontic therapies. *(J Endod 2008;34:1474–1479)*

Key Words

Biocompatibility, regenerative endodontics, smear layer, stem cells

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The dental pulp contains a population of stem cells called dental pulp stem cells (DPSCs) (1, 21) or, in the case of immature teeth, stem cells from human exfoliated deciduous teeth (SHED) (3, 4). Sometimes the progeny of the DPSCs are called odontoblastoid cells because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells they replace (5). After severe pulp damage or mechanical or caries exposure, the odontoblasts are often irreversibly injured beneath the wound site (6, 7). Odontoblasts are postmitotic terminally differentiated cells and cannot proliferate to replace subjacent irreversibly injured odontoblasts (8). The source of the DPSCs that produce odontoblastoid cells that secrete reparative dentin bridges has proven to be controversial. It was initially proposed that the cells within the subodontoblast cell–rich layer or zone of Höhl adjacent to the odontoblasts differentiate into odontoblastoid cells (9). However, the purpose of these cells appears to be limited to an odontoblast-supporting role because the survival of these cells was linked to the survival of the odontoblasts and no proliferative or regenerative activity was observed (6, 7). Radiographic studies provided the first evidence for the theory that the DPSCs are resident undifferentiated mesenchymal cells within the pulp core of teeth (10–13). The origins of these cells may be related to the primary odontoblasts because during tooth development only the neural crest– derived cell population of the dental papilla is able to specifically respond to the basement membrane–mediated inductive signal for odontoblast differentiation (14, 15). The ability of both young and old teeth to respond to injury by induction of reparative dentinogenesis suggests that a small population of competent DPSC may exist within the dental pulp throughout life (16).

The potential benefit of transplanting tissue-engineered dental pulp into root canals is that it will revitalize teeth. Restoring the natural state of the tooth will allow it to repair itself and to respond to dental injuries (17). Regenerative endodontic procedures may be defined as biologically based procedures designed to predictably replace damaged, diseased, or missing structures, including dentin and root structures as well as cells of the pulp-dentin complex, with live viable tissues preferably of the same origin that restore the normal physiologic functions of the pulp-dentin complex (18). In order to accomplish regenerative endodontic therapy, it is necessary to stimulate DPSC attachment or the attachment of other types of dental stem cells or systemic stem cells to root canal dentin after treatment. The presence of a smear layer on root canal walls may inhibit the adherence of implanted DPSCs, potentially causing the regenerative endodontic treatment to fail. The smear layer is a 1- to 5-μm thick layer (19) of denatured cutting debris produced on instrumented cavity surfaces and is composed of dentin, odontoblastic processes, nonspecific inorganic contaminants, and microorganisms (20, 21). There have been no studies to date that have investigated how irrigating solutions and the presence of the smear layer influence stem cell attachment to root canal dentin or how these treatments influence DPSC survival during *in vitro* culture. The removal of the smear layer from the instrumented root canal walls has become less controversial in clinical practice (22). Its removal provides better sealing of the endodontic filling material to root canal walls (23); however, it is uncertain what effect the smear layer or its removal will have on DPSC attached to root canal dentin.

The purpose of this study was to measure the cytotoxicity and to compare the effect of ten different endodontic irrigation and chelating treatments on DPSC attachment to root canal surfaces. These results are essential to the development of some regenerative endodontic treatments.

Materials and Methods

A preexisting archive of extracted human teeth was used for this project after institutional review board approval. Thirty-eight extracted, intact permanent teeth, which had not been stored in antibacterial or fixative solutions and had not received root canal medicaments, were selected. Radiographs of the teeth were taken before inclusion to ensure that all the teeth had a single root canal, and the root lengths were approximately 18 mm. The teeth were sterilized before experimentation using a steam autoclave at 250°C for 15 minutes. The teeth were decrowned at the cementoenamel junction using a diamond rotary bone-cutting saw (Materials Science, NW Ltd, Settle, England, UK). Before instrumentation, the samples were randomly divided into four control (n = 8) and six experimental groups (n = 30). The teeth were cleaned and shaped using Protaper and ProFile rotary instrumentation (Tulsa Dentsply, Tulsa, OK). The root canals were instrumented using a combination of the passive step-back technique and rotary instruments to an apical ISO size of 40 as described by Ferreira et al. (24).

