



## Overview:

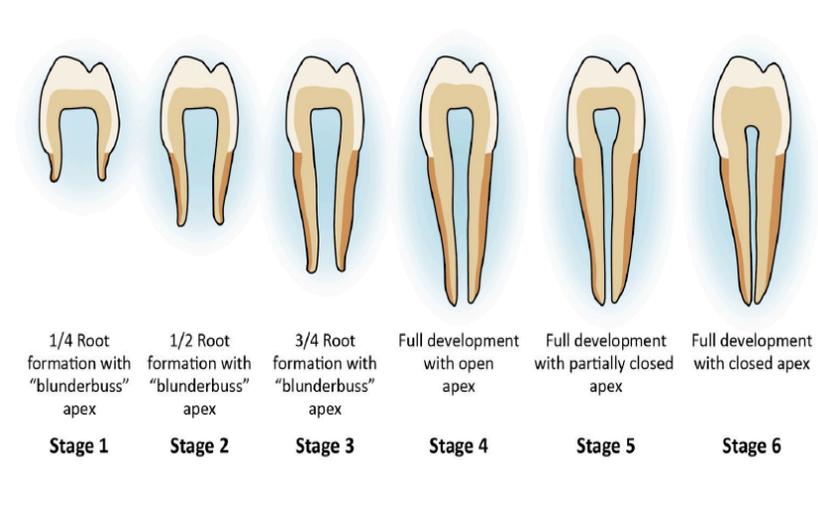
- ✓ The management of immature permanent teeth with pulpal necrosis is challenging as the root canal system is often difficult to debride and the thin dentinal walls are at an increased risk of a subsequent cervical fracture. This results in a restorative problem since implants are generally contraindicated in young patients with a growing craniofacial skeleton. Regenerative endodontic therapy provides an alternative treatment approach that builds on the principles of regenerative medicine and tissue engineering. The aim of the therapy is to successfully treat these challenging cases by regenerating functional pulpal tissue utilizing protocols referred to as regenerative endodontic procedures (REPs) (**Colleagues for Excellence**)
  
- ✓ Different pre-, intra- and post-operative factors have been associated with the outcome of regenerative endodontic treatment. Common features of cases with successful clinical outcomes after REPs are:
  - ✓ **Young patient**
  - ✓ **Necrotic pulp and immature apex**
  - ✓ **Minimal or no instrumentation of the dentinal walls**
  - ✓ **Placement of an intracanal medicament**
  - ✓ **Creation of a blood clot or protein scaffold in canal**
  - ✓ **Effective coronal seal**

## Success of regenerative endodontic treatment:

The degree of success of regenerative endodontic procedures is largely measured by the extent to which it is possible to attain **primary, secondary, and tertiary** goals:

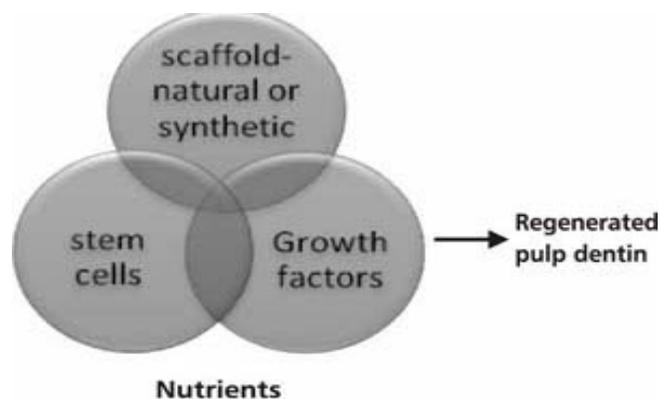
- ✓ **Primary goal: The elimination of symptoms and the evidence of bony healing.**
- ✓ **Secondary goal: Increased root wall thickness and/or increased root length (desirable, but perhaps not essential)**
- ✓ **Tertiary goal: Positive response to vitality testing (which if achieved, could indicate a more organized vital pulp tissue)**

- ✓ Regenerative endodontics is not a new topic. **Nygaard-Ostby (Acta Odont Scand 1961)** for the first time investigated the role of the blood clot in the endodontic therapy. They proposed that the organization of the **blood clot** in the apical periodontium and in the root canal can be directly compared with **wound healing**.
- ✓ In a most recent study, **Diogenes and Ruparel (2016, Dent Clin North Amr)** investigated the preference of operators in performing the regenerative treatment versus apical barrier apexification based on the different stages of tooth development. It was shown that up to stage 4, 73% of participants will choose regenerative treatment. But this rate will drop to 46% at stage 5.



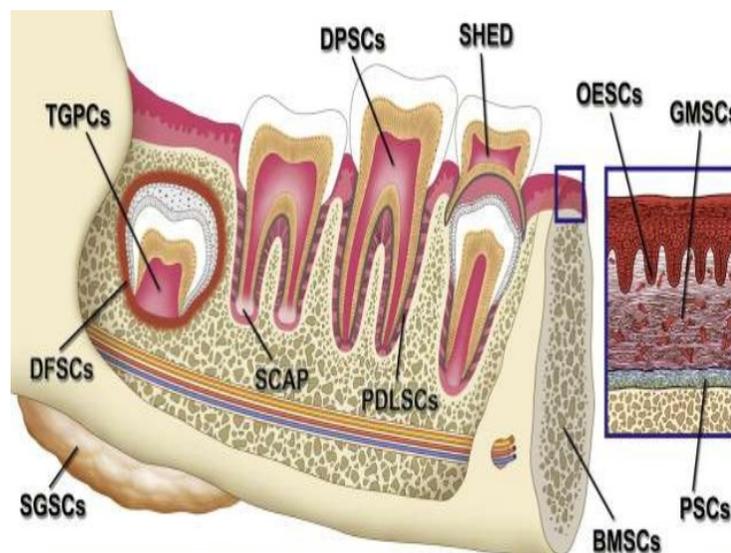
courtesy of Dr. Anibal Diogenes

- ✓ Following figure illustrates the core principles of tissue engineering. Tissue regeneration requires an appropriate source of **stem/progenitor cells, growth factors and scaffolds** in order to control the development of the targeted tissue (**J Endod. 2013 Mar, Hargreaves**).



