
بسم الله الرحمن الرحيم

Regenerative Endodontics

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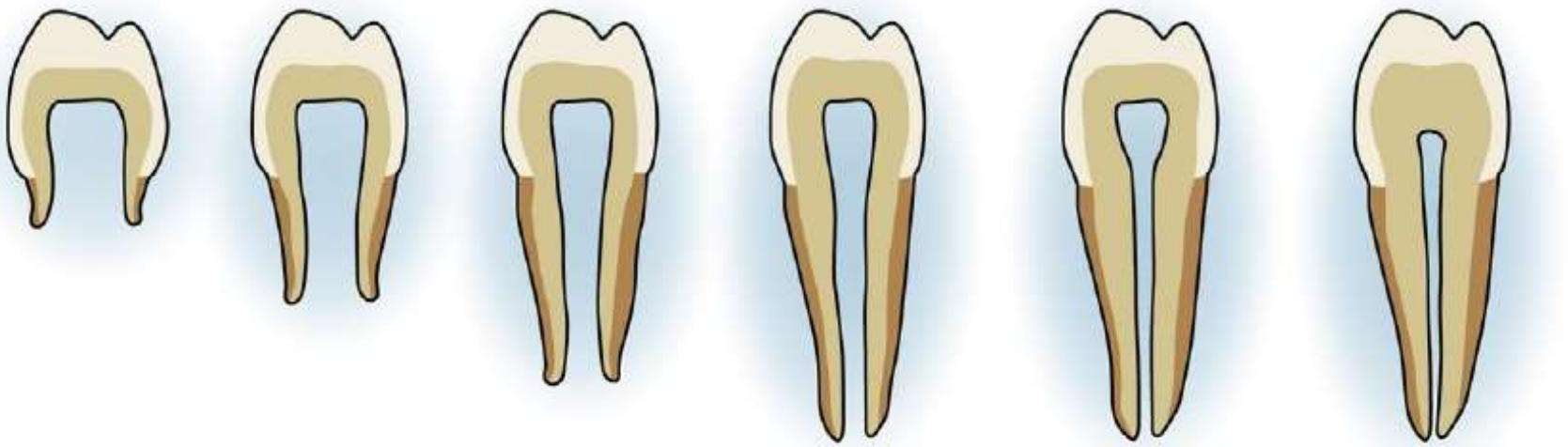


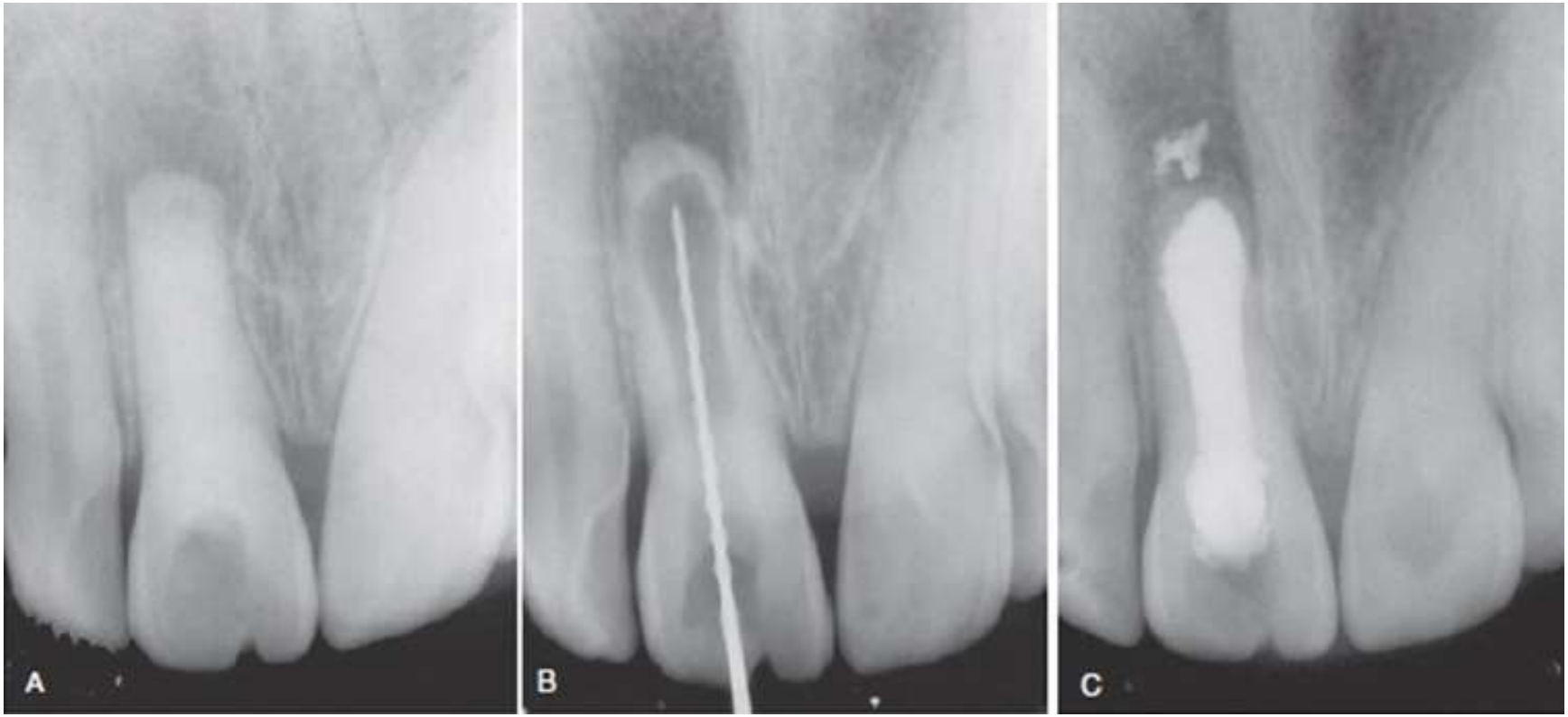
1/4 Root formation with "blunderbuss" apex	1/2 Root formation with "blunderbuss" apex	3/4 Root formation with "blunderbuss" apex	Full development with open apex	Full development with partially closed apex	Full development with closed apex
Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**Stage 6**

Problem List?

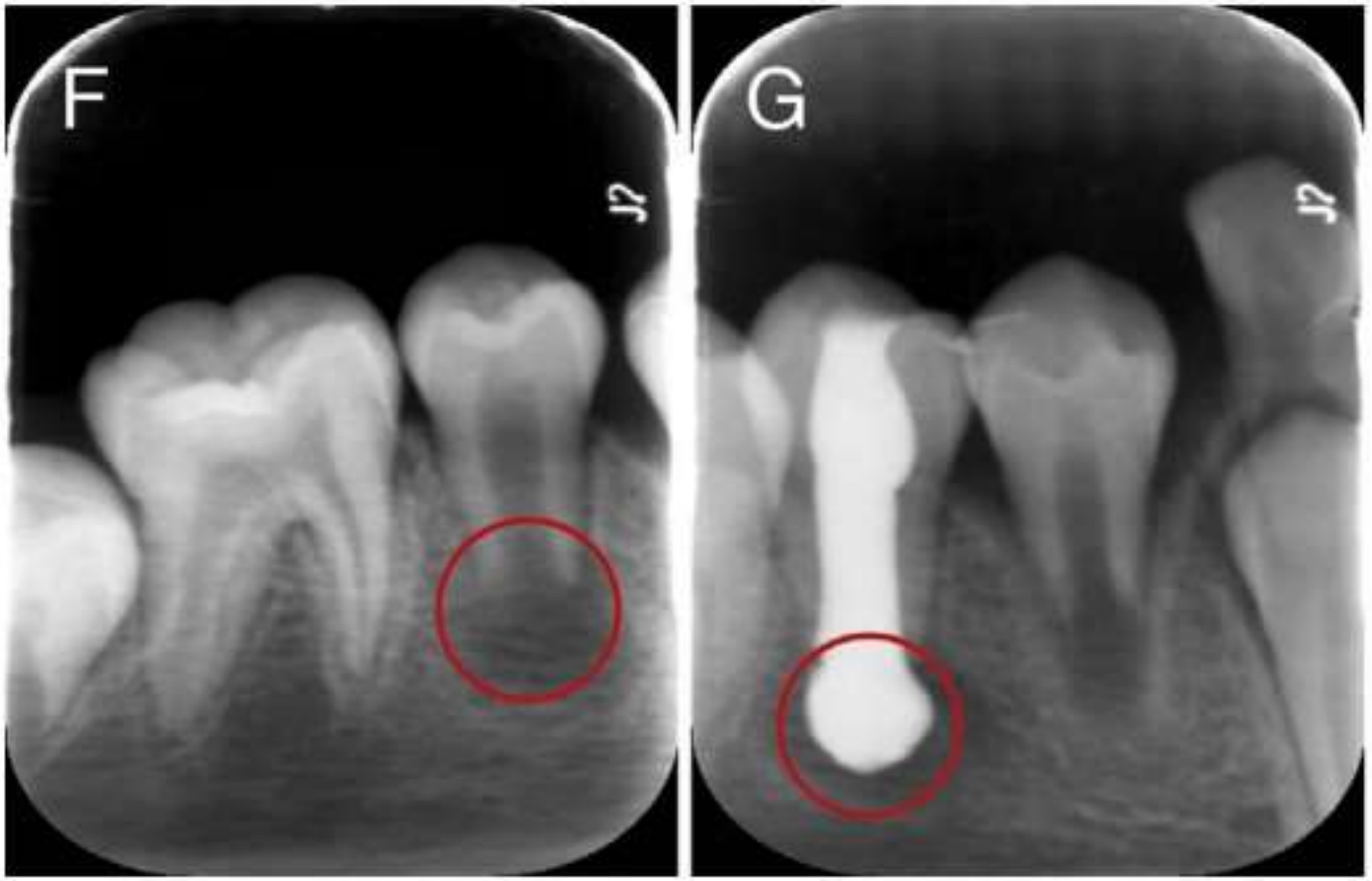
- Difficult to establish correct working length in immature teeth
- Risky to file a tooth with thin root canal walls
- Obturation of a tooth with wide open apex may result in overfilling
- Possibility of root fracture (due to the thin dentin walls)
- Unfavorable crown-root ratio

Treatment plans

- Apexification by calcium hydroxide
- Apexification by calcium silicate-based cements (known as MTA plug)
- Regenerative Endodontic Procedures



Hargreaves K.M, Berman L.H. Cohen's pathways of the pulp. 2016; Chapter 24



Lin J, Zeng Q, Wei X, Zhao W, Cui M, Gu J, et al. Regenerative Endodontics Versus Apexification in Immature Permanent Teeth with Apical Periodontitis: A Prospective Randomized Controlled Study. J Endod. 2017 Nov;43(11):1821-7.