During cleaning and shaping, 1 mL of irrigating solution was used after each instrument size. In all groups, a total of 5 to 6 mL of irrigation solution was used during the biomechanical preparation using small plastic needles (Ultradent Products, South Jordan, UT). All the teeth were handled with sterile gloves and sterile forceps to prevent contamination. In the first treatment group, the canals of five instrumented teeth were irrigated with 6% NaOCl (Clorox, Oakland, CA). In the second treatment group, the canals of five instrumented teeth were irrigated with 6% NaOCl followed by a 15-second rinse with 17% EDTA (PulpDent, Watertown, MA), and a final flush with 6% NaOCl. In the third treatment group, the canals of five instrumented teeth were irrigated with 6% NaOCl followed by the addition of MTAD (Dentsply Tulsa Dental) into the root canal for 5 minutes. This was followed by a 15-second rinse with MTAD. In the fourth treatment group, the canals of five instrumented teeth were irrigated with 2% CHX (Vista Dental, Racine, WI) followed by a 15-second rinse with 17% EDTA and a final flush with 2% CHX. In the fifth treatment group, the canals of five instrumented teeth were irrigated with aquatine endodontic cleanser (Aquatine*EC*; Sterilox, Puricore, Malvern, PA) followed by a 15-second rinse with 17% EDTA and a final flush with Aquatine*EC*. In the sixth treatment group, the canals of five instrumented teeth were irrigated with *Morinda citrifolia* juice (MCJ; Tahitian Noni International, Provo, UT) followed by a 15-second rinse with 17% EDTA and a final flush with MCJ (25). In the negative control groups, 7 and 8, the canals of four instrumented teeth were irrigated with saline (VWR, Suwanee, GA). In the positive control groups, 9 and 10, the canals of four instrumented teeth were irrigated with saline followed by a 15-second rinse with 17% EDTA and a final flush with saline. These methods are congruent with those of Shabahang et al. (26).

A preexisting DPSC line (SHED) that was obtained through a material transfer agreement with the National Institutes of Dental and Craniofacial Research (3, 4) was used in this study to avoid a potential controversy over the most appropriate cell culture to use. This cell line was selected because it is the best studied of the human DPSC lines available. The SHED was cultured in Dulbecco's Modified Eagles Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin antibiotics, and Fungizone (Lonza Walkersville Inc., Walkersville, MD) was maintained at 37°C in a humidified atmosphere of 5% CO_2 with the culture media being replenished every second day. Confluent SHED cultures were collected by trypsinization (0.2% trypsin/EDTA) and subcultured in T-75 culture flasks (BD Biosciences, Franklin Lakes, NJ). Rat fibroblast L929 cells (ATTC, Manassas, VA) were maintained in cell culture and harvested using the same methods. An aseptic handling technique inside a laminar flow hood was used to deliver the cells into the root canals of the cleaned and shaped teeth. The SHED were added to the cleaned and shaped root canals of groups 1 to 7 and 9 shown in Table 1. L929 was added to the two cleaned and shaped root canals of group 10 shown in Table 1. The cells were added to the root canals at a density of 1×10^6 cells per mL of DMEM culture media using a microsyringe (17). The teeth containing the cells were individually placed upright in sterile 1.8-mL Eppendorf tubes and maintained at 37°C in a humidified atmosphere of 5% CO_2 with the culture media being replenished every second day (17). After 7 days of cell culture, the teeth were removed and processed for scanning electron microscopy. During the 7-day period of cell culture, the cytotoxicity of the irrigated root canal treatments shown in Table 1 were measured using a lactate dehydrogenase assay (LDH) at 2, 4, and 7 days. The assay measured the release of LDH from cells that have a damaged plasma membrane. The LDH released into the culture medium was measured with a 10-minute coupled enzymatic assay that results in the conversion of resazurin to a fluorescent resorufin product (27). The LDH assay (CytoTox-ONE; Promega Corporation, Madison, WI) was prepared from the supplied substrate mix and assay buffer, equilibrated to room temperature, and added to the media removed from the tooth cell cultures according to the manufacturer's instructions. The concentration of LDH was measured using a spectrophotometer at fluorescence of 560 nm. The culture media from the teeth without cells (group 6) was used as a control to determine the background fluorescence and was subtracted from the measurements of the treatment groups containing cells. The attachment of SHED to the dentin of root canals was assessed with high-power micrograph images of the root canals using a scanning electron microscope (SEM). The teeth were prepared for use in the SEM by fixing the tooth tissues in 10% neutral-buffered formalin solution at 18°C for 24 hours. The teeth were then dehydrated in a graded series of ethanol solutions (20%, 50%, 70%, and 90% for 2