## First Step; Stem cells

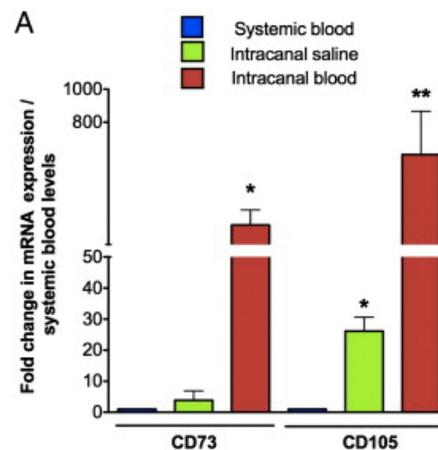
- ✓ What are stem cells? “Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells” (NIH) These cells should have two qualities: **1- Self-Renewal 2-Differentiate into more specialized cells**
- ✓ **What are sources of stem cells?** It has been suggested that regenerative endodontic is a stem-cell based treatment. There are different sources of stem cells. The most investigated cell involved in the REPs are Stem Cell of Apical Papilla (SCAP). Epi hertwig sheet need to communicate with meshanchial stem cell and the apical papilla to complete the root development.



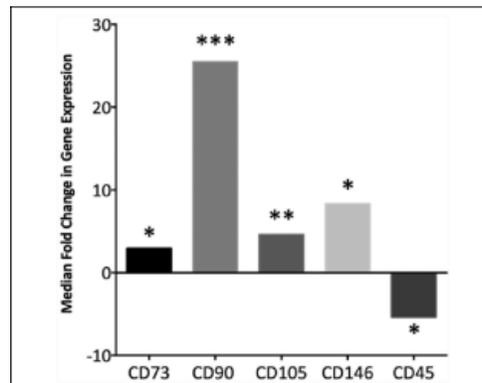
Diogenes et al., Endodontic Topics 2013

- ✓ **George T.-J. Huang JOE, 2008:** Apical papilla is apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp. Importantly, **there are stem/progenitor cells located in both dental pulp and the apical papilla, but they have somewhat different characteristics.** Because of the apical location of the apical papilla, this tissue may be benefited by its collateral circulation, which enables it to survive during the process of pulp necrosis.

- ✓ **Are SCAP distinct from dental pulp stem cells (DPSCs)?** It has been reported that SCAP showed a significantly greater bromodeoxyuridine uptake rate, number of population doublings, tissue regeneration capacity, and number of STRO-1–positive cells when compared with DPSCs. In addition, SCAP express a higher level of survivin (antiapoptotic protein) than DPSCs and are positive for hTERT (human telomerase reverse transcriptase that maintains the telomere length) activity, which is usually negative in MSCs. These lines of evidence suggest that **SCAP derived from a developing tissue may represent a population of early stem/progenitor cells, which may be a superior cell source for tissue regeneration.** Additionally, these cells also highlight an important fact that developing tissues may contain stem cells distinctive from that of mature tissues (PLoS ONE 2006;1:e79).
  
- ✓ **Are stem cells delivered into the root canal system?** The mesenchymal stem cell markers CD105, CD73 were detected in cells collected from the canal space after the evoked-bleeding step. These findings demonstrate that the **evoked-bleeding step in regenerative procedures triggers the significant accumulation of undifferentiated stem cells into the canal space where these cells might contribute to the regeneration of pulpal tissues seen after antibiotic paste therapy of the immature tooth with pulpal necrosis.** The figure shows that saline irrigation with no introducing of blood can also increase the number of cells with CD73, CD105 surface markers (indicator of mesenchymal stem cells) (J Endod. 2011 Feb;37(2):133-8, Lovelace TW).

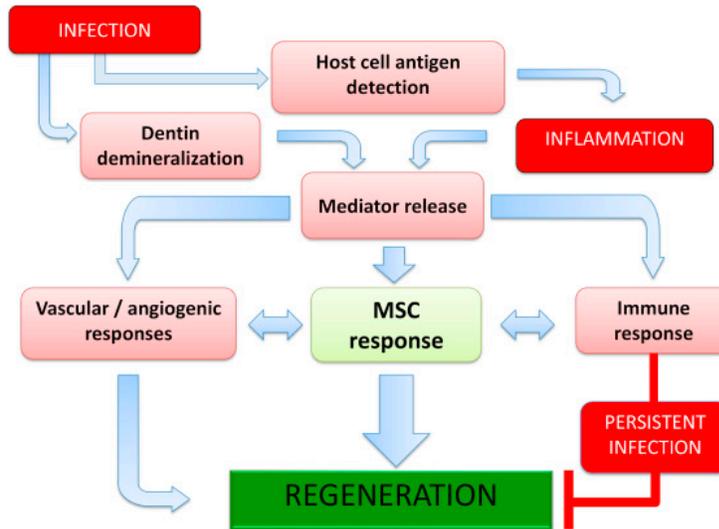


- ✓ **Can we recruit the SCAPs in to the root canal of teeth with mature apex? V. Chrepa and Diogenes (JDR, 2015)** evaluated whether evoked bleeding from the periapical tissues elicits the influx of MSCs into the root canal system in mature teeth with apical lesions. It was shown that **evoked-bleeding technique delivers MSCs into the root canal system in mature teeth with apical lesions.**



Comparison of the levels of MSC markers observed in the systemic blood with those identified in blood obtained from the root canal system by the evoked-bleeding step revealed a median 4.4-fold intracanal increase in CD73, a median 32.6- intracanal increase in CD90, a median 4.3-fold increase of CD105, and a median 6.9-fold increase in CD146 transcripts