Adding *Regenerative Endodontics* to the Table of Contents

Table 1. Top 20 Cited Papers in *JOE* from 2011–2015

Authors	Title	Year	No. citations
Lovelace et al	Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure	2011	76
Nosrat et al	Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of 2 cases with a new biomaterial	2011	74
Torabinejad and Turman	Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report	2011	74
Kim et al	Cyclic fatigue and torsional resistance of 2 new nickel-titanium instruments used in reciprocation motion: Reciproc versus WaveOne	2012	59
Ruparel et al	Direct effect of intracanal medicaments on survival of stem cells of the apical papilla	2012	56
Shen et al	Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development	2011	56
Jeeruphan et al	Mahidol study 1: Comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: A retrospective study	2012	53
Nowicka et al	Response of human dental pulp capped with biodentine and mineral trioxide aggregate	2013	52
Burklein and Schafer	Apically extruded debris with reciprocating single-file and full-sequence rotary instrumentation systems	2012	52
Galler et al	Dentin conditioning codetermines cell fate in regenerative endodontics	2011	51
Cehreli et al	Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: A case series	2011	51
Trevino et al	Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips	2011	48
Aguilar and Linsuwanont	Vital pulp therapy in vital permanent teeth with cariously exposed pulp: A systematic review	2011	48
Yamauchi et al	Tissue engineering strategies for immature teeth with apical periodontitis	2011	44
Belobrov and Parashos	Treatment of tooth discoloration after the use of white mineral trioxide aggregate	2011	43
Zanini et al	Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization	2012	42
Berutti et al	Canal shaping with waveone primary reciprocating files and protaper system: A comparative study	2012	42
Paque and Peters	Micro-computed tomography evaluation of the preparation of long oval root canals in mandibular molars with the self-adjusting file	2011	41
Dai et al	The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris	2011	41
Liang et al	Endodontic outcome predictors identified with periapical radiographs and cone-beam computed tomography scans	2011	40

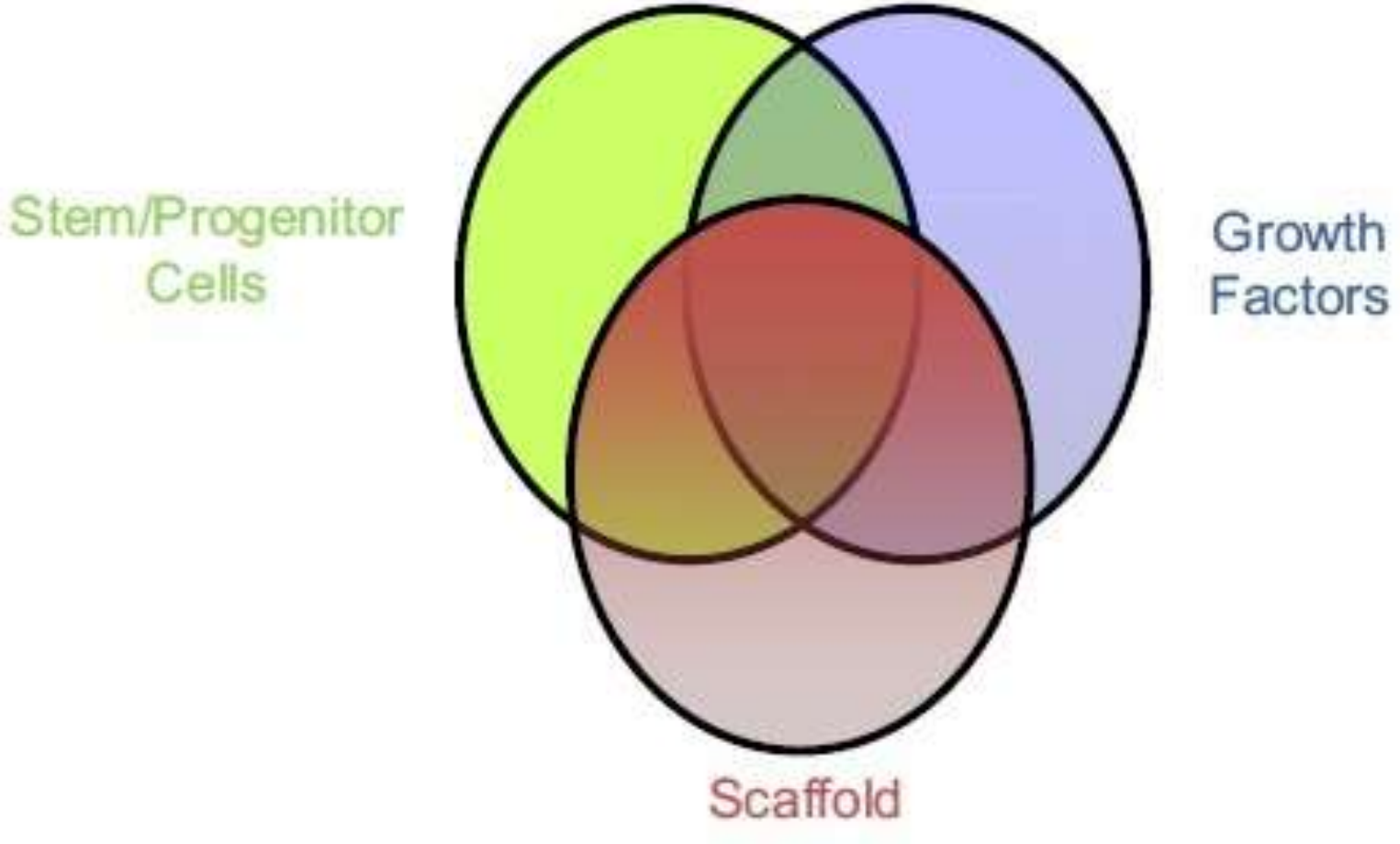
Available at: www.Scopus.com. Accessed October 29, 2015.

Regenerative Endodontic Procedures (REPs)

- REPs have been defined as biologically based procedures designed to replace damaged structures such as dentin, root structures, and cells of the pulp-dentin complex.

- In 1960 Professor Nygaard-Østby evaluated a revascularization method for reestablishing a pulp-dentin complex in permanent teeth with pulpal necrosis.
- He hypothesized that a blood clot could be the first step in the healing of a damaged dental pulp, similar to the role of the blood clot in the healing process observed in other areas (e.g., alveolar bone following extraction). To test the hypothesis that the presence of a blood clot within a root canal system promotes healing, mature teeth diagnosed with either vital or necrotic pulp received pulp space débridement followed by foraminal enlargement, medicament dressing for the necrotic cases, evoked intracanal bleeding, and a kloroperka obturation placed coronal to the formed blood clot.

Regenerative Endodontic Procedures



Stem Cells

- Stem cells are defined as a distinct subpopulation of undifferentiated cells with the ability of self-renewal and differentiation into cell lineages of their tissue of origin

Types of Stem Cells

Stem cell type	Cell plasticity	Source of stem cell
Totipotent	Each cell can develop into new potential	Cell from early (1-3 days) embryos
Pluripotent	Cells can form vary (over 2000) cell types	Some cell of blastocyst
Multipotent	Cells differentiated, but can form a number of other tissue	Fetal tissue, cord blood, post natal stem cell including dental pulp stem cell

Multipotential Cells

Cells	Description
Embryonic Stem Cells (ESCs)	A small group of inner cells within the blastocyte are pluripotent
Induced Pluripotent Stem Cell (iPSCs)	The reprogramming technology applied to human cells
Hematopoietic Stem Cells (HSCs)	Blood progenitor cells
Mesenchymal Stromal Cells (MSCs)	Key support cells in the cellular niches 1. Different sources 2. Different function in various organ

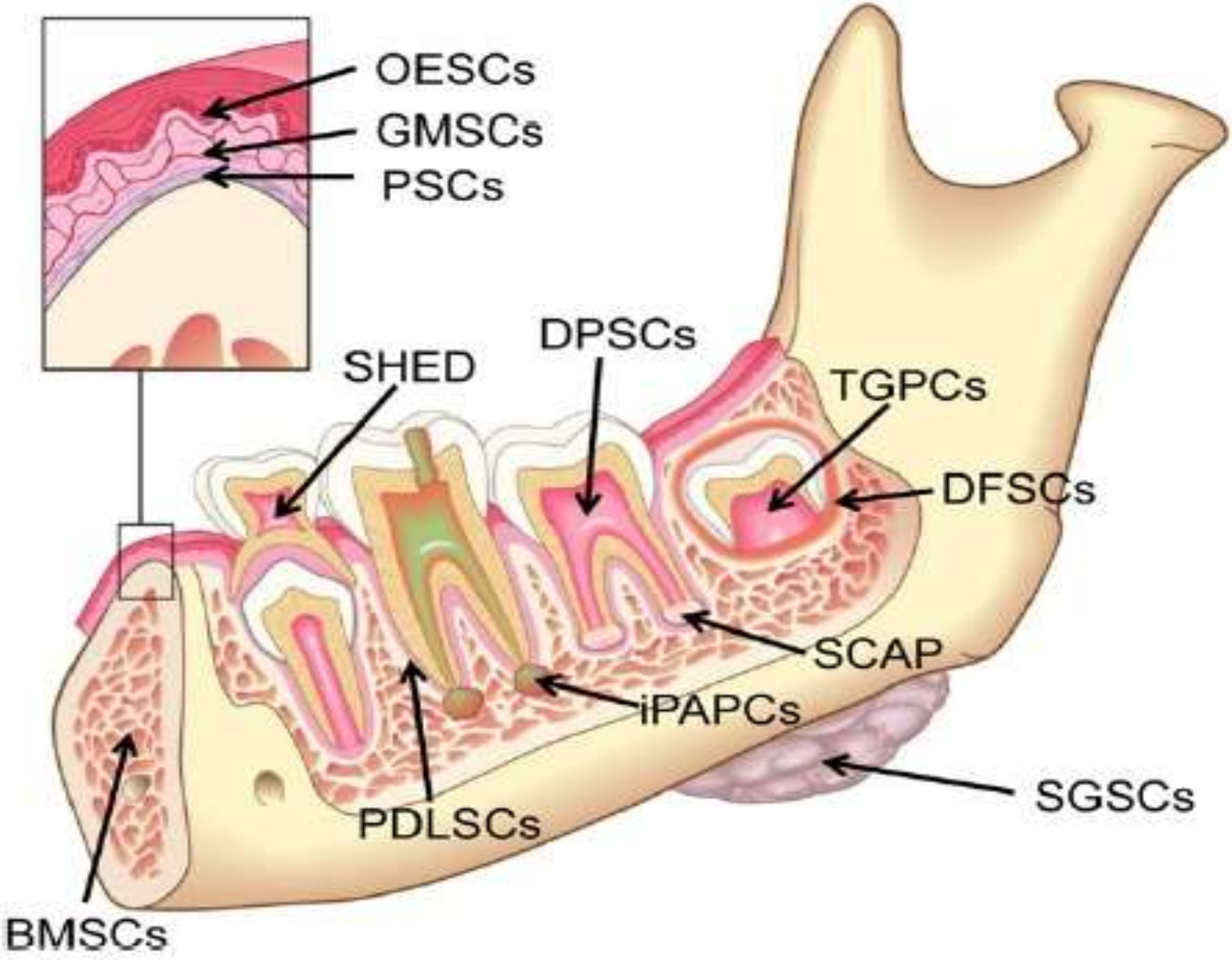
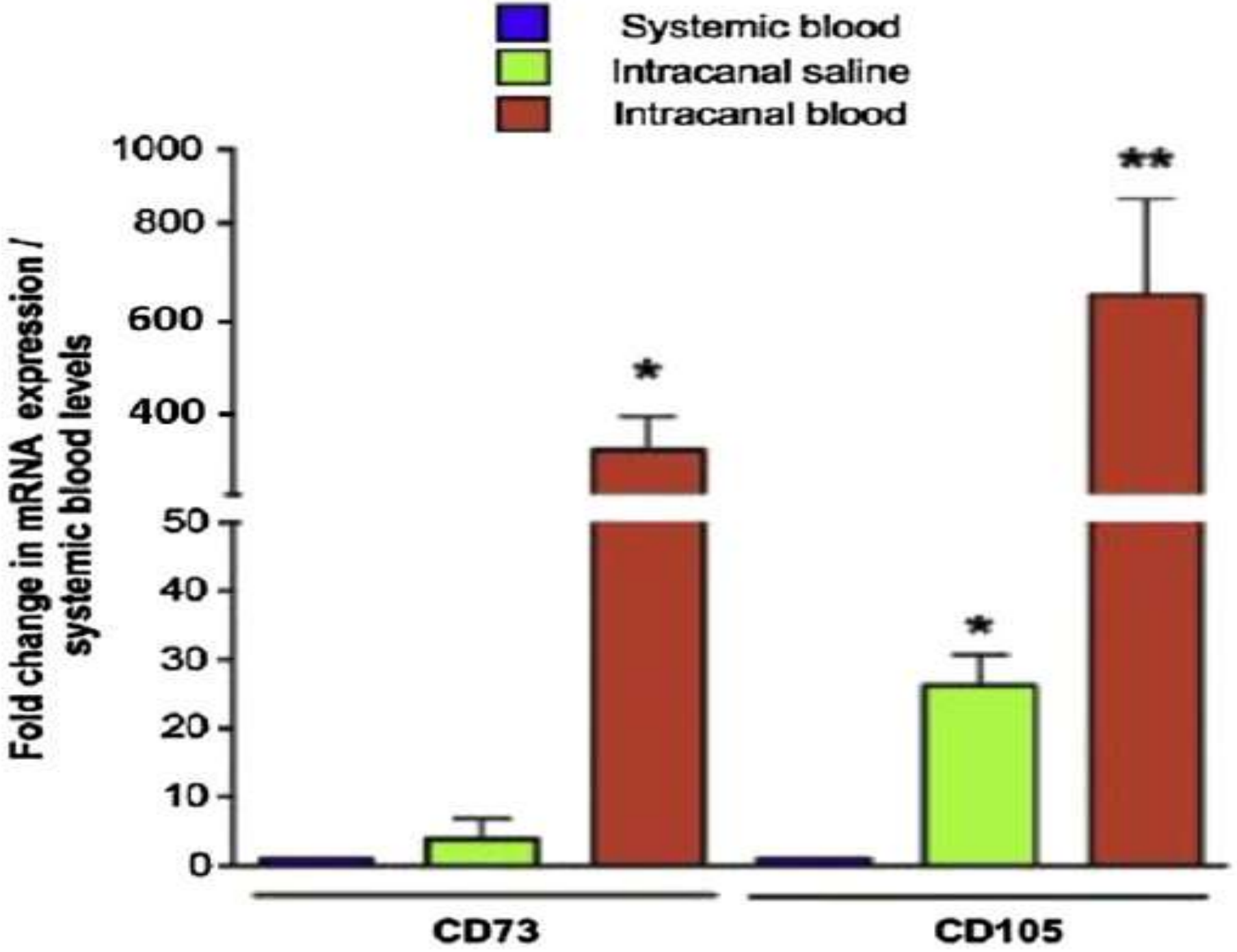