TABLE 1. Treatment Groups

#	Group	Irrigant Used During Root Canal Cleaning and Shaping	Chelating Agent	Final Flush	Cell Type	Number of Teeth
1	NaOCl	6% sodium hypochlorite	None	None	Pulp stem cells	5
2	NaOCl + EDTA	6% sodium hypochlorite	17% EDTA	6% sodium hypochlorite	Pulp stem cells	5
3	MTAD	6% sodium hypochlorite	MTAD	MTAD	Pulp stem cells	5
4	CHX	2% Chlorhexidine	17% EDTA	2% Chlorhexidine	Pulp stem cells	5
5	Sterilox	Aquatine*EC*	17% EDTA	Aquatine*EC*	Pulp stem cells	5
6	Morinda citrifolia	Morinda citrifolia	17% EDTA	*Morinda citrifolia*	Pulp stem cells	5
7	Negative Control	Saline	None	None	Pulp stem cells	2
8	Negative Control	Saline	None	None	None	2
9	Positive Control	Saline	17% EDTA	Saline	Pulp stem cells	2
10	Positive Control	Saline	17% EDTA	Saline	L929 fibroblast cells	2

hours each followed by 1 day of 100% ethanol). They were removed from the solutions and placed in hexamethyldisilazane for 5 minutes to fix the dehydrated specimens. The teeth were dried on filter paper. The dried specimens were fractured longitudinally using a chisel and mounted onto aluminum stereoscan stubs with rapid set Araldite (Devcon Ltd, Shannon, Ireland). The dried mounted specimens were coated with a 20- to 30-nm thin metallic layer of gold/palladium in a Polaron E5000 sputter coater (BioRad, Hercules, CA). SEM micrographs were obtained at 2,000× magnification using digital image analysis software (25). All specimens were examined and the micrograph images stored as digital files in an Acer Computer connected to the SEM. Each of the root canals was scanned in its entirety to obtain an overview of the general surface topography. Micrographs were taken of representative areas characteristic of the general surface topography of each specimen. Micrographs were taken of three areas of each specimen including the apical, middle, and coronal aspects of the root canal. The dentin root canal surfaces were assessed for the presence of smear layer by two double-blind independent reviewers using semiquantitative visual criteria (25) as follows: (0) complete removal of smear layer and all dentinal tubules visible, (1) some removal of smear layer and more than 50% of tubules visible, (2) some removal of smear layer and less than 50% of tubules visible, and (3) no removal of smear layer and no dentinal tubules visible.

The number of cells (L929 and SHED) attached to the root canal walls per SEM micrograph field of view were assessed by two double-blind independent reviewers using semiquantitative criteria (17). The images of cells in the SEM micrographs were colorized using the "magnetic loop" and "color fill" functions of imaging software (Photoshop; Adobe, San Jose, CA). The morphology of the cells in the SEM micrographs was assessed using phenotypic criteria (score 0–2) described by Al-Nazhan (28): (0) round cells, (1) oblong cells, and (2) flattened cells. The SEM phenotypic criteria were analyzed statistically using chi-square tests. The numbers of attached cells were counted and analyzed using analysis of variance (ANOVA) statistical tests. The raw data were evaluated using STATview software (SAS Inc, Gary, NC) at a confidence level of 95%.