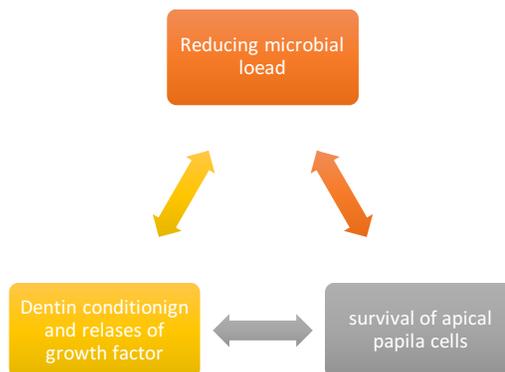
- ✓ **What is the most common procedure for recruiting stem cells in the root canal system?** In the review of protocols by Kontakiotis, JOE 2015 it was reported that 87% of clinical studies induce blood clot in hope of maximizing stem cell in the root canal system.
- ✓ **Smith A J Endod 2016 :** There is a **delicate balance between persistent pathosis and healing in the root canal system.** A variety of microbial sensors have been described as being present on these cell types, and the best characterized are the **Toll-like receptor** family that can detect microbial components ranging from their nucleic acids to cell wall constituents. After detection of the infection by host cells, cytokines such as interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , tumor necrosis factor alpha, IL-4, IL-6, IL-8, and IL-10 are secreted, and depending on levels and temporality, they can lead to several cellular and tissue outcomes.



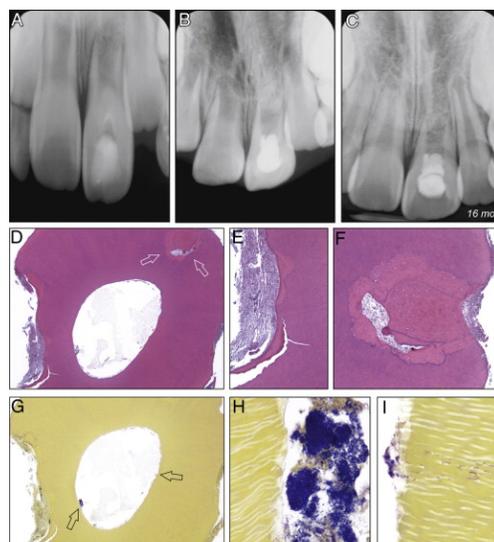
Schematic of potential steps and interactions between dental tissue infection, inflammation, vascular responses, and regeneration. An inflammatory response occurs generally after carious bacterial infection, leading to mediator release from host cells and demineralized dentin. This cocktail of cytokines, growth factors, and other signaling molecules acts on MSCs, immune cells, and the vascular system. There is significant crosstalk between these systems, and ultimately if the immune cell response is able to contain or remove the infection, potentially with the involvement of clinical intervention, then dental tissue regeneration can occur

## Second step; Dis-infection:

- ✓ Use of irrigation and intra-canal medicament is a broad topic in the regenerative endodontics. In choosing the appropriate irrigation 3 areas should be considered:
- ✓ **Efficacy of irrigation in reducing the microbial load**
- ✓ **Effect of irrigation of dentin structure and release of growth factor**
- ✓ **Effect of irrigation on the survival of apical papilla stem cells**



- ✓ **Is the micro-flora of the necrotic immature tooth similar to primarily infected permanent teeth? J Endod. 2014; Nagata:** The microbial profile of infected immature teeth is similar to that of primarily infected permanent teeth. The greatest bacterial reduction was promoted by the irrigation solutions. The revascularization protocols that used the tested intracanal medicaments were efficient in reducing viable bacteria in necrotic immature teeth.
  
- ✓ **How deep bacteria can penetrate into the dentinal tubules? Fouad; J Endod 2009:** The mean depth of bacterial invasion in the young and the old group was approximately 420  $\mu\text{m}$  and 360 $\mu\text{m}$ , respectively. Bacteria penetrated the young radicular dentin to a significantly deeper level than the old dentin ( $p = 0.033$ ).
  
- ✓ **Mente J Endod 2009** Reported that presence of **apical periodontitis can affect the outcome of apexification**. Teeth without or with preoperative periapical radiolucency had a healed rate of 100% and 78%, respectively.
  
- ✓ **What is the most common cause of failure following regenerative treatments?** In studies (Fouad 2016-JDR; Ricucci JOE) which have investigated the reasons of failure following regenerative treatments, **persistent biofilm was associated with the lack of regeneration**. Presence of bacteria in the cases that healed but the regeneration process failed indicates that bacterial load is not enough to cause AP but hinder the regeneration



### Third step; Growth factors and dentin conditioning

- ✓ **Smith in 2003 (JDE)** elaborated the **role of dentin matrix in releasing the growth factors**. The origin of these growth factors in dentin matrix is probably largely the odontoblast cell, and after secretion, they interact with extracellular matrix or mineral components of the dentin thereby becoming incorporated or sequestered within the matrix. Growth factors may be released from the dentin matrix as a result of both injury events to the tissues and clinical restorative procedures. During caries, diffusion of acidic plaque bacterial metabolites into the tissue will lead to demineralization and release of soluble extracellular matrix components, including growth factors.
- ✓ **What is the role of dentin in the regenerative endodontics?** A number of reports (**Nakashima**) of placement of exogenous growth factors, particularly TGF- $\beta$ s and Bone Morphogenetic Proteins (BMPs), on exposed pulps have demonstrated the potential of these molecules to signal reparative dentinogenic events. Application of TGF-1 and BMP-7 to the odontoblasts of unexposed pulps in cultured tooth slices has also shown the ability of these growth factors to signal reactionary dentinogenesis. It has become increasingly evident that BMPs play an important role in dentinogenesis and in dentin regeneration (**Nakashima**). In a study by **L. Casagrande (JDR, 2010)** it was shown that **release of BMP by dentin is essential for expressing of dentinogenesis markers by dental pulp stem cells**.



The above pictures shows that all dentinogenesis markers such as DMP and DSPP are highly expressed in the cells which are implanted in the dentin scaffolds which have been treated with EDTA.