OESCs
GMSCs
PSCs
SHED
DPSCs
TGPCs
DFSCs
SCAP
iPAPCs
SGSCs
PDLSCs
BMSCs

Table 1: Dental and non-dental stem cell characterstics

Stem cells		Markers	Differentiation potential	Applications in dentistry/ regenerative medicine
Dental stem cells	Dental pulp stem cell	CD29, CD34, CD44, CD45, CD73, CD105, CD106, CD117, CD146, 3G5, STRO1, Oct4, Nanog, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4	Osteoblast, odontoblast, adipocyte, chondrocyte, neural cells, muscle cells, melanoma cells, hepatocytes, endothelial cells	Mandibular bone defects regeneration Scaffold-based dentin/pulp regeneration Acute myocardial infarction repairment Cerebral artery occlusion improvement Angiogenesis induction
	Stem cell from human exfoliated deciduous teeth	CD29, CD73, CD90, CD146, Nanog, Oct4, SSEA-3, SSEA-4, TRA1-60 and TRA1-81, nestin	Osteoblast, odontoblast, neural cells, adipocyte, hepatocytes, endothelial cells	Critical-sized cranial defects regeneration Scaffold based pulp regeneration Muscular dystrophy improvement spinal cord injury improvement Reconstruction of the corneal epithelium
	Stem cells from apical papilla	CD24, CD73, CD90, CD105, CD146, STRO-1, nestin, survivin	osteoblast, odontoblast, neuron, adipocyte	Tooth regeneration Complete root formation
	Periodontal ligament SC	CD44, CD90, CD105, CD146, STRO-1, scleraxis	adipocytes, chondrocytes, osteoblast neural cells, cementoblast, periodontium	Treatment of periodontitis Periodontium regeneration Tooth regeneration
	Dental follicle progenitor cells	CD29, CD44, CD105, Notch-1, nestin	Osteoblast, adipocyte, chondrocyte, neural cells, cementoblast, periodontal ligament, fibroblast, hepatocyte- like cells (HLCs)	Critically-sized bone defects regeneration Scaffolds-based root/dentin/pulp formation
	Tooth germ progenitor cells	CD29, CD44, CD73, CD90, CD105, CD106, CD166, STRO-1, Nanog, Oct4, Sox2, C- myc, Klf4	Osteoblast, odontoblast, adipocyte, chondrocyte, neural cells, hepatocytes	Treatment of liver diseases

Khazaei M, Bozorgi A, Khazaei S, Khademi A. Stem cells in dentistry, sources, and applications. Dent Hypotheses 2016;7 (20): 42-52.

- In 2011 a study was conducted to evaluate the presence of MSCs following the evoked-bleeding step in regenerative procedures. It was found that there is a substantial influx of MSCs into root canals during regenerative procedures resulting in an increase greater than 700-fold in the expression of MSC markers .


Systemic blood
Intracanal saline
Intracanal blood
Fold change in mRNA expression / systemic blood levels
1000
800
600
400
50
40
30
20
10
0
*
*
**
CD73
CD105

Uncertainty in the source of stem cells

- Although this study did not evaluate whether the MSCs detected in REPs are derived from the apical papilla, it was assumed that these cells were SCAPs because the evoked-bleeding step lacerated the apical papilla. However, these MSCs are a heterogeneous population of cells that could come from any of the periradicular tissues after the mechanical step of evoking bleeding into the root canal system.

Growth factors

- Dentin is composed of collagen fibers (90%, collagen type I) and noncollagenous matrix molecules (proteoglycans, phosphoproteins, and phospholipids). The collagen fibers act as a grid or matrix, and this structure behaves as a scaffold upon which mineralization can occur. Dentin phosphoprotein (DPP) and DSP are the most abundant dentin-specific proteins among the noncollagenous proteins of organic matrix.
- DSP resembles other sialoproteins such as bone sialoprotein, but its precise function is still unclear; it may have a role in matrix mineralization. Both DSP and DPP are a part of the small integrin-binding, ligand N-linked glycoproteins (SIBLINGs) which include dentin matrix acidic phosphoprotein 1 (DMP-1), bone sialoprotein, osteopontin, osteocalcin, and osteonectin. These proteins are only a small part of the whole cocktail of noncollagenous proteins which form components of the dentin

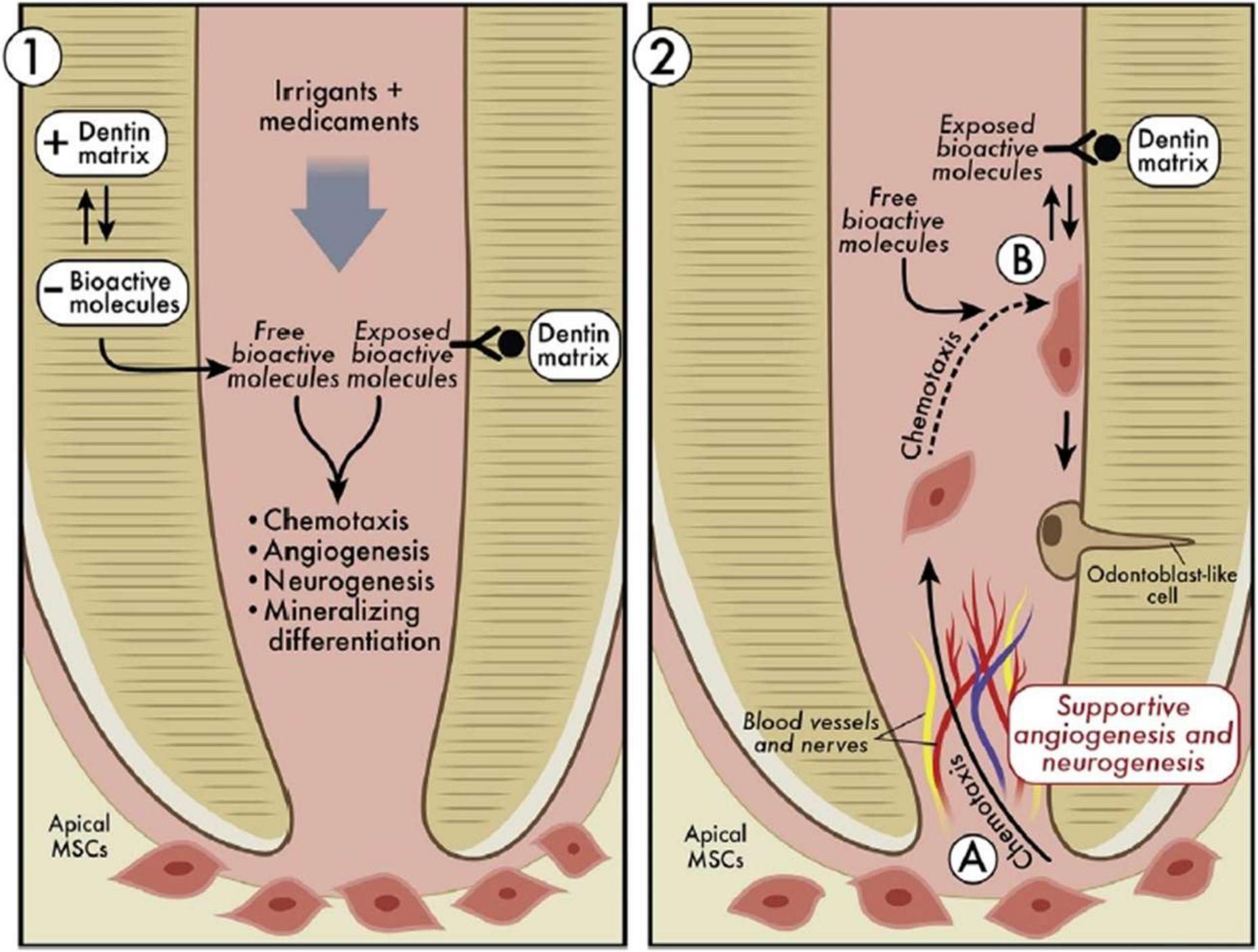
List of growth factors found into the mineralized dentin matrix