Results

The numbers of DPSC attached to the root canal surfaces after cleaning and shaping appeared to vary according to the use of different irrigating and chelating treatments (ANOVA, $p < 0.0001$). The highest average numbers of DPSCs (5.7 per SEM micrograph field) were attached to the root canals irrigated with Aquatine*EC* and EDTA (Fig. 1*A*), and the lowest average numbers of cells (1.5 per SEM micrograph field) were attached to the root canals irrigated with NaOCl and MTAD (Fig. 1*B*). The average difference between the numbers of cells attached in these two cleaning and shaping treatments was (1.5 vs 5.7 per SEM micrograph field) was 371% (Fig. 2). Four saline irrigation treatments (groups 7–10) shown in Table 1 helped to validate the accuracy of the results of the other irrigation treatments (groups 1–6) shown in Table 1 as follows: no cells were observed attached to the root canal walls where no DPSCs were added (group 8, Fig. 2). DPSCs were also observed attached to root canals after irrigation with saline (group 7, Fig. 2) and after a rinse of EDTA (group 9, Fig. 2). The L929 cells (group 10, Table 1) were observed attached to root canal walls similar to the DPSCs (group 7, Table 1) (Fig. 2). The rank order of cleaning and shaping treatments from the lowest to the highest mean numbers of attached DPSCs was NaOCl/MTAD, CHX/EDTA, NaOCl, NaOCl/EDTA, MCJ/EDTA, and Aquatine*EC*/EDTA (Fig. 2).

The absence and degree of the presence of the smear layer on the root canal surface did not appear to have much effect on cell attachment (ANOVA, $p > 0.05$) because the DPSC attached to the dentinal tubules (Figs. 1a and 1b) in addition to smear layer (Fig. 1*C*). Only one of the two-hundred and forty-nine SEM micrographs had the smear layer completely removed. The presence of smear layer and distribution of the numbers of DPSC attached to the root canal surfaces is shown in Figure 3.

The cleaning and shaping treatments appeared to present different levels of cytotoxicity to the DPSC added to the root canals (ANOVA, $p > 0.01$). The LDH absorbance was highest in the first four treatment groups: NaOCl, NaOCl/EDTA, NaOCL/MTAD, and CHX/EDTA, suggesting these were the most toxic cleaning and shaping treatments to DPSC (groups 1–4, Fig. 4). The LDH absorbance was lowest after the use of Aquatine*EC*/EDTA and MCJ/EDTA cleaning and shaping treatments (groups 5 and 6, Fig. 4). The Aquatine*EC*/EDTA and MCJ/EDTA treatments had a much lower LDH absorbance (ANOVA, $p < 0.001$) to the first four treatment treatments: NaOCl, NaOCl/EDTA, NaOCL/MTAD, and CHX/EDTA (groups 1–4, Fig. 4). The Aquatine*EC*/EDTA and MCJ/EDTA treatments had a similar LDH absorbance to the four saline control treatments (ANOVA, $p > 0.05$) (groups 7–10, Fig. 4).

The morphology of the DPSC's attached to the root canals appeared to be influenced by the cleaning and shaping treatments (chi-square test, $p<0.01$). In the first four treatments, NaOCl, NaOCl/EDTA, NaOCL/MTAD, and CHX/EDTA (groups 1–4, Fig. 5), most of the DPSCs had morphologies that ranged from round (Fig. 1*B*) to oblong (Fig. 1*C*). These DPSCs had a different morphology compared with the DPSCs attached to canals after the Aquatine*EC*/EDTA and MCJ/EDTA treatments (chi-square test, $p < 0.01$). Although the DPSCs attached to root canals after Aquatine*EC*/EDTA and MCJ/EDTA treatments had morphologies that ranged from oblong to flattened (Fig. 1*A*), similar to the saline control treatments (chi-square test, $p > 0.05$) (groups 7, 9, and 10, Fig. 5), the presence or absence of smear layer had no or little influence on the morphology of attached DPSCs (chi-square test, $p > 0.05$).