- ✓ **Dentin Conditioning Codetermines Cell Fate in Regenerative Endodontics, Kerstin M. Galler JOE 2011:** In dentin treated with NaOCl, resorption lacunae were found at the cell-dentin interface created by multinucleated cells with clastic activity. **After conditioning with EDTA, DPSCs adjacent to the dentin formed an intimate association with the surface, differentiated into odontoblasts-like cells that expressed dentin sialoprotein, and extended cellular processes into the dentinal tubules. A vascularized soft connective tissue similar to dental pulp was observed inside the dentin cylinder.**

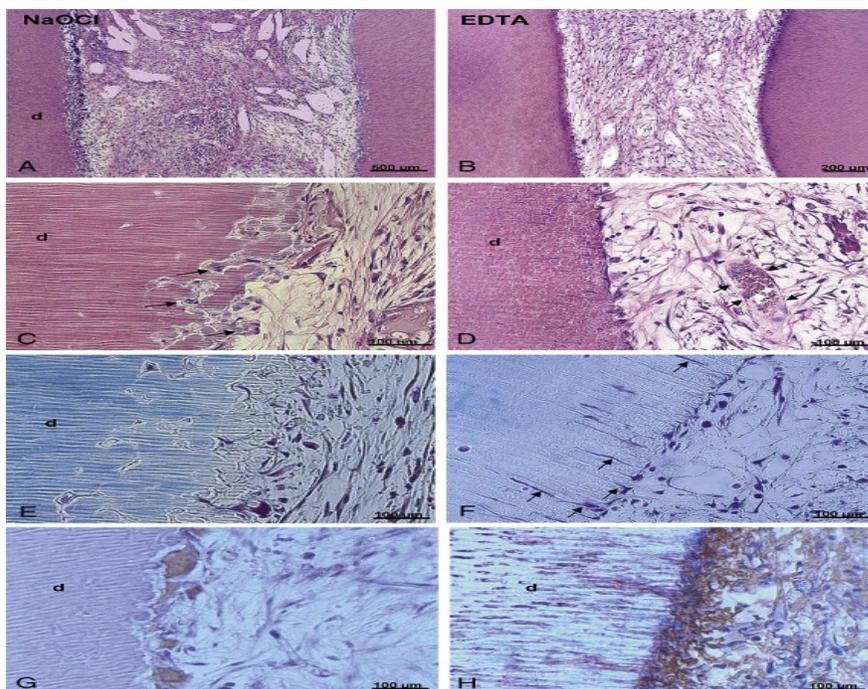


Figure: The dentin (d) cylinder with soft connective tissue where a resorption front  
 B: Soft connective tissue formation in dentin cylinders pretreated with EDTA; resorption cannot be observed  
 C: resorption lacunae with multinucleated cells  
 D: issue similar to dental pulp has formed.  
 G: clastic activity can be observed  
 H: Odontoblastic cell like marginating the dentin

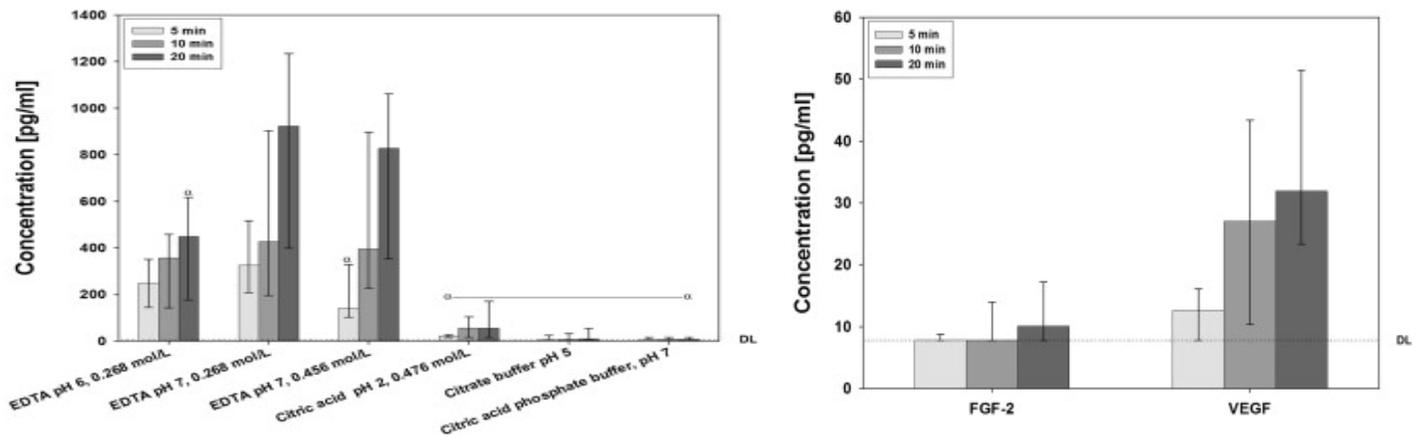
- ✓ **Ultrastructural localization of TGF-beta exposure in dentine by chemical treatment Histochem J. 2000; Zhao** EDTA treatment provided good exposure of TGF-beta1 on the dentine surface, whilst citric acid and sodium hypochlorite treatments revealed lesser amounts of this isoform. Naocl degenerated the growth factor therefore in this study TGF was not released from dentin samples which were treated by Naocl.

**Table 1.** Gold particle density (mean  $\pm$  SEM per 100  $\mu\text{m}^2$  from 10 random fields) for TGF- $\beta$  isoform immunolabelling after various chemical treatments of the dentine specimens and in control specimens.

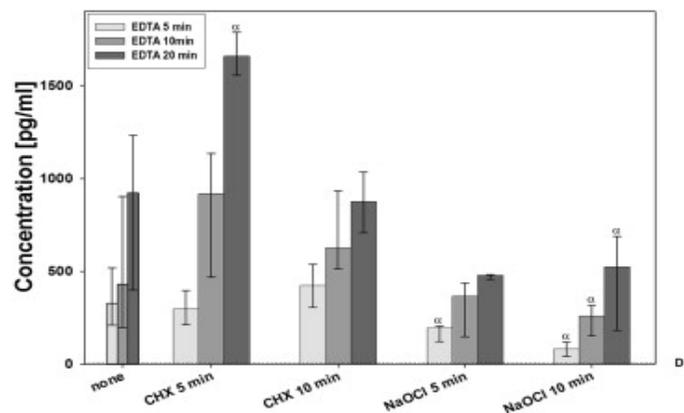
Treatment	TGF- $\beta$ 1	TGF- $\beta$ 2	TGF- $\beta$ 3
EDTA	7.1 $\pm$ 0.41	0	0
Hypochlorite	3.7 $\pm$ 0.28	0.1 $\pm$ 0.09	0.1 $\pm$ 0.09
Citric acid	3.0 $\pm$ 0.24	0	0.1 $\pm$ 0.09

Naocl degenerated the growth factor

- ✓ **Influence of Root Canal Disinfectants on Growth Factor Release from Dentin Galler;**  
**J Endod. 2015:** This study aimed to identify a demineralizing solution suitable for growth factor release directly from dentin and to evaluate whether commonly used disinfectants for endodontic treatment will compromise this effect.

