Title: Long-term survival of indirect pulp treatment performed in primary and permanent teeth with clinically diagnosed deep carious lesions

Author: Gruythuysen R et al.

Journal: JOE; 36(9): 1490-3

Reviewer: Daniel Cassis, DDS

Purpose: To investigate the 3-year survival rate of functional (clinically and radiographically) primary and permanent teeth treated with indirect pulp treatment (IPT).

Materials and Methods:
- Radiographic and clinical records were reviewed from procedures performed from 2000 to 2004.
- Sixty-six uncooperative children (4-18 years old) with at least one tooth clinically diagnosed with deep caries were included. The lesion depth was greater than two thirds of the dentin thickness.
- Incomplete excavation was performed leaving infected carious dentin at the center of the cavity. A layer of resin-modified glass ionomer was placed as a liner, and then the teeth were restored.
- A 3-year survival analysis (Kaplan-Meier) was performed. Failure was determined as the presence of either a clinical symptom (pain, swelling, or fistula) or radiologic abnormality at recall.
- 86 of 125 (69%) of treated primary molars and 34 of 45 (76%) treated permanent teeth were available for both clinical and radiographic evaluation.

Results: The survival rate was 96% for primary molars and 93% for permanent teeth.

Discussion: Previous studies indicate deep carious lesions are normally accompanied by pulpal involvement. Despite this, 93% of permanent teeth and 96% of primary teeth remained symptomless and free of intraradicular/periapical radiolucencies for up to 3 years following IPT. An adequate sealing of remaining infected dentine is of the utmost importance in IPT. Studies have shown that when a restoration is placed over carious dentin: (1) viable bacteria decreases (2) a shift occurs towards a less cariogenic microflora (3) the level of bacterial colonization is equal to complete caries removal. Some bacteria may survive but not in sufficient quantity to advance disease.

Conclusion: This study shows that IPT performed in primary and permanent teeth of young patients may result in a high 3-year survival rate.

LOE: 4
Title: Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation and surfactant.

Author: Stojicic S et al.

Journal: JOE, Vol 36, No.9:1558

Reviewer: Arwa Siyam, DDS

Purpose: To test the effect of concentration, temperature, agitation and surfactant on the tissue dissolving capacity of NaOCl.

Materials and Methods:

- Three different NaOCl solutions
  1. Regular 1 (6% NaOCl): EMD chemicals Inc, Gibbstown NJ
  2. Regular 2 (5.8% NaOCl): Inter-Med, Inc/Vista Dental products, Racine, WI
  3. Chlor-Xtra™ (A Sodium Hypochlorite solution that has been enhanced with powerful wetting agents and proprietary surface modifiers. Alkylating agents have also been added to increase the electrical capacity of the solution.): Vista Dental
- Four different concentrations of each solution were prepared using distilled water. The concentrations were 1%, 2%, 4% and 5.8%. The two controls were distilled and sterilized water.
- Bovine meat of similar shape, size and weight was used as a tissue sample. (4x4x2 mm and 68mg).
- The samples were weighed before and after the experiments, and the percentage of weight loss was calculated.
- To test the effect of temperature; the three different solutions were tested each with four different concentrations at room temperature (RT), 37 and 45º.( the experiments were carried out in a temp. controlled water bath for 5 min)
- To test the effect of agitation: Three agitation methods (ultrasonic, sonic and pipet) were used and compared to each other and to no agitation at RT and at 45º at selected concentrations. (2% and 5.8%)
- The samples were agitated for 15 seconds each minute for 5 min.
- The effect and duration of pipetting alone was tested using the 2% and 5.8% solution of Reg2 at RT. The agitation protocols were 1 or 2, 15 seconds for each minute or continuously for 5 min.
- Contact angle measurement: A 1.5 µl droplet of the 1% and 5.8% of the 3 solutions was placed on highly polished root dentine. The contact angle was measured within 30 seconds using a NRL contact angle goniometer.