Discussion

Some previous studies have shown that cells can attach and survive on dentin surfaces (29) and endodontic materials (30); other studies have shown that cells can attach to tissue-engineering scaffolds (17). However, no previous studies have investigated the attachment of DPSC to root canal dentin after different endodontic cleaning and shaping treatments. The present study investigated the cleaning and shaping of *ex vivo* human nondiseased single-canal teeth using ProTaper and ProFile rotary instrumentation and compared the effect of 10 different endodontic irrigation and chelating treatments on DPSC attachment to root canal surfaces. The present study investigated the traditional irrigating solutions, 6% sodium hypochlorite and 2% chlorhexidine gluconate, in conjunction with EDTA or MTAD. The present study also investigated the novel irrigants: MCJ and Aquatine*EC*. *Morinda citrifolia* is an exotic plant often called Noni that has been used in folk remedies by Polynesians for over 2,000 years. MCJ is reported to have a broad range of therapeutic effects, including antimicrobial activity, and has been suggested as a natural endodontic irrigating solution (25). In August 2006, the US Food and Drug Administration cleared Aquatine*EC* for use as an endodontic irrigating solution. Aquatine*EC* is intended to irrigate, cleanse, and debride the root canal system (510k number K061689). The active component in Aquatine*EC* is hypochlorous acid (HOCl). HOCl is produced by the body's immune cells, via a chain of aerobic reactions called the oxidative burst pathway, to kill invading pathogens and to fight infection (31). HOCl is biocompatible and antimicrobial against a broad range of microorganisms (32). The irrigation treatments were used in conjunction with EDTA chelating agent to replicate common endodontic practice. The present study used four saline irrigation treatments as control groups for comparison with