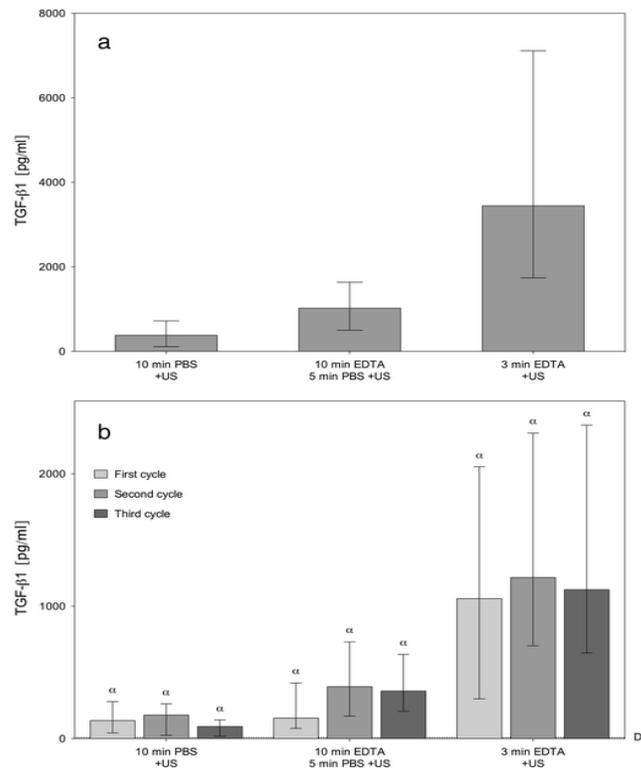


Release of TGF- $\beta$ 1, FGF and VEGF from dentin disks after treatment with different demineralizing solutions for 5, 10, or 20 minutes.



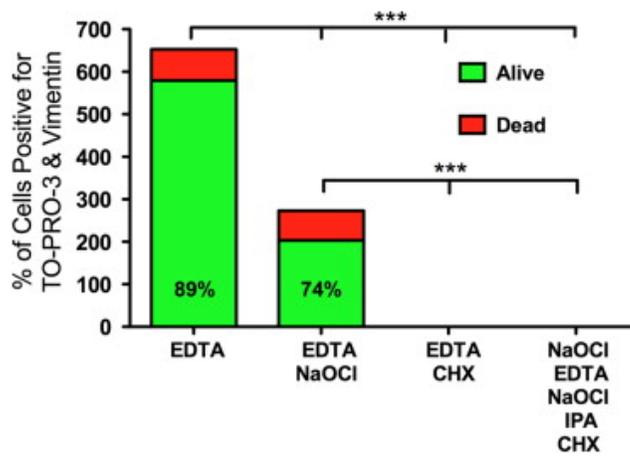
Release of TGF- $\beta$ 1 from dentin by EDTA conditioning after pretreatment with irrigation solutions chlorhexidine digluconate (CHX) and sodium hypochlorite (NaOCl) for 5 or 10 minutes.

- ✓ It was suggested that Irrigation with chlorhexidine before EDTA conditioning increased TGF- $\beta$ 1 release; sodium hypochloride had the opposite effect.
- ✓ Ultrasonic activation of irrigants increases growth factor release from human dentine Clin Oral Investig. 2016 Apr 25; Widbiller M. This study suggested that ultrasonic activation enhances growth factor release from human dentine.



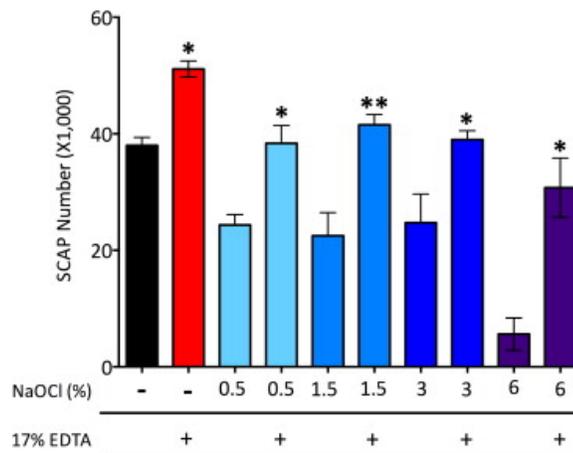
The above figure depicts the effect of ultrasonic activation on release of growth factor from dentin following EDTA conditioning.

- ✓ Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips; J Endod. 2011; Trevino EG This study aimed to test the hypothesis that different root canal irrigation protocols alter survival of stem cells from the apical papilla (SCAP). It was reported that Irrigation with 17% EDTA best supported cell survival followed by irrigation with 6% NaOCl/17% EDTA/6% NaOCl. Conversely, protocols that included 2% CHX lacked any viable cells.

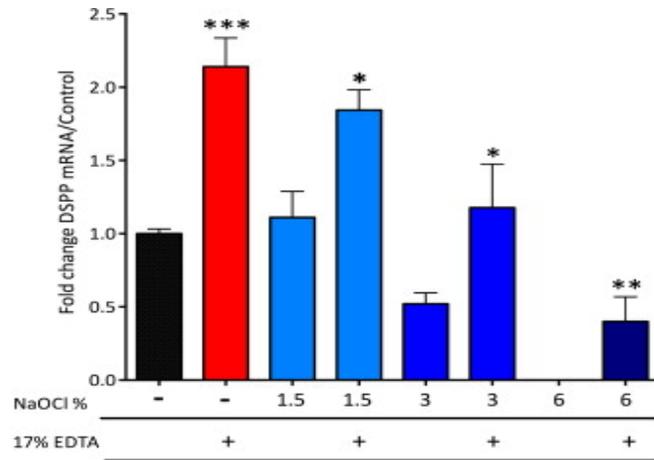


✓ It can be concluded that pre-treatment with Naocl can significantly reduce the vitality of stem cells. Also following CHX there is no viable stem cells.