Results:

- Weight loss increased with increasing concentrations of NaOCl.
- A significant difference in weight loss was observed after exposure to 2% Chlor-Xtra and 4% and 5.8 % of Regular 1 and Regular 2 compare with controls at RT.
- Heating the hypochlorite solutions greatly increased tissue dissolution.
- Chlor-Xtra™ dissolved significantly more tissue than the other two in all temperature/concentration groups.
- Tissue wt. loss was significantly higher when the solution was agitated at both tested temperatures than without agitation.
- Under agitation at RT tissue wt. loss was significantly higher with 5.8 % Chlor-Xtra™ than with both 5.8% Regular 1 and 2.
- Agitation experiments with increased time of active agitation showed continuous increase in tissue dissolution.
- Tissue weight loss was significantly higher after simultaneous action of temperature and agitation than by either one alone.
- Chlor-Xtra™ 5.8% had the lowest contact angle of the three hypochlorite solution. There was no significant difference in the contact angle between the 1% solutions.

Conclusion: The increase in concentration and temperature of NaOCl greatly increased the efficacy in tissue dissolution. Refreshing the solution at the site of dissolution by agitation, preferably continuous resulted in a marked increase in tissue dissolution. High temperature and agitation had an additive effect on tissue dissolution. The solution with added surface active agent was the most effective in tissue dissolution at all temperatures and concentrations.

LOE: 5
Title: Frequency of nonodontogenic pain after endodontic therapy: a systemic review and meta-analysis

Author: Nixdorf D et al

Journal: JOE, Vol.36, 9:1494

Reviewer: Chaiwing Hsiao, DMD

Purpose: To conduct a systemic review of the prospective studies that reported the frequency of non-odontogenic pain in patients who have undergone endodontic procedure

Materials and Methods: Studies were searched in four databases electronically (Medline, Cochrane library, trip database and Google scholar), complemented by hand searching. Eligible for inclusion in this review were endodontic procedure articles published in any language before June 5, 2009 that reported on postoperative tooth pain after at least a 6 months follow up. Qualifying endodontic procedures included initial root canal treatment or retreatment, surgical or non-surgical, but not pulpotomy, partial pulpotomy or pulp capping. The study outcome was the presence of dentoalveolar pain that did not have an odontogenic etiology.

Results: Seven hundred seventy articles were retrieved and reviewed, 10 met inclusion criteria and 9 had data on both odontogenic and non-odontogenic causes of pain status. In the 10 studies 3343 teeth were enrolled, 1125 teeth were followed up for 6 months at least. Among them 48 (4.3%) in seven studies were reported to have pain without an identifiable odontogenic source. In these 9 studies 44 non-odontogenic pain cases (56%) of the 78 all cause pain cases were identified.

Conclusion: Pain of non-odontogenic origin after root canal therapy was found in 3.4% of patients, a number that likely represents about half of all persistent “tooth pain” therefore the outcome of nonodontogenic pain is not as rare as commonly assumed. Studies with a shorter follow up (6-12 months) had a greater frequency of persistent non-odontogenic pain than those with a longer follow up, which is an important finding and may suggest that such persistent pain improves with time. It is important to differentiate non-odontogenic pain from those of local etiology because tooth based pathology is amenable to endodontic retreatment and non-odontogenic pain would be best treated if recognized.

LOE: 2
Comparison of tetraacetylethylendiamine + sodium perborate and sodium hypochlorite cytotoxicity on L929 fibroblasts.

Simbula G et al.

JOE, Volume 36, number 9 : 1516-1520

Ferras Mashtoub, DDS

To test and compare the cytotoxicity of TAED+P and NaOCl on fibroblasts.