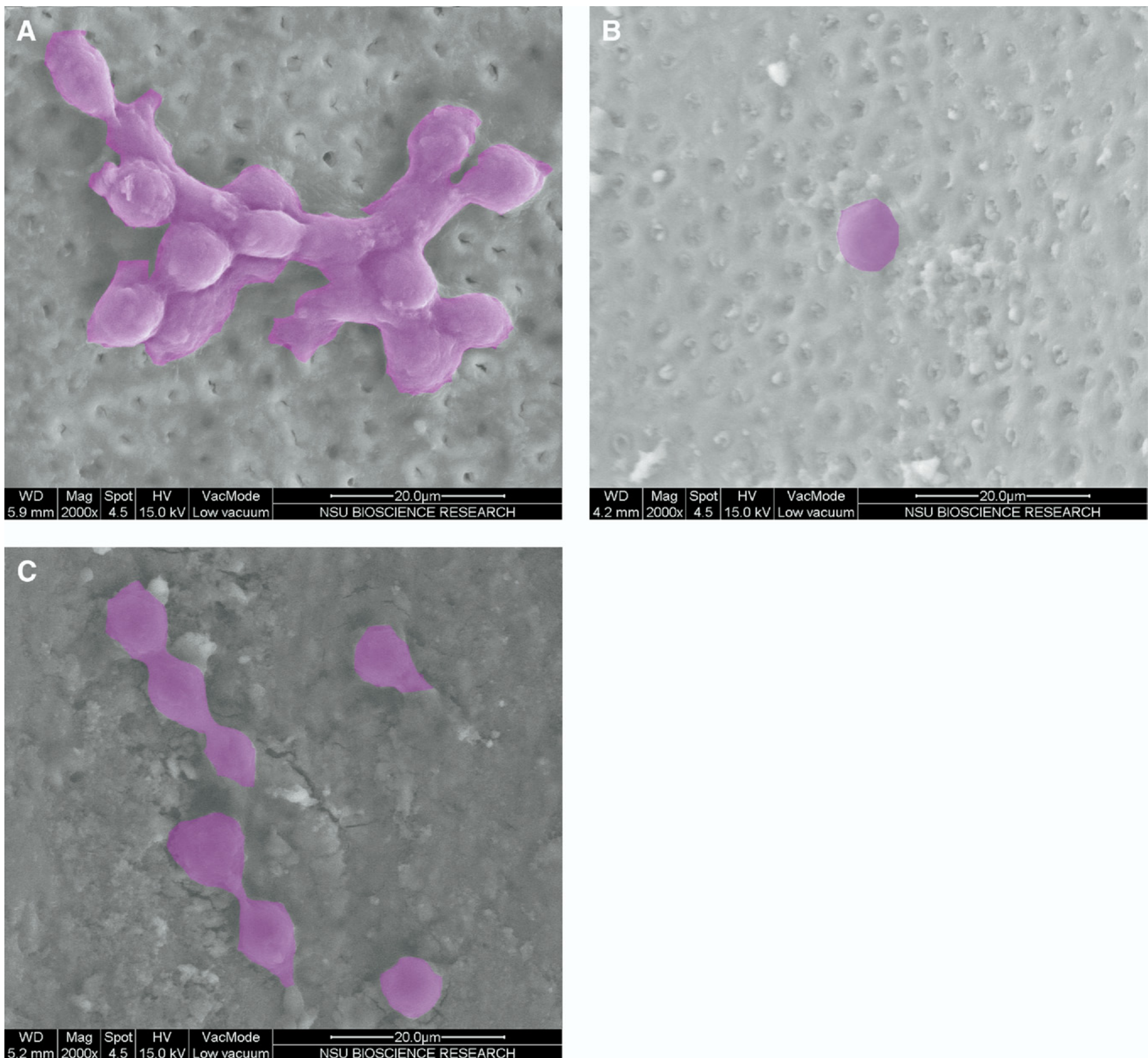

Figure 1. (*A*) Flattened pulp cells attached to cleaned and shaped dentin after the use of Aquatine EC and MTAD. (B) Rounded pulp cells attached to cleaned and shaped dentin after the use of NaOCl and MTAD. (C) Oval pulp cells attached to the root canal smear layer after the use of NaOCl.

the six traditional and novel irrigation treatments. The instrumented teeth were immediately placed in cell culture with confluent DPSCs for 1 week to investigate the reactions of the cells in response to the various root canal irrigation treatments. One week was selected because pilot studies showed that it provided sufficient time for the cells to be seen attached in SEM micrographs; at shorter time intervals, less cells were seen. One week was selected, rather than 1, 2, or 3 days, to prolong the effect of the irrigation treatments and to help exclude the possibility of short-term effects.

Future regenerative endodontic procedures may be routinely used to revitalize immature permanent teeth (33–35) and, with continued research and development, eventually even mature teeth (18). Regenerative endodontic procedures may replace damaged, diseased, or missing dentin and root structures as well as cells of the pulp-dentin complex with live viable tissues (18). In order to accomplish regenerative endodontic therapy, it may be necessary to stimulate DPSC attachment to root canal dentin; however, information is scarce about how various irrigating solutions used during cleaning and shaping can influence DPSC activity. The present study found that the numbers of DPSCs attached to the root canal surfaces after cleaning and shaping appeared to vary according to the use of different irrigating and chelating solutions. The highest average numbers of DPSCs were attached to the root canals irrigated with Aquatine*EC* and EDTA, and the lowest average numbers of cells were attached to the root canals irrigated with NaOCl and MTAD. The rank order of irrigation and chelation treatments from the lowest to the highest mean numbers of attached DPSCs was NaOCl/MTAD, CHX/EDTA, NaOCl, NaOCl/EDTA, MCJ/EDTA, and Aquatine EC/EDTA (Fig. 2). Unexpectedly, given the importance of smear layer removal to the success of root canal treatments (22, 23), the absence and degree of presence of smear layer on the root canal surface did not appear to have much effect on DPSC attachment because the cells were attached to the dentinal tubules in addition to smear layer (Fig. 3). These results indicate that it will not be necessary to remove 100% of the smear layer from root canal walls for DSPC attachment to occur as part