Growth factors in dentin Matrix	Authors/Year
TGFβ-I	Cassidy et al., 1997
TGFβ-2	Cassidy et al., 1997
TGFβ-3	Cassidy et al., 1997
BMP-2	Thomadakis et al., 1999
BMP-4	About et al., 2000
BMP-7	Thomadakis et al., 1999
IGF-I	Finkleman et al., 1990
IGF-II	Finkleman et al., 1990
HGF	Tomson et al., 2013
VEGF	Roberts-Clark and Smith, 2000
Adrenomedullin	Musson et al., 2010

Effects of Selected Growth Factors on the Differentiation of Odontoblast-Like Cells

Growth Factors	Cell Source	Phenotype	Condition	Authors
Dexamethasone	Human dental pulp	Odontoblast-like	In vitro × 8 weeks	Huang et al., 2006[102]
Dexamethasone and Vitamin D_3	Human dental pulp	Odontoblast-like	In vitro × 8 weeks	Huang et al., 2006[102]
Dexamethasone and Ascorbate-2-phosphate and β-Glycerophosphate	Human or rat dental pulp	Odontoblast-like	In vitro × 3 weeks	Wei et al., 2007[244] Zhang et al., 2005[268]
Insulin and Indomethacin and 3-Isobatyl-1-methylxanthine (IMBX)	Human dental pulp	Adipocyte	In vitro × 19 days	Wei et al., 2007[244]
Dexamethasone and Insulin and Ascorbate-2-phosphate and Sodium pyruvate and TGF-β1	Human dental pulp	Chondrocyte	In vitro × 8 weeks	Wei et al., 2007[130]
Growth/differentiation factor 11 (Gdf11)	Dental pulp	Odontoblast-like	In vitro/in vivo 10 days	Nakashima et al., 2004[75]
Simvastatin (statins)	Human dental pulp	Odontoblast-like	In vitro/in vivo	Okamoto et al., 2009[88]
LIM mineralization protein 1 (LMP-1)	Human dental pulp	Odontoblast-like	In vitro/in vivo	Wang et al., 2007[129]
Bone morphogenetic proteins	Dental pulp	Odontoblast-like	In vitro	Saito et al., 2004[99] Sloan et al., 2000[107] Chen et al., 2008[19]
TGF-β1-3	Rat/monkey dental pulp	Odontoblast-like	In vitro	Sloan et al., 1999[109]
Demineralized dentin	Human or rodent pulp	Odontoblast-like	In vitro/in vivo	Smith et al., 1990[111] Smith et al., 2001[110] Tziafas, 2004[123]
Nerve growth factor (NGF)	Immortalized apical papilla	Odontoblast-like	In vitro	Arany et al., 2009[11]
Fibroblast growth factor 2	Human dental pulp	Odontoblast-like	In vitro	He et al., 2008[43]
Dentin matrix protein 1	Rat dental pulp	Odontoblast-like	In vivo	Almushayt et al., 2006[1]

- **The selection** of growth factors, the variation in their **dosage**, and the **time** to deliver them into target sites may exert entirely different biological effects on cellular behaviors, ranging from induction to inhibition and even cell apoptosis.


1
Irrigants +
medicaments
+ Dentin matrix
– Bioactive molecules
Free bioactive molecules
Exposed bioactive molecules
Dentin matrix
• Chemotaxis
• Angiogenesis
• Neurogenesis
• Mineralizing differentiation
Apical MSCs
2
Exposed bioactive molecules
Dentin matrix
Free bioactive molecules
B
Chemotaxis
Odontoblast-like cell
Blood vessels and nerves
Supportive angiogenesis and neurogenesis
Chemotaxis
A
Apical MSCs

Scaffold

- (1) Provide a spatially correct position of cell location and
- (2) Regulate differentiation, proliferation, or metabolism while promoting nutrient and gaseous exchanges.

Matrix

- Fibronectin
- Hyaluronan
- Heparan sulfat
- Tenascin
- Laminins
- Collagen type I, III, VI

Extracellular matrix

- Glycoproteins
  - Fibronectine
  - Tenascin
- Proteoglycan
  - Hyaluronic acid
  - Heparan sulfat

Fibronectin

- FN is a 450-kDa fibril-forming glycoprotein composed of two subunits that is a major component of the hematopoietic microenvironment. FN is produced by both marrow stromal (endothelial cells and fibroblasts) and blood cells, and is implicated in marrow homing of hematopoietic cells.
- HSCs display multiple integrins and their engagement contributes to cell survival and/or expansion. For example, *ex vivo culture* of human CD34+ cells on FN maintains the repopulating capacity of HSCs, whereas growing the cells in suspension obliterates their ability to repopulate hematopoiesis.

Hyaluronan

- Another stromal cell matrix glycoprotein is hyaluronan, which binds to two hematopoietic cell-surface receptors, RHAMM and CD44. Although most CD34+ marrow cells express CD44, only a fraction of them adhere to hyaluronan, a process that can be mediated by cytokines, as a result of either increased surface expression of CD44 or an alteration in its conformation

Heparan Sulfate

- LTCs that support hematopoiesis develop a heparan sulfate proteoglycan layer. Immunochemical analysis has shown that marrow stromal cell lines synthesize and secrete numerous members of the syndecan family of heparan sulfate, including glypican, betaglycan, and perlecan. Evidence is accumulating that heparan sulfate-containing proteoglycans may be vital components of the stem cell niche.

Tenascin

- Tenascins are large, extracellular matrix (ECM) glycoproteins found in several tissues, synthesis of which is **upregulated in response to tissue regeneration**. Tenascins are multimeric proteins composed of numerous modules. For example, tenascin-C is composed of six subunits linked like spokes in a wheel by their C-terminal fibrinogen-like domains, each subunit being composed of multiple epidermal growth factor (EGF)-like and FN type III modules.

- Marrow cells can adhere to tenascin-C within the fibrinogen-like domain and to two sets of the FN type III-like repeats, and when so engaged, they undergo a proliferative response. Genetic elimination of tenascin leads to modest deficiencies in marrow hematopoietic progenitor cells, although as the levels of FN in such mice are also reduced, it is unclear if direct tenascin engagement of hematopoietic cells is responsible, or the defect is a result of the secondary reduction of FN engagement of *β1 integrins.*

Laminins

- Laminins are heterotrimeric (αβγ) extracellular proteins that regulate cellular function by adhesion to integrin and nonintegrin receptors

Collagen Types I, III, IV, and VI

- Collagen types I, III, IV, and VI have been identified in LTC or in situ from marrow sections by a number of methods. Most of the marrow-derived collagen types are assembled into long fibrils, which form the fine, background reticulin staining seen on marrow biopsies, although type IV collagen is assembled into a meshwork seen most commonly as part of basement membranes. Collagens also interact with laminins in the marrow. Collagen types I and VI are strong adhesive substrates for various hematopoietic cell lines and marrow mononuclear cells, including committed myeloid and erythroid progenitors. Classic collagen receptors on blood cells are of two types, the β1 integrins (α1β1 and α2β1) and the nonintegrin glycoprotein VI, present predominantly on platelets.

Scaffold

1. Natural
2. Synthetic polymer
3. Hydroxyapatite, Tricalcium phosphate, dentin, Self-assembling peptid hydrogels

NATURALLY OCCURRING BIOMATERIALS APPLIED AS SCAFFOLDS IN DENTAL PULP TISSUE ENGINEERING

Scaffold	*Setup*
Collagen	Evaluation of dentin formation following 70 days of transplantation of a collagen matrix combined with human recombinant BMP-2, BMP-4, and TGF-β1 in the pulp cavity of dogs.
	In vivo generation of pulp-like tissue after 6 weeks of subcutaneous implantation of a dentin slice containing collagen type I/ceramic powder, DPSCs, and dentin matrix protein 1 in immunodeficient mice.
	Evaluation of dentin matrix formation following 6 or 10 weeks of autologous transplantation of collagen combined with swine DPSCs in a postextraction tooth socket of a minipig.
	Assessment of vascularized tissue formation after 4 and 8 weeks of transplantation of an emptied root canal containing collagen type I, rat DPSCs, and growth factors in a rat femur or a tooth socket.
	Regeneration of pulp/dentin-like tissue following 35 days of subcutaneous transplantation of emptied human root canals containing human recombinant collagen type I and human SHEDs in immunodeficient mice.
Fibrin	Evaluation of vascularized tissue formation following 5 weeks of transplantation of dentin disks containing PEGylated fibrin and DPSCs in immunocompromised mice.
	Assessment of complete tooth regeneration after 36 weeks of transplantation of a fibrin glue–platelet-rich fibrin scaffold containing swine tooth bud cells in a minipig tooth socket.
	Stimulation of pulp-like tissue ingrowth following 12 weeks of subcutaneous implantation of an emptied human root canal filled with fibrin gel on the calvarial bone of rats.
Hyaluronic acid	Regeneration of dentin/pulp-like tissue, cementum, and periodontal ligament following 36 weeks of autologous transplantation of a gelatin–chondroitin–hyaluronan tricopolymer scaffold containing swine tooth bud cells in a swine tooth socket.
	Assessment of dental pulp proliferation and vessel ingrowth following 1 and 3 weeks of transplantation of hyaluronic acid sponges onto the amputated pulp of pathogen-free rats.

Synthetic Polymers Applied as Scaffolds in Dental Pulp Tissue Engineering

Scaffold	*Setup*
Poly(-L-)lactic acid P(L)LA	Regeneration of tooth structures following 20–30 weeks of transplantation of PGA/PLLA scaffolds seeded with porcine tooth bud cells in the omentum of rats. Evaluation of dental pulp tissue engineering after 14, 21, or 28 days of subcutaneous implantation of PLLA tooth slice/scaffolds seeded with SHEDs in immunodeficient mice.
Polyglycolic acid (PGA)	In vitro engineering of dental pulp-like tissue following 60 days of culturing of PGA matrices seeded with dental pulp fibroblasts. Production of extracellular matrix after 3 weeks of subcutaneous implantation of dental pulp fibroblasts combined with PGA scaffolds in immunocompromised mice. Regeneration of tooth structures following 12 weeks of transplantation of PGA scaffolds seeded with rat tooth bud cells in the omentum of rats.
Poly(lactic-co-glycolic acid) (PLGA)	Regeneration of tooth structures following 20–30 weeks of transplantation of PLGA scaffolds seeded with porcine tooth bud cells in the omentum of rats. Evaluation of dentin/pulp-like tissue regeneration after 2 or 6 weeks of autologous transplantation of PLGA sheets seeded with DPSCs in rabbits. De novo regeneration of dentin/pulp-like tissue following 12 weeks of subcutaneous implantation of an emptied root canal containing human DPSCs or SCAPs combined with a PLGA scaffold in immunocompromised mice. Assessment of dentin matrix formation following 6 or 10 weeks of autologous transplantation of PLGA combined with swine DPSCs in a postextraction tooth socket of a minipig. Regeneration of pulp-like tissue following 8 weeks of implantation of PLGA/TCP scaffolds in the mesentery of rats.