✓ **Concentration-dependent Effect of Sodium Hypochlorite on Stem Cells of Apical Papilla Survival and Differentiation; J Endod 2014 Jan;40(1):51-5; Martin DE** The present study aimed to assess the effect of various concentrations of NaOCl on the stem cells of the apical papilla (SCAPs) survival and dentin sialophosphoprotein (DSPP) expression. Authors reported that there was **a significant reduction in survival and DSPP expression in the group treated with 6% NaOCl** compared with the untreated control group. Comparable survival was observed in the groups treated with the lower concentrations of NaOCl, but greater DSPP expression was observed in the 1.5% NaOCl group. In addition, 17% EDTA resulted in increased survival and DSPP expression partially reversing the deleterious effects of NaOCl.



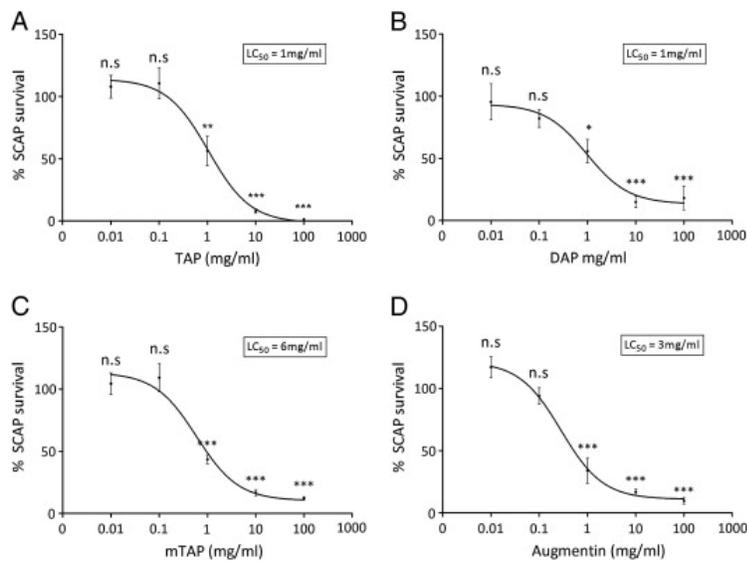
✓ NaOCl decreases the SCAP survival. In the figure it is clear that Naocl irrigation can significantly reduce the number of SCAP cells. However, treatment with EDTA after Naocl irrigation can bring the number of viable cells to the control level.



✓ NaOCl decreases the expression of the odontoblastic marker DSPP.

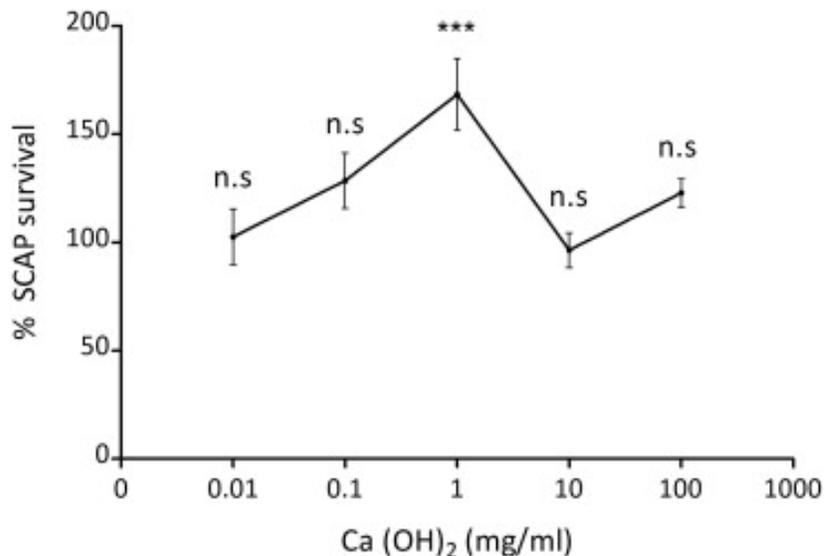
### Intra-canal medicament

✓ **Direct Effect of Intracanal Medicaments on Survival of Stem Cells of the Apical Papilla; J Endod. 2012;Ruparel** This study aimed to investigate the effect of commonly used intra-canal medicaments on the survival of apical papilla stem cells. It was shown that all 4 antibiotics significantly reduced SCAP survival in a concentration-dependent fashion. Interestingly, **Ca(OH)(2) was conducive with SCAP survival at all concentrations.**



TAP at the concentrations 1, 10, and 100 mg/mL resulted in  $58.0\% \pm 12.4\%$ ,  $8.0\% \pm 1.8\%$ , and  $1.3\% \pm 0.5\%$  SCAP survival, respectively. Conversely, the lower concentrations of 0.1 and 0.01 mg/mL had no detectable effect on SCAP survival. It worth mentioning that for past consistency you need 1000mg /ml of the antibiotic. However, based on **Sabrah et al findings (JOE, 2013)** the effective concentrations for TAP and DAP are 0.3 and 0.14 mg/ml, respectively.

- ✓ Another study (**Clin Oral Investig. 2015; Sabrah**) evaluated the antimicrobial efficacy of diluted antibiotic paste along with its cytotoxicity on the papillary stem cells. It was reported that “**effect of diluted antibiotic paste 1mg/ml has minimal cytotoxicity against stem cells and excellent effectiveness against bacteria = “more selective concentration”**”
- ✓ **Ruparel:** Application of calcium hydroxide had no detrimental effect on the SCAP survival at any of the concentrations tested. Instead, it significantly increased the proliferation/survival of SCAPs at the concentration of 1 mg/mL, resulting in an increase of  $68.3\% \pm 15\%$  in the viable SCAP number



- ✓ **Berkhoff (2014, JOE)** assessed the effectiveness of current irrigation techniques EndoActivator (Dentsply, Tulsa, OK), EndoVac (SybronEndo, Coppel, TX), or using a standardized irrigation protocol in a closed system in removal of TAP. It was shown that Approximately 88% of the TAP was retained in the root canal system regardless of the irrigation technique used (no difference among groups). Furthermore, approximately 50% of the radiolabeled TAP was present circumferentially up to 350  $\mu\text{m}$  within the dentin. Conversely, up to 98% of the radiolabeled intracanal calcium hydroxide was removed, and most residual medicament was found present in the initial 50  $\mu\text{m}$  of dentin. This study suggested that TAP residuals after irrigation may be detrimental for SCAP.