Materials and Methods:

Primary:
- L929 Fibroblasts were selected for this study.
- Fibroblasts were seeded in a 96-well microtrite plate at a concentration of 1x10⁴ cells/well. Each experiment was conducted using six cultures for each group.
- 2% TAED+P and 5% NaOCl were dissolved in distilled water and added to the culture mediums to obtain the final concentrations specified for each group (ranged from 0.0025% to 0.5%).
- The cytotoxicity of each disinfectant was evaluated after 2, 4, 6, and 24 hour incubation periods using the neutral red uptake (NRU) and MTT assays.
- Control were untreated (no disinfectant) cells.

Secondary:
- Fibroblasts were also examined under a microscope for morphologic changes (data described but not formally shown in paper)
- To mimic tissue damage in vivo, the concentration of FCS (Fetal calf serum) was changed from 0%-10% and an MTT (more sensitive than NRU) assay was done.
- Finally, fibroblasts were treated with increasing concentrations of both disinfectants for 30 minutes and then medium was replaced with fresh medium (no disinfectants) to evaluate recovery of cells 24 hours later.

Results:
- The viability of untreated (control) cells remained unchanged throughout the experimental period
- Both disinfectants induced a dose-related inhibitory effect on the cell viability of L929 fibroblasts
- Drop seen in cell viability for both disinfectants after 2 hours at 0.025% concentration, but drop in viability was greater with NaOCl.
- TAED+P was less cytotoxic than NaOCl at every examined time point at concentrations less than or equal to 0.025%.
- The most pronounced difference in cytotoxicity was at the 24 hour mark
- 50% inhibition dose for NaOCl was estimated 50 micrograms/ml (0.005%) and for TAED+P it was 350 micrograms/ml (0.035%).
- When examined under a microscope, the TAED+P treatment in 0.0025%-0.025% dose range induced fewer morphologic changes in the fibroblasts than did NaOCl.
- Differing the FCS concentrations to mimic tissue injury did not change the efficacy of either chemical at any concentration
- The cells treated for 30 minutes, then refreshed with new medium showed an ability to recover from TAED+P in concentrations up to 0.025%, after that the cells were not able to recover. With NaOCl, the recovery rate was much smaller and at lower concentrations

Discussion and Conclusion: The TAED+P showed lower cytotoxicity than NaOCl, which is a desirable trait in an irrigating solution. However, further tests still need to be done to determine if other traits of TAED+P (such as anti-microbial activity, ability to dissolve tissue, prevent or remove a smear layer from forming, etc.) are comparable to NaOCl and would warrant its use as an irrigating solution.

LOE: 5
Title: Physicochemical properties of methacrylate resin-based root canal sealers

Author: Sousa-Neto M et al

Journal: JOE, Vol. 36, No.9:1531

Reviewer: Nicole Vu, DMD

Purpose: To assess the physicochemical properties of 2 methacrylate resin-based sealers (Epiphany® SE and Hybrid Root Seal) and compare the results to epoxy resin-based sealer, AH Plus®.

Materials and Methods:

- Group 1 (AH Plus®); Group 2 (Epiphany® SE); Group 3 (Hybrid Root Seal)
- Setting time: Materials were placed into plasters of cast rings. Group 2 and 3 were light-cured for 40 sec. A Gilmore-type needle was used to make indentations. Time from the start of mixing to this point was recorded.
- Flow test: Materials were placed on glass plates. Another plate was placed on top of the materials, and was removed after 10 min. Major and minor diameters of the compressed disc were measured.
- Radiopacity test: Sealers were placed in wells on acrylic plates and incubated. Another acrylic plate containing an aluminum step wedge was placed in front of phosphor plate. Samples were placed next to aluminum step wedge and in front of phosphor plate. Radiographic images were obtained using Spectro 70x x-ray machine.
- Dimensional change after setting: Teflon molds were filled with sealers and incubated. Samples were removed, measured with digital caliper, and then stored in distilled and deionized water. After 30 days, samples were measured again for length.
- Solubility: Teflon molds filled with materials. Nylon threads were placed inside the cements, the molds incubated, weighted after setting. Samples suspended on the threads were placed in distilled and deionized water for 7 days, and then weighted.
- SEM examination: Teflon molds were filled with sealers and incubated. Sections were exam under SEM