HYDROXYAPATITE, TRICALCIUM PHOSPHATE, DENTIN, AND SELF-ASSEMBLING PEPTIDE HYDROGELS APPLIED AS SCAFFOLDS IN DENTAL PULP TISSUE ENGINEERING

Scaffold	*Setup*
Hydroxyapatite (HA)/tricalcium phosphate (TCP)	Regeneration of dentin/pulp-like tissue after 6 or 8 weeks of subcutaneous transplantation of human DPSCs mixed with HA/TCP ceramic particles in immunocompromised mice.
Treated dentin matrix fragments (TMD)	Promotion of dentin matrix formation after 4 weeks of omental transplantation of rat FSC-TMD constructs in rats. In vivo regeneration of a tooth root complex after 8 weeks of transplantation of TMD and FSCs in the alveolar fossa of rats. Evaluation of dental pulp and tooth root formation following 8 weeks of subcutaneous implantation of human FSC-based cell sheets combined with TMD in immunodeficient mice. Formation of a vascularized dentin/pulp-like complex with a continuous layer of dentin after 6 weeks of subcutaneous implantation of SCAP-based cell sheet-derived pellet and TMD in immunodeficient mice.
Self-assembling peptide hydrogels	In vivo generation of vascularized soft connective tissue following 5 weeks of subcutaneous transplantation of dentin cylinders containing human DPSCs encapsulated in growth factor-laden self-assembling peptide hydrogels in immunocompromised mice. Assessment of dental pulp regeneration after 35 days of subcutaneous transplantation of emptied human root canals containing commercially available Puramatrix™ and human SHEDs in immunodeficient mice. Regeneration of a vascularized dentin/pulp complex following 4 weeks of subcutaneous implantation of emptied human root canals comprising a self-assembling peptide hydrogel and DPSC-human umbilical cord vein endothelial cells cocultures in immunodeficient mice.

Clinical Procedures

- Traditionally, an immature tooth with an open apex is treated by apexification, which involves creating an apical barrier to prevent extrusion. In many cases, this entails an involved, long-term treatment with Ca(OH)2, resulting in the formation of a hard-tissue apical barrier. However, a disadvantage of the traditional apexification procedures is that the short-term or long term use of Ca(OH)2 has the potential to reduce root strength. This finding is consistent with a large case series using the traditional apexification protocol; it showed that a major reason for tooth loss following apexification was root fracture.

The advent of one-step apexification, by creation of artificial barriers (i.e., apical plugs) using materials such as MTA, has greatly decreased the number of appointments and time to completion.

Importantly, the one-step apexification has been shown to have as high success rate as apexification with calcium hydroxide in resolving apical periodontitis (both symptoms and radiographic presentation). However, apexification procedures do not generally result in further root development.

First treatment visit for REPs

1. Informed consent, including explanation of risks and alternative treatments or no treatment.
2. After ascertaining adequate local anesthesia rubber dam isolation is obtained.
3. The root canal systems are accessed and working length is determined (radiograph of a file loosely positioned at 1 mm from root end).
4. The root canal systems are slowly irrigated first with 1.5% NaOCl (20 mL/canal, 5 min) and then irrigated with saline (20 mL/canal, 5 min), with irrigating needle positioned approximately 1 mm from root end.
5. Canals are dried with paper points.
6. Calcium hydroxide or an Antibiotic Paste or solution (combined total of 1–10 mg/mL) is delivered to canal system.
7. Access is temporarily restored.

Final (second) treatment visit for REPs (Typically 2–4 weeks after the first visit)

1. A clinical exam is first performed to ensure that that there is no moderate to severe sensitivity to palpation and percussion. If such sensitivity is observed, or a sinus tract or swelling is noted, then the treatment provided at the first visit is repeated.
2. After ascertaining adequate local anesthesia with 3% mepivacaine (no epinephrine) rubber dam isolation is obtained.
3. The root canal systems are accessed; the intracanal medicament is removed by irrigating with 17% ethylenediaminetetraacetic acid (EDTA) (30 mL/canal, 5 min) and then a final flush with saline (5 mL/canal, 1 min).
4. The canals are dried with paper points.
5. Bleeding is induced by rotating a precurved K-file size #25 at 2 mm past the apical foramen with the goal of having the whole canal filled with blood at least to the level of the cementoenamel junction, but preferably coronal to it.

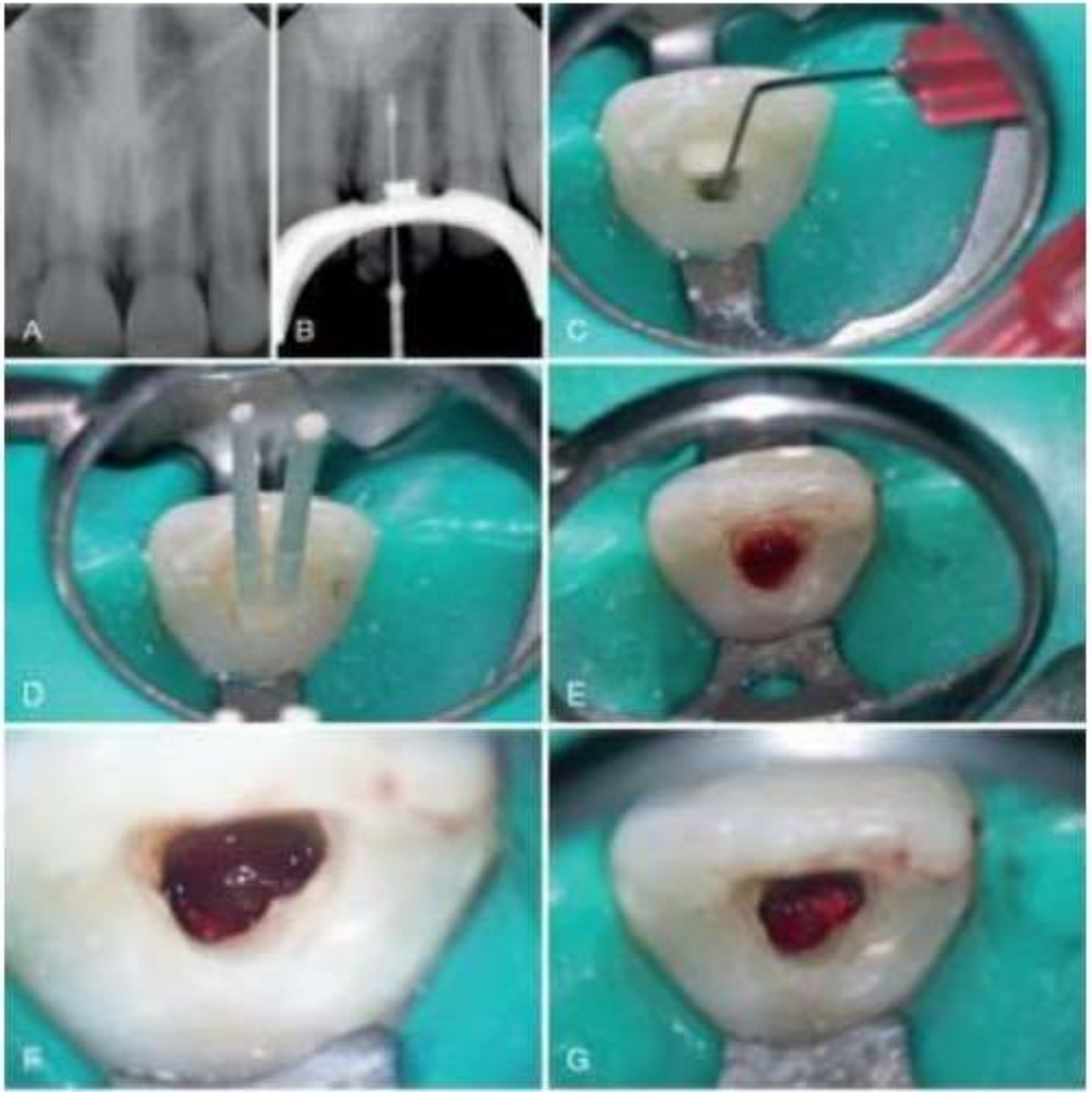
Final (second) treatment visit for REPs (Typically 2–4 weeks after the first visit)

6. Once a blood clot is formed, a premeasured piece of Collaplug (Zimmer Dental Inc., Warsaw, IN) is carefully placed on top of the blood clot, preferably above the cementoenamel junction, to serve as an internal matrix for the placement of approximately 2 mm of a tricalcium silicate biomaterial such as Biodentine (Septodont) or Endosequence BC Root Repair Material-Putty (ES) (Brasseler).

7. A (3 mm) layer of glass ionomer layer (e.g., Fuji IX, GC America, Alsip, IL; or other) is flowed gently over the bioactive coronal barrier and light cured for 40 s.