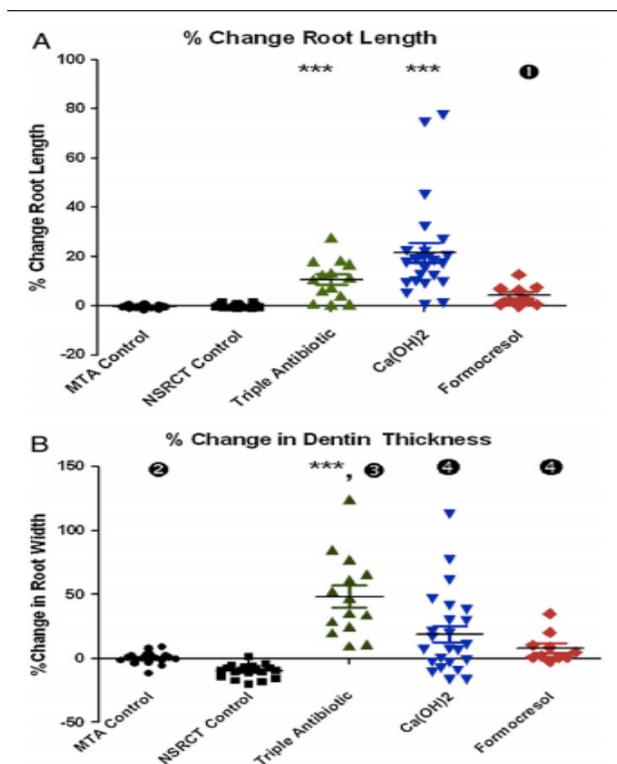
**In conclusion, the recent guidelines suggest:**

- ✓ **TAP and DAP medicaments should not be used as as “thick” slurry. Instead, concentrations of 1 to 10mg/ml should be used, if required.**
- ✓ **So far,  $\text{Ca(OH)}_2$  appears to be the most compatible antibacterial intracanal medicament for stem-cell based therapies.**

**Fourth step; Evaluation of outcomes**

- ✓ **A Retrospective Evaluation of Radiographic Outcomes in Immature Teeth With Necrotic Root Canal Systems Treated With Regenerative Endodontic Procedures; Hargreaves, JOE 2009** The present study aimed to compare the effect of various regenerative and apexification protocols on the width and length of immature teeth. It was shown that regenerative endodontic treatment with triple antibiotic paste and  $\text{Ca(OH)}_2$

produced significantly greater increases in root length than either the MTA apexification or NSRCT control groups. The triple antibiotic paste produced significantly greater differences in root wall thickness than either the Ca(OH)<sub>2</sub> or formocresol groups. The position of Ca(OH)<sub>2</sub> also influenced the outcome. **When Ca(OH)<sub>2</sub> was radiographically restricted to the coronal half of the root canal system, it produced better results than when it was placed beyond the coronal half.** e. **When Ca(OH)<sub>2</sub> was radiographically restricted to the coronal half of the root canal system, the median percentage increase in dentinal wall thickness was 53.8%, as compared with a 3.3% increase when it was placed beyond coronal half (ie, into the apical half of the root canal system).**



- ✓ This study also showed that increase in the width and length are time depended. The minimum time for 30% root width gain and 30% root length gain was 18 months and 36 months respectively

- ✓ **Comparison of Radiographic and Survival Outcomes of Immature Teeth Treated with Either Regenerative Endodontic or Apexification Methods: A Retrospective Study' J Endod 2012 Jeeruphan T** Present study aimed to evaluate radiographic and clinical outcomes of immature teeth treated with regeneration, MTA apexification and CaOH apexification methods. It was shown that the percentage change of root width was significantly greater in the revascularization group (28.2%) compared with the MTA apexification (0.0%) and calcium hydroxide apexification groups (1.5%). In addition, the percentage increase of root length was significantly greater in the revascularization group (14.9%) compared with the MTA (6.1%) and calcium hydroxide apexification groups (0.4%). The survival rate of the revascularization-treated teeth (100%) and MTA apexification–treated teeth (95%) were greater than the survival rates observed in teeth treated with calcium hydroxide (77.2%). Also teeth treated with the revascularization protocol had rates of complete healing (80%) similar to teeth treated with either the MTA (68%) or calcium hydroxide (77%) apexification methods. There was no trend for differences in outcome for either increased root width or increased root length regarding the sex and age.

The Survival Rate of Immature Permanent Teeth after Treatment

Outcome	Treatment					
	Revascularization (n = 20)		MTA (n = 19)		Calcium hydroxide (n = 22)	
	Teeth	%	Teeth	%	Teeth	%
Survival rate	20	100.00	18	94.70	17	77.30

- ✓ **Mohamed M. Nagy (JOE, 2014)** in a prospective study evaluated the effectiveness of 3 different methods in treating necrotic immature teeth,
  - 1) *The MTA group*: MTA apical plug
  - 2) *The REG group*: The regenerative endodontic protocol (blood clot scaffold)
  - 3) *The FGF group*: The regenerative endodontic with a blood clot and an injectable hydrogel scaffold impregnated with bFGF