Results:

- Setting time: AH Plus®>Hybrid Root Seal>Epiphany® SE
- AH Plus® set longer than the other two, Hybrid Root Seal was intermediate, Epiphany® SE exhibited inferior setting time.
- Flow test: Hybrid Root Seal>AH Plus®=Epiphany® SE
- AH Plus® set longer than the other two. All groups conformed to ANSI/ADA standards.
- Radiopacity test: AH Plus®=Epiphany® SE>Hybrid Root Seal
- All materials had radiopacity above 3mm aluminum recommended by standards.
- Dimensional change after setting: Hybrid Root Seal>AH Plus®=Epiphany® SE
- All sealers exhibited dimensional change was greater than acceptable values.
- Solubility: AH Plus®=Epiphany® SE>Hybrid Root Seal
- All results showed agreement with the standards
- Distilled and deionized water was analyzed for the ions released by each sealer; AH Plus® released high amount of Ca²⁺, Hybrid Root Seal high in K⁺, Epiphany® SE high Zn
- SEM: AH Plus® and Epiphany® SE seemed better compacted and organized than Hybrid Root Seal.

Discussion:

Before the clinical use of any materials, it is necessary to perform standardized tests to check for their physiochemical and biological properties. AH Plus® had the setting time 9 times than the other two sealers, possibly because of the slow polymerization reaction of epoxy resin amines. Hybrid Root Seal showed the highest flowability because of it composition of small spherical particles that form less compact and viscous structure. Hybrid Root Seal has only one radiopacifying agent therefore was the least opaque material. The presence of hydrophilic radicals in methacrylate-based sealers resulted in higher water absorption and consequently higher expansion.

Conclusion: The study disclosed that the physicochemical properties of the 3 sealers conformed to ANSI/ADA standardization, except the setting time of Hybrid Root Seal and the dimensional change of all sealers.

LOE: 5
Title: Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth

Author: Vijayendran, G et al.

Journal: JOE, Vol. 36, No. 9:1504

Reviewer: Christian Kecht, DDS

Purpose: To compare proliferation rate, gene expression profile, and lineage propensity of stem cells from deciduous teeth (SCD) and stem cells from permanent teeth (DPSCs).