8. A bonded reinforced composite resin restoration is placed over the glass ionomer.

9. The case needs to be followed-up at 3 months, 6 months, and yearly after that for a total of 4 years.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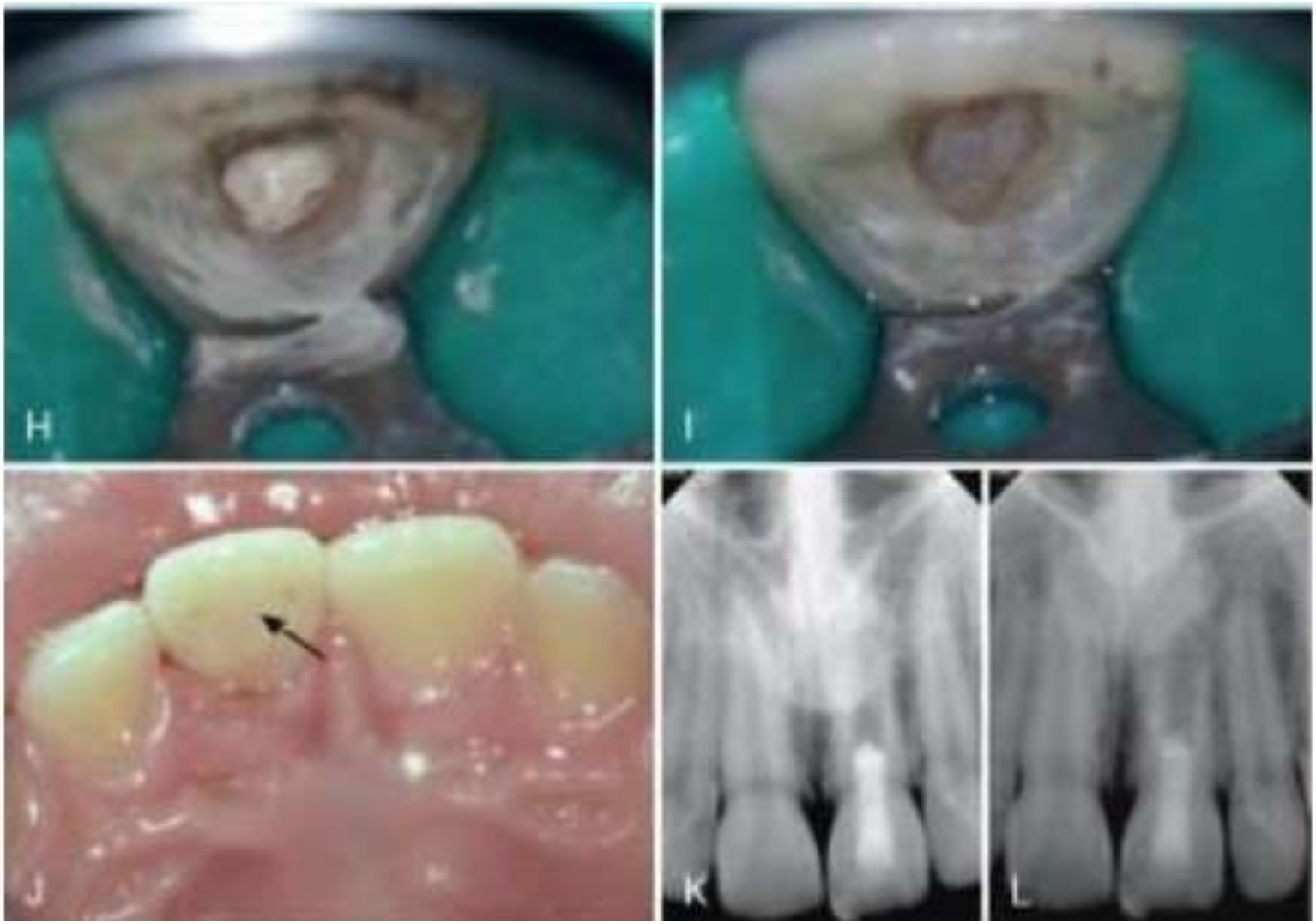
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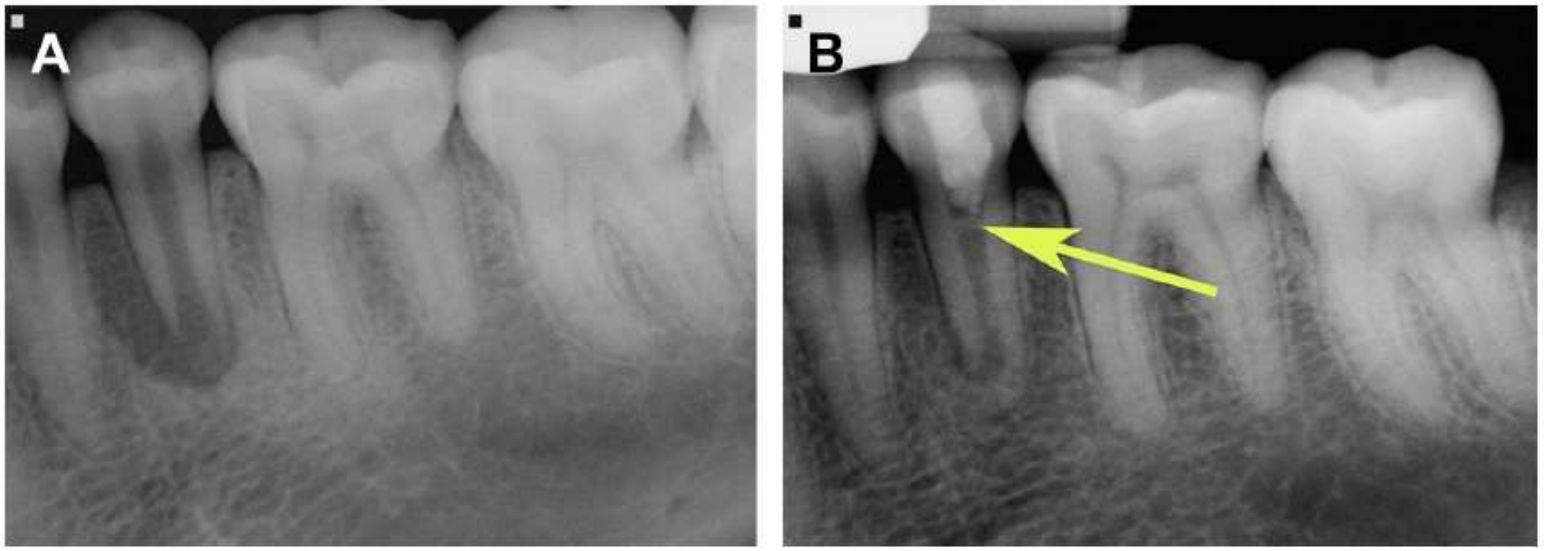
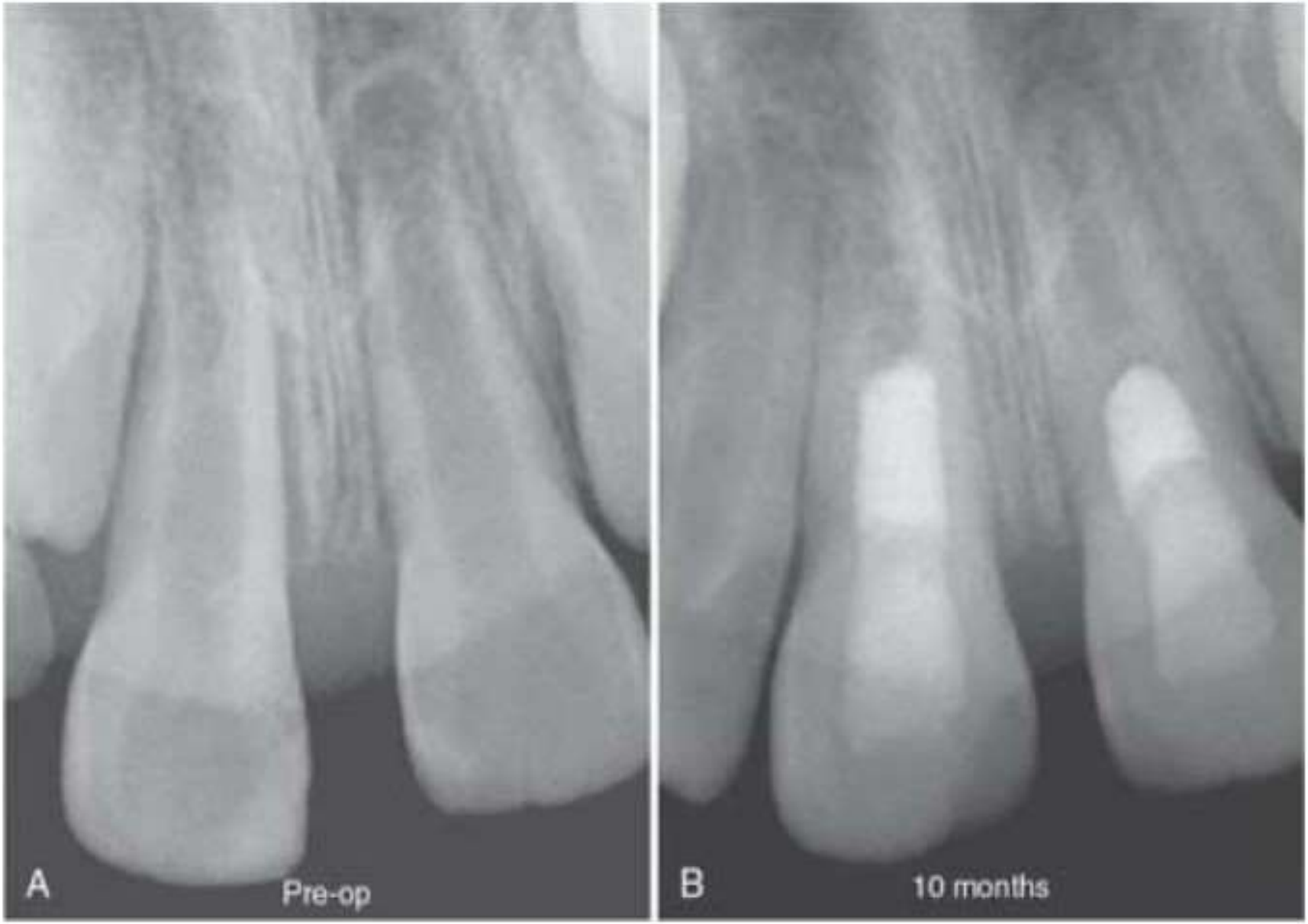
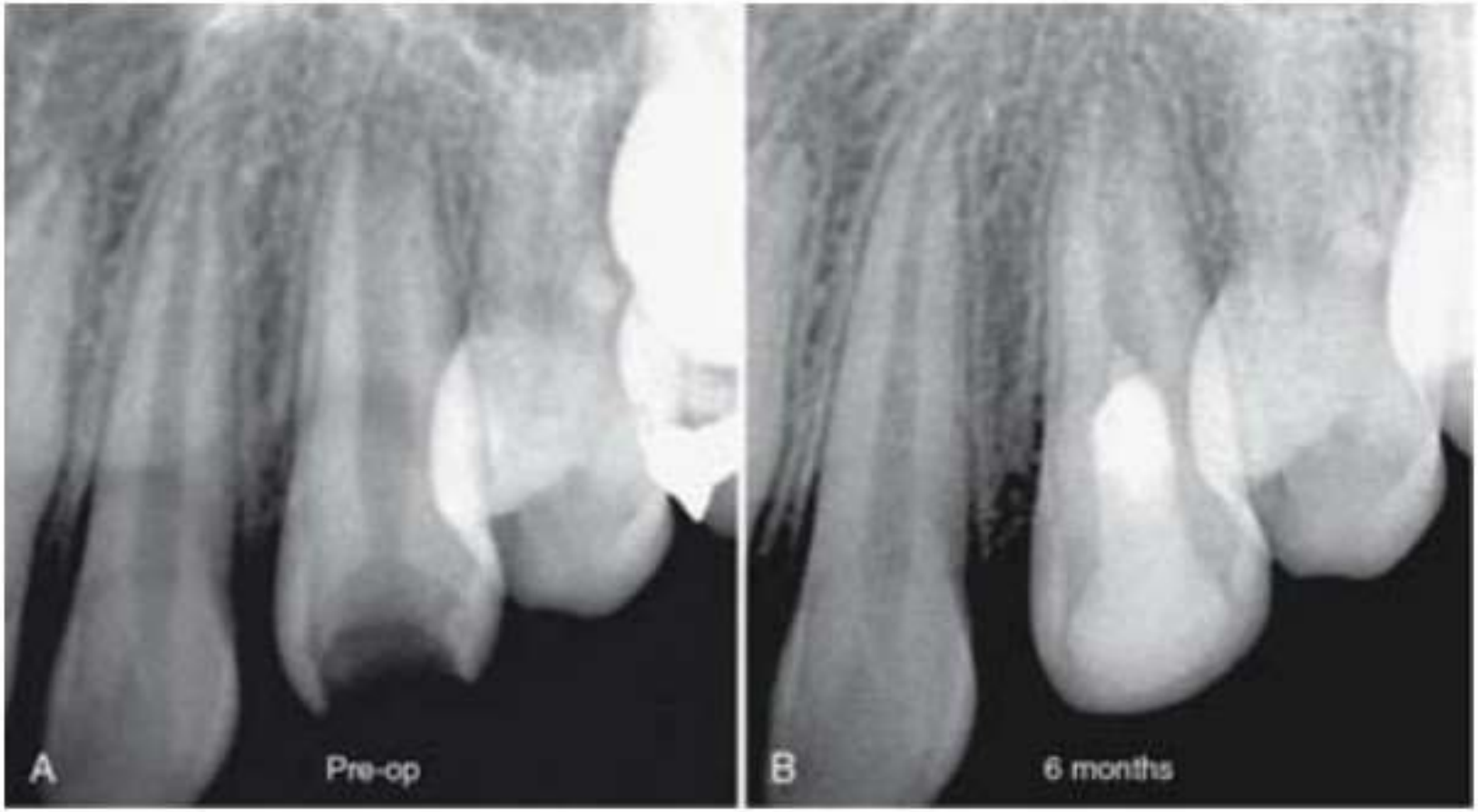
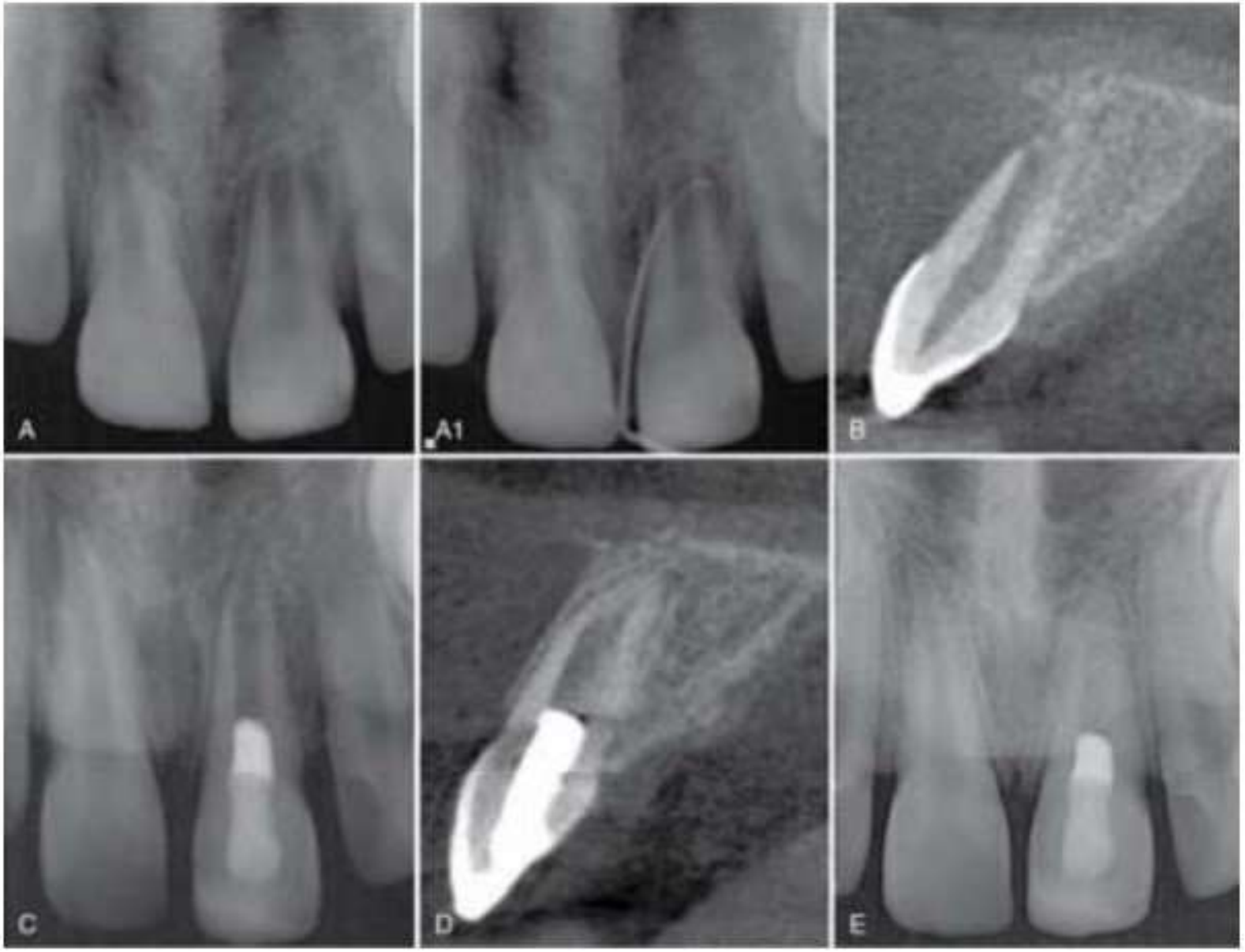