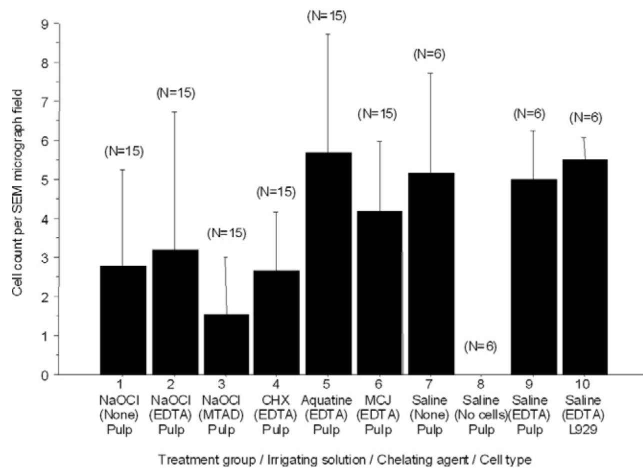

Figure 2. Cell attachment to root canal surfaces after cleaning and shaping. The error bars represent the standard deviation of the mean. The N is the number of root canal SEM micrographs assessed for each group.

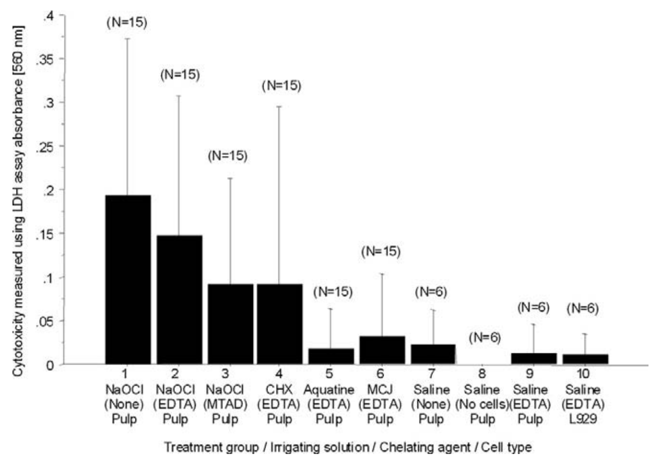

Figure 4. The cytotoxicity of endodontic cleaning and shaping treatments. The error bars represent the standard deviation of the mean. The N is the number of root canal SEM micrographs assessed for each group.

of regenerative endodontic treatment. Given the difficulty of removing 100% of smear layer from root canals, this finding suggests new more effective smear layer removal treatments are not needed to allow regenerative endodontic therapies to become a reality. However, the development of more effective smear layer removal treatments may be advantageous to improve the effectiveness of root canal cleaning and shaping.

It has already been well established that the common endodontic irrigating solutions; NaOCl and CHX are cytotoxic to pulp cells and oral tissues. This can be beneficial for necrotic tissue dissolution (36). However, little attention has been given to the possible cytotoxic effects of irrigating solutions when used as part of regenerative endodontic therapy, such as the addition of DPSC to the root canal, as described in the present study. This study evaluated Aquatine*EC* and MCJ as irrigants because of their biocompatibility and low cytotoxicity in order to measure their effects on DPSC attachment to the root canal. The inclusion of these novel irrigating solutions was fortuitous because the selection of irrigating solution during cleaning and shaping appeared to present different levels of cytotoxicity to the DPSC added to the root canals (Fig. 4). The LDH absorbance was highest in the first four irrigation and chelation treatments: NaOCl, NaOCl/EDTA, NaOCl/MTAD, and CHX/EDTA, suggesting that these were the most toxic cleaning and shaping treatments to DPSC. The cytotoxicity of the root canals irrigated with NaOCl/MTAD was slightly less than NaOCl and NaOCl/EDTA, indicating that MTAD is more biocompatible than NaOCl, which is in agreement with a previous study (37). The Aquatine*EC*/EDTA and MCJ/EDTA treatments had a much lower average LDH absorbance, similar to the saline control treatments (Fig. 4), suggesting that Aquatine*EC*/EDTA and MCJ/EDTA are the least cytotoxic of the treatments examined.