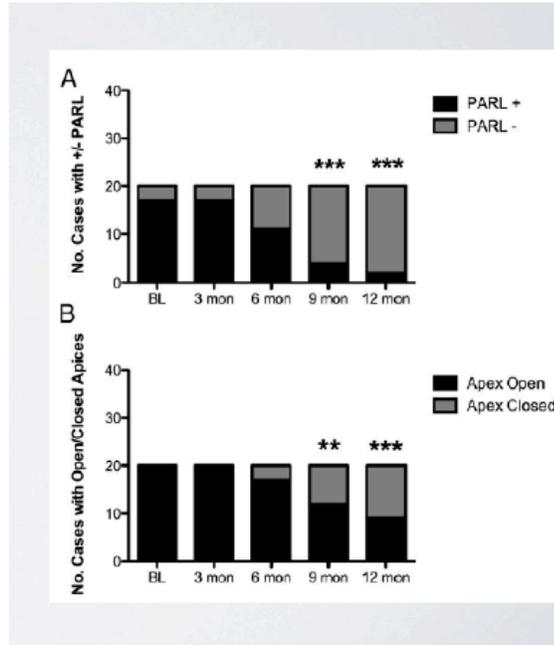
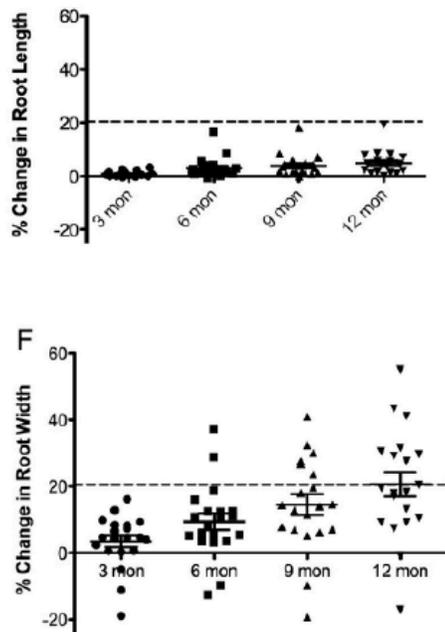
courtesy of Dr. Anibal Diogenes

✓ Findings:

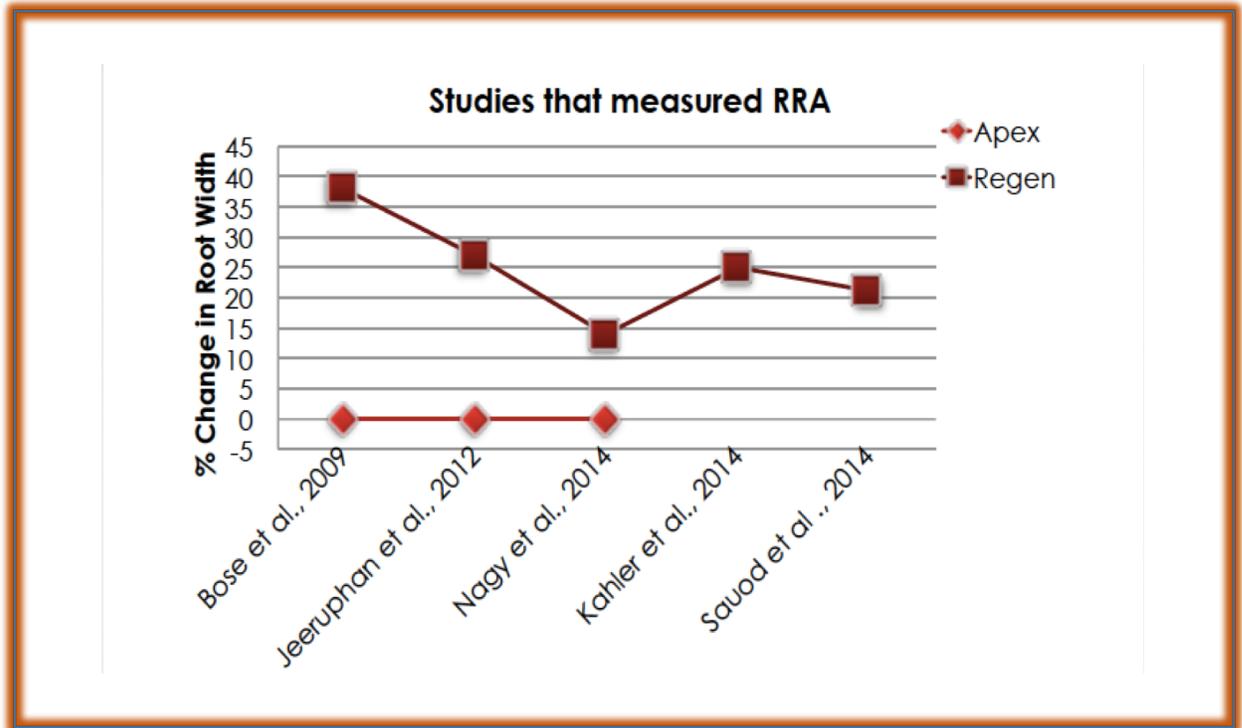
- 1) A significant improvement in bone density was found after 12 months of follow-up in all groups. No significant difference was found between all groups through the whole follow-up period.
- 2) Groups 2 and 3 showed a progressive increase in root length and width and a decrease in apical diameter.

- ✓ **Anibal Diogenes (JOE, 2016)** evaluated the effect of age and the apical diameter on the outcomes of the regenerative procedures. It was reported that revascularization procedures can be implemented in any age ranging from 9 to 18 years; however, younger age groups were better candidates for revascularization procedure than older ones. Regarding the apical diameter, regeneration procedures were successful with apical diameters as small as 0.5 mm. However, **teeth with preoperative wider diameters ( $\geq 1$  mm) demonstrated greater increase in root thickness, length, and apical narrowing.**

- ✓ **Tarek Mohamed A.** evaluated the Clinical and Radiographic Outcomes of Traumatized Immature Permanent Necrotic Teeth after Revascularization/Revitalization Therapy. It was concluded that the proportion of subjects with a periapical radiolucency decreased throughout the study period and became significantly different from baseline after 9 months. B) The proportion of subjects with an open apex began to decrease after 6 months, and became significantly different from baseline at 9 months. In this study all the included samples were Traumatized cases. **Comparing the results of the present study with other studies it might be speculated that traumatized cases seem to have less predictable root development**



✓ Summary of studies which have assessed continued root development



courtesy of Dr. Anibal Diogenes