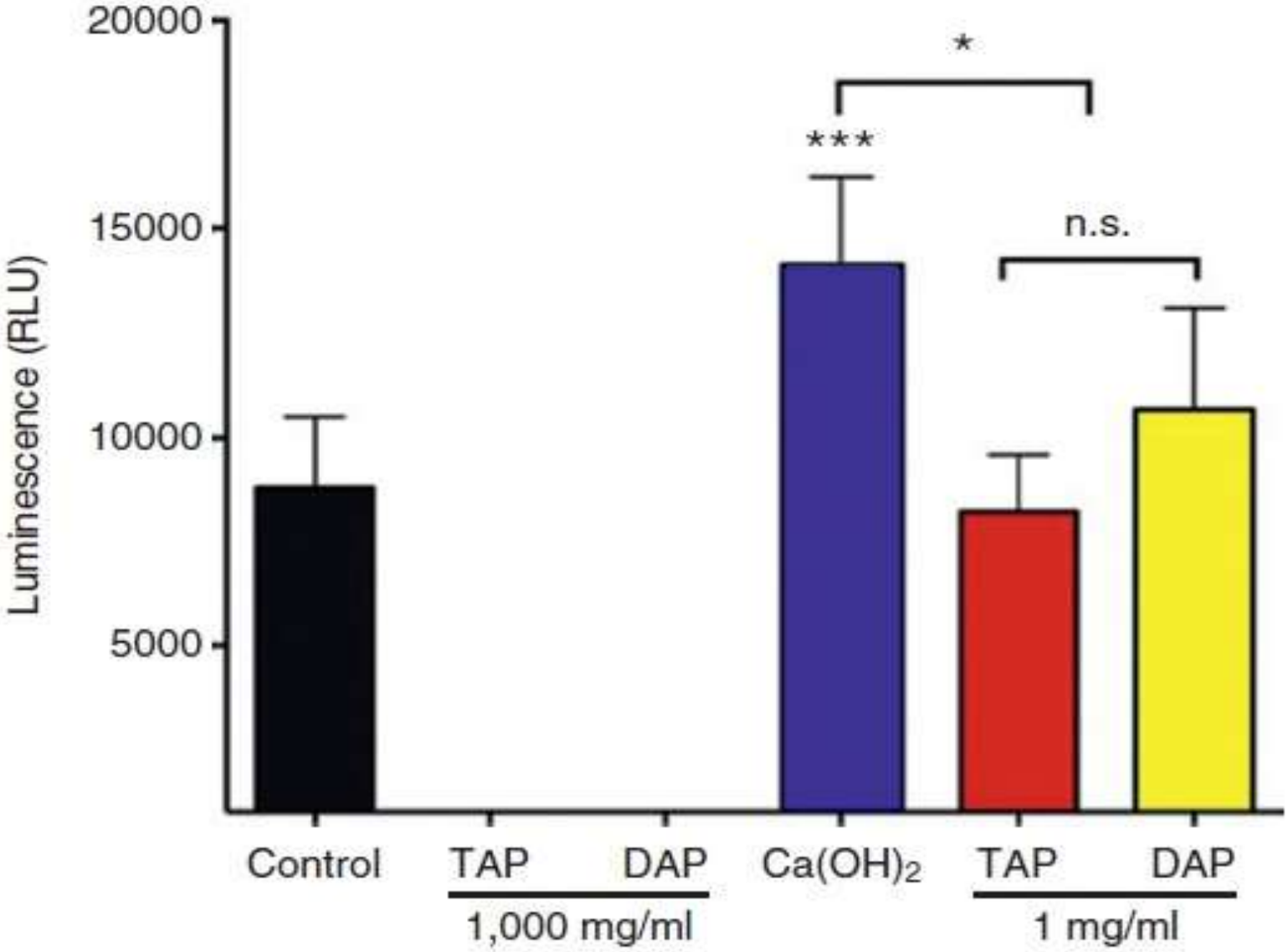
Fig. 3. A 27-year-old woman patient presented with sinus tract associated with tooth #20 diagnosed with dens evaginatus and pulpal necrosis and chronic apical abscess. The patient reported discomfort on the tooth for several years. A large radiolucency associated with the open apex of the immature root with thin dentinal walls was observed in the preoperative radiograph (*A*). The tooth was treated with a REP following AAE-recommended guidelines. The patient presented asymptomatic and with resolution of the sinus tract 30 days after the first visit for completion of the treatment. At the 2-year follow-up appointment, the patient was still asymptomatic, and the tooth responded repeatedly to electric pulp testing. In addition, there was evidence of complete resolution of the radiolucency, thickening of the dentinal walls, and the presence of a mineralized bridge immediately below the MTA layer on the postoperative radiograph (*yellow arrow, B*). (*Courtesy of* Dr Blake Ishikawa, DDS, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.)