The morphology of the DPSCs attached to the root canals appeared to be influenced by selection of cleaning and shaping irrigation and chelating treatments. In the first four treatment groups, NaOCl, NaOCl/EDTA, NaOCl/MTAD, and CHX/EDTA, most of the DPSCs had morphologies that ranged from round (Fig. 1*B*) to oblong (Fig. 1*C*). Meanwhile, the DPSCs attached to root canals after the Aquatine*EC*/EDTA and MCJ/EDTA treatments had morphologies that ranged from oblong to flattened (Fig. 1*A*), similar to the saline control treatments (Fig. 5). The morphology of the DPSCs appeared to be correlated with the LDH absorbance measure of cytotoxicity. However, the presence or absence of smear layer had no or little influence on the morphology of attached DPSCs. The analysis of morphology of the DPSCs attached to the cleaned and shaped root canals is important because the cells are round when attaching to the substratum, they then become oblong with the radial growth of filopodia and cytoplasmic webbing, and eventually when fully

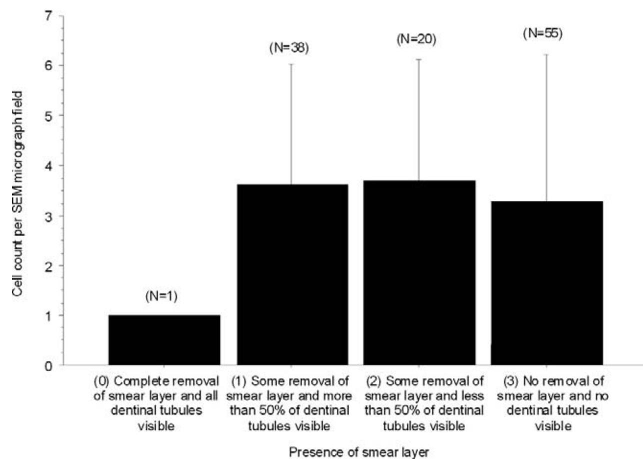

Figure 3. Cell attachment to root canal surfaces after cleaning and shaping. The error bars represent the standard deviation of the mean. The N is the number of root canal SEM micrographs assessed for each group.

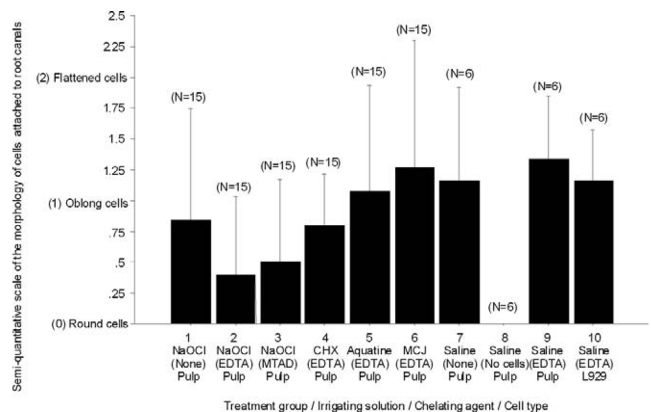

Figure 5. The morphology of cells attached to root canals. The error bars represent the standard deviation of the mean. The N is the number of root canal SEM micrographs assessed for each group.

attached they are flattened (38). These cellular changes explain the selection of the cell morphology (score 0-2) criteria described by Al-Nazhan (28): (0) round cells, (1) oblong cells, and (2) flattened cells. The present findings of more flattened cells (DPSCs) in the most biocompatible irrigation treatments, Aquatine*EC*/EDTA and MCJ/EDTA, is in agreement with a previous study that measured the morphology of periodontal ligament fibroblasts attached to MTA as a measure of material cytotoxicity (30). These results suggest that Aquatine*EC*/EDTA and MCJ/EDTA are among the most optimal of the irrigating solutions to help maintain the survival and attachment of DPSCs to be used as part of regenerative endodontic treatment.

The use of saline is not recommended as an irrigating solution because it lacks antimicrobial and tissue dissolution activity. It was strictly used in the control protocols (groups 7–10) of the present study for a comparison of its effects. However, its good biocompatibility suggests that saline could be used in regenerative endodontic treatments as a final rinse after irrigation with NaOCl, CHX, or MTAD to help promote DPSC attachment to root canal walls.

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