Materials and Methods:
- Isolation and Culture: Stem cells from permanent teeth (DPSCs) obtained from sound and intact human third molars from adults (age 24-35 yrs). Stem cells from deciduous teeth (SCD) obtained from sound and intact deciduous teeth (age 5-8 yrs). The root surfaces of the teeth were cleaned with povidone-iodine and the pulps were extirpated within 2 hours after extraction and then processed. Pulp tissue was minced into small fragments then digested in a collagenase solution for 40 minutes. The cells were centrifuged and seeded in culture flasks with culture medium. When primary cultures became subconfluent (10-14 days) the cells were collected by trypsinization and processed for subsequent passages. Human bone marrow mesenchymal stem cells (BM-MSCs) and cultures were established from three donors (age 18-25 yrs).
- Colony-forming Units: Number of colony-forming units (CFU) was determined by plating 100 cells in 35mm dishes, culturing them for 14 days, then counting colonies more than 2mm in diameter.
- Growth Kinetics: Cells were plated in culture flasks, three replicates performed for each passage and time point, and cells counted and assessed for viability by means of trypan blue dye. Subsequent passages were performed for a total of five passages. Growth kinetics analyzed by calculating population doubling (PD) time.
- Cell Cycle Analysis: Cells were seeded and cultured until reaching 90% confluence. Cells were detached, fixed, and DNA content analyzed.
- Flow Cytometric Analysis: Immunophenotyping done at passage 5. All analyses were standardized against negative control cells incubated with isotype-specific immunoglobulin.
- Differentiation of DPSCs: Cultures initiated, grown to confluence, and subjected to differentiation into adipogenic, chondrogenic, and osteogenic lineages. Adipogenic differentiation assessed by lipid droplet visualization using oil red O staining. Chondrogenic differentiation assessed by visualizing proteoglycan accumulation with alcian blue staining. Osteogenic differentiation was assessed by visualization of calcium accumulation with von Kossa staining.
- Human Taqman Low Density Array: Human Taqman Low Density Array (TLDA) containing a well-defined set of validated gene expression markers to characterize embryonic stem cell identity was used for analyzing the expression of a focused panel of pluripotent and stem cell markers.
- Reverse Transcription PCR and Real-time Reverse Transcription PCR: Total RNA extracted, reverse transcribed into cDNA, followed by amplification. PCR products resolved on agarose gel.
- Neurogenic Differentiation: SCD and DPSCs cultured in Neurobasal-A medium supplemented with EGF and bFGF for 15 days. Neurosphere-like bodies generated after 15 days were counted. Neurospheres were titrated and seeded onto gelatin-coated dishes in neurodifferentiation medium one. Immunocytochemical analysis was performed 21 days after cultivation.
- Karyotype Analysis: Cultures treated with colcemid, detached, and fixed with Conroy’s solution. 20-30 separate metaphase spreads were examined for each culture.
- Data and Statistical Analysis: Data analyzed using two-way analysis of variance (ANOVA). Significance level was set at P=.05. Tukey post hoc multiple comparison tests were done to determine differences between groups. Differences between SCD and DPSCs were visualized by applying a novel approach based on principle component analysis (PCA). PCA is a mathematical algorithm that describes the data on the basis of their dissimilarity.

Results:
- Isolation and Characterization of SCDs and DPSCs: Morphologic characteristics of SCD and DPSCs displayed indistinguishable fibroblast morphology resembling that of BM-MSCs. The CFUs were higher in SCD compared to DPSCs. The number and size of colonies were more in SCD than in DPSCs, indicating SCD has higher proliferation rate than DPSCs.
- Cell Surface Antigen Profile of SCD and DPSCs: Immunophenotyping showed that SCD and DPSCs were negative for hematopoietic markers, but 90% of the cells were positive for mesenchymal stem cell markers.
- Differentiation of SCD and DPSCs into Classic Lineages: mRNA expression of two osteoblast markers, osteocalcin and osterix was found to be higher in SCD as compared with DPSCs. Also, higher number of SCD than that of DPSCs featured an adipogenic differentiation capacity. Both SCD and DPSCs demonstrated a cartilage-like phenotype.
- Cytogenetic Stability of SCD and DPSCs: Both showed normal karyotypes at passage 5.
• Pluripotent Gene Array Analysis between SCD and DPSCs: The pluripotency was highly maintained in the SCD as compared with DPSCs and BM-MSCs over the course of culturing. SCD also expressed some endoderm and mesoderm markers as compared with DPSCs. However, DPSCs expressed higher neuron/ectoderm markers.
• Neuronal Differentiation in SCD and DPSCs: Both SCD and DPSCs capable of forming neurospheres. The DPSCs had a significant increased number of neuritis as compared with SCD.

Discussion:
The use of SCD and DPSCs is easy for several reasons including ease of isolation and noninvasive collection with less or no ethical issues as compared with BM-MSCs. The growth kinetics revealed that SCD possessed a higher proliferation rate than DPSCs. Both SCD and DPSCs were able to differentiate into osteoblasts adipocytes, and chondrocytes, thus qualifying the minimum requirements of MSCs.

Conclusion: Gene variations occurred within the different sources of the same stem cells, and that these variations determine their lineage propensity toward a specific destination. SCD retained their plasticity over the passages, whereas DPSCs lost their plasticity and were shown to be more committed toward neuronal lineage. Both SCD and DPSCs could act as useful candidates for regenerative medicine in various diseases, emphasizing the usage of DPSCs for neurologic diseases.

LOE: 5