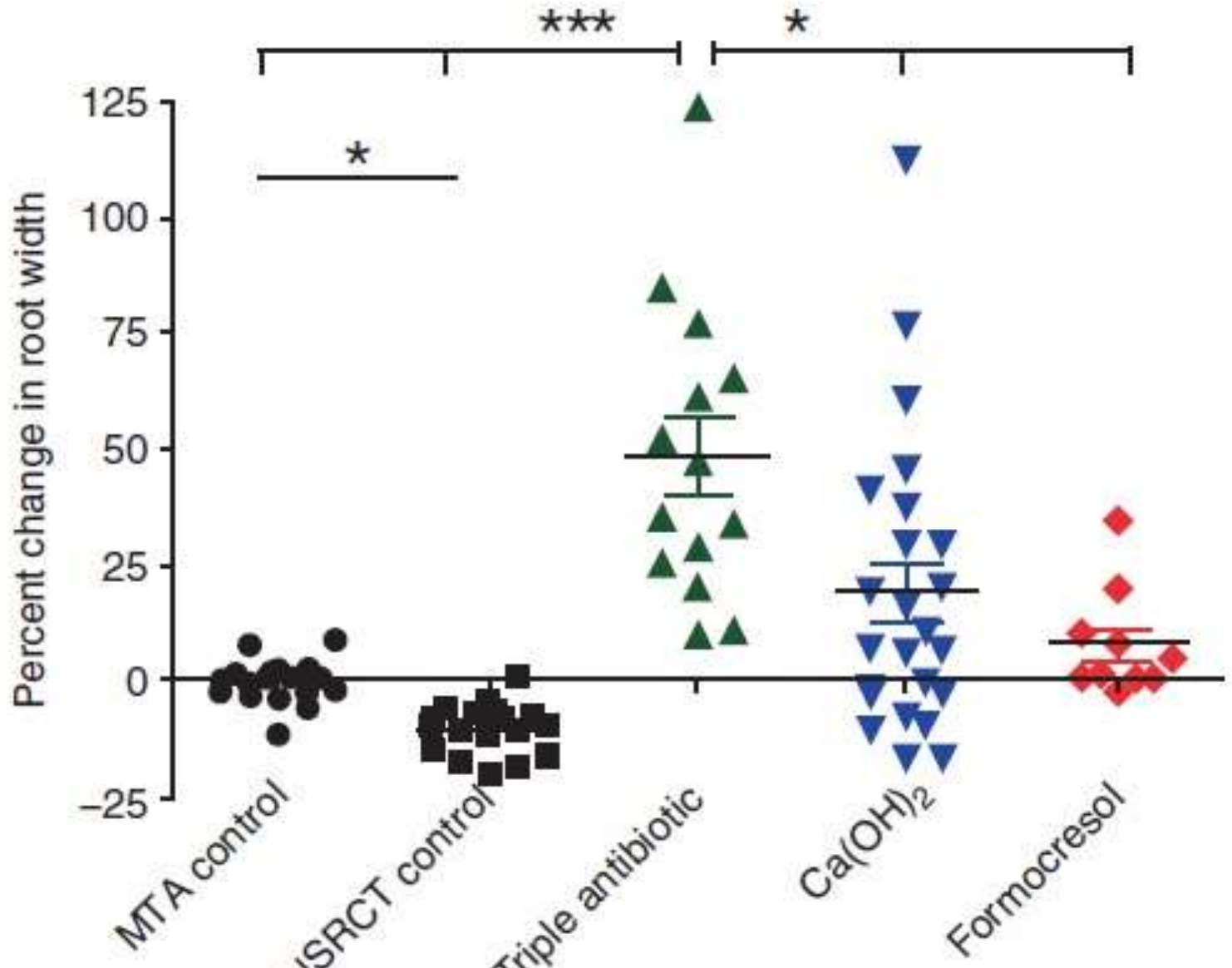

A
Pre-op
B
10 months

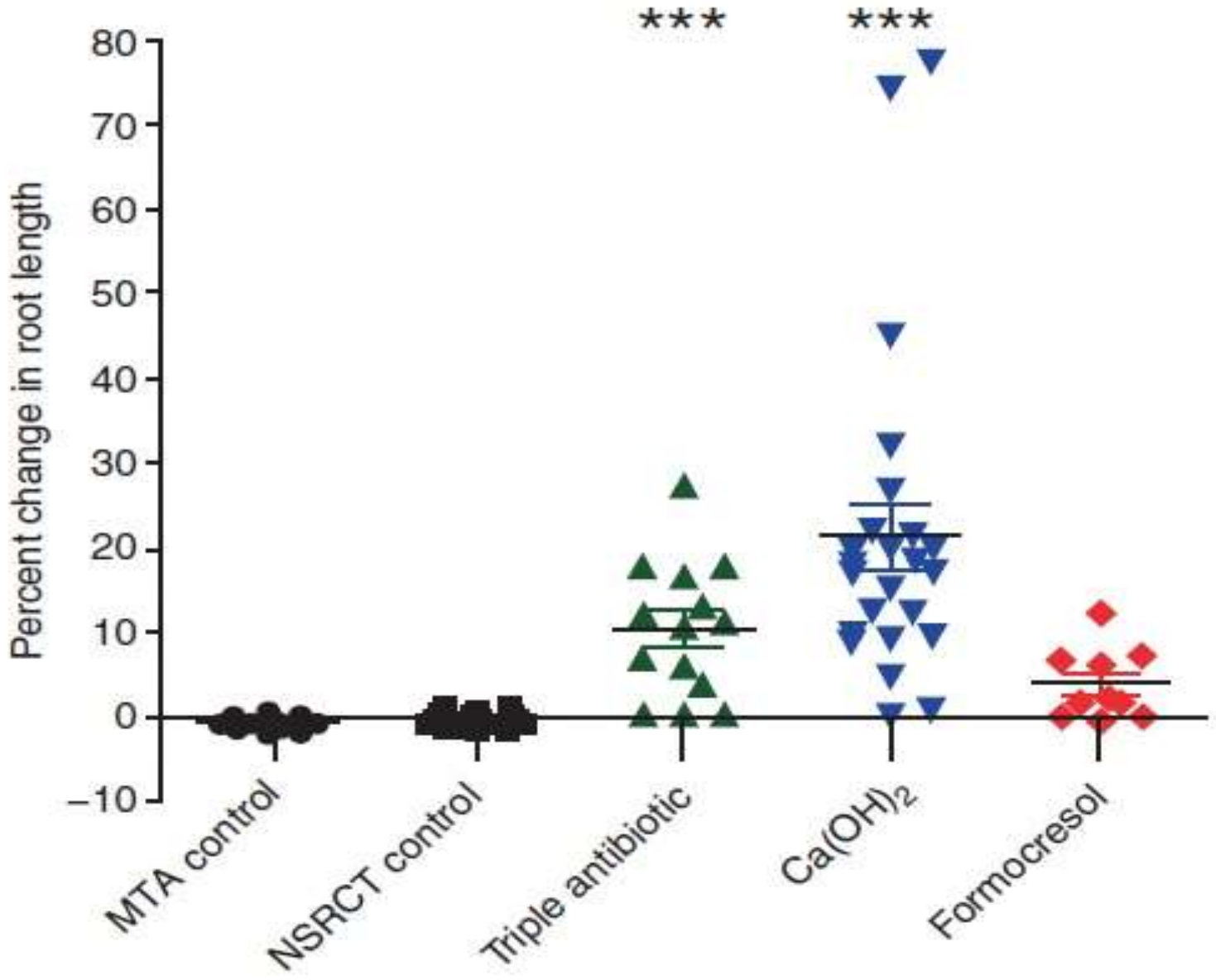

A
Pre-op
B
6 months


A
A1
B
C
D
E

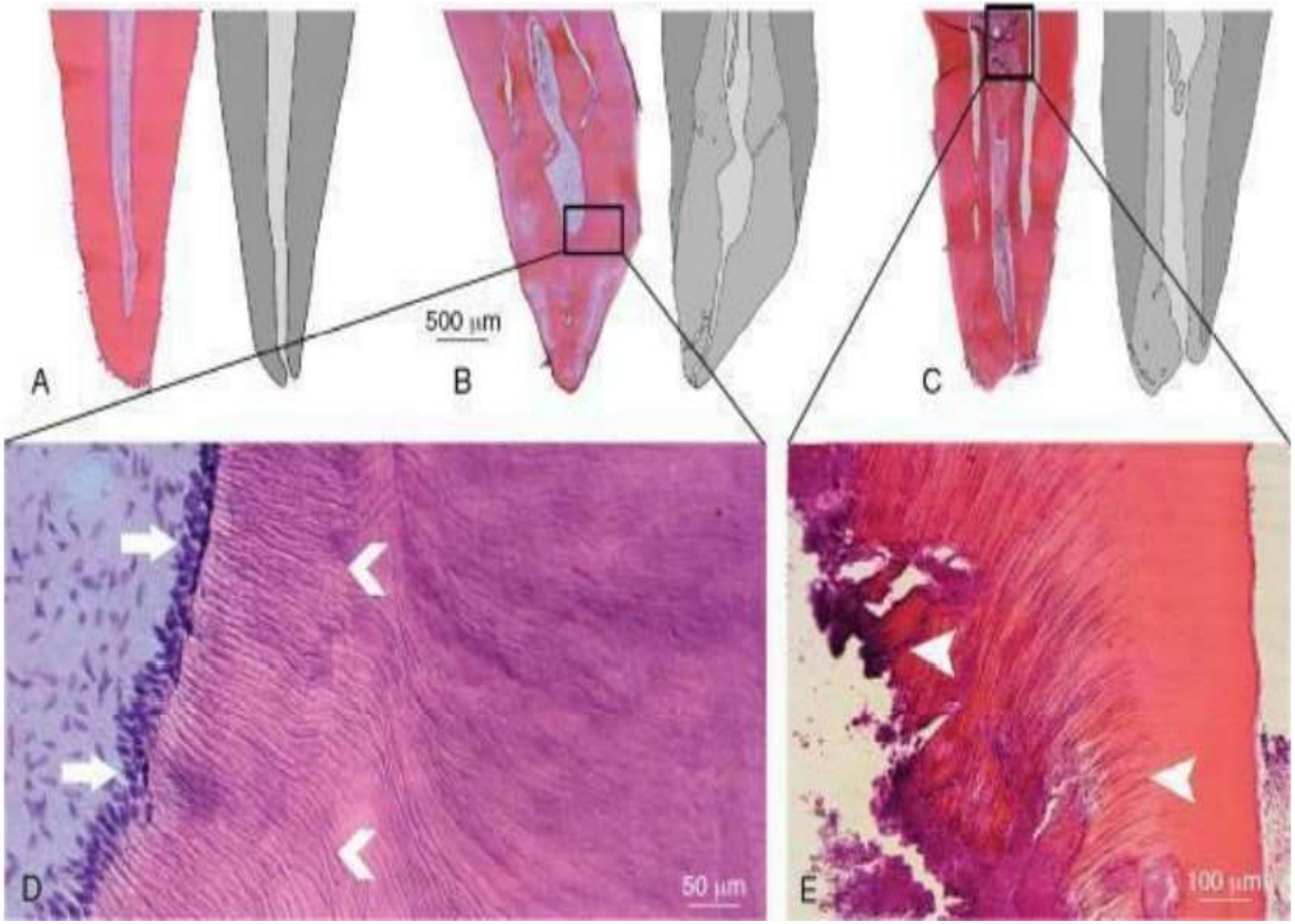
Dentin conditioning for 7 days with medicaments used in REPs has a profound effect on SCAP survival. Standardized dentin disks were treated for 7 days with TAP or double antibiotic paste (DAP) (concentrations of 1000 mg/mL or 1 mg/mL), Ca(OH)2 (Ultracal), or sterile saline (control). SCAP in a Matrigel scaffold (BD Biosciences; Bedford, MA) was seeded into the lumen of the disks after the medicaments were removed and cultured for 7 days. Cell viability (survival) was determined using a luminescent assay. SCAP culture on dentin treated with TAP or DAP at the concentration of 1000 mg/mL resulted in no viable cells. Conversely, dentin conditioning with TAP or DAP at the concentration of 1 mg/mL supported cell viability with no difference from untreated dentin disks (control). Greater survival and proliferation were detected in the group treated with Ca(OH)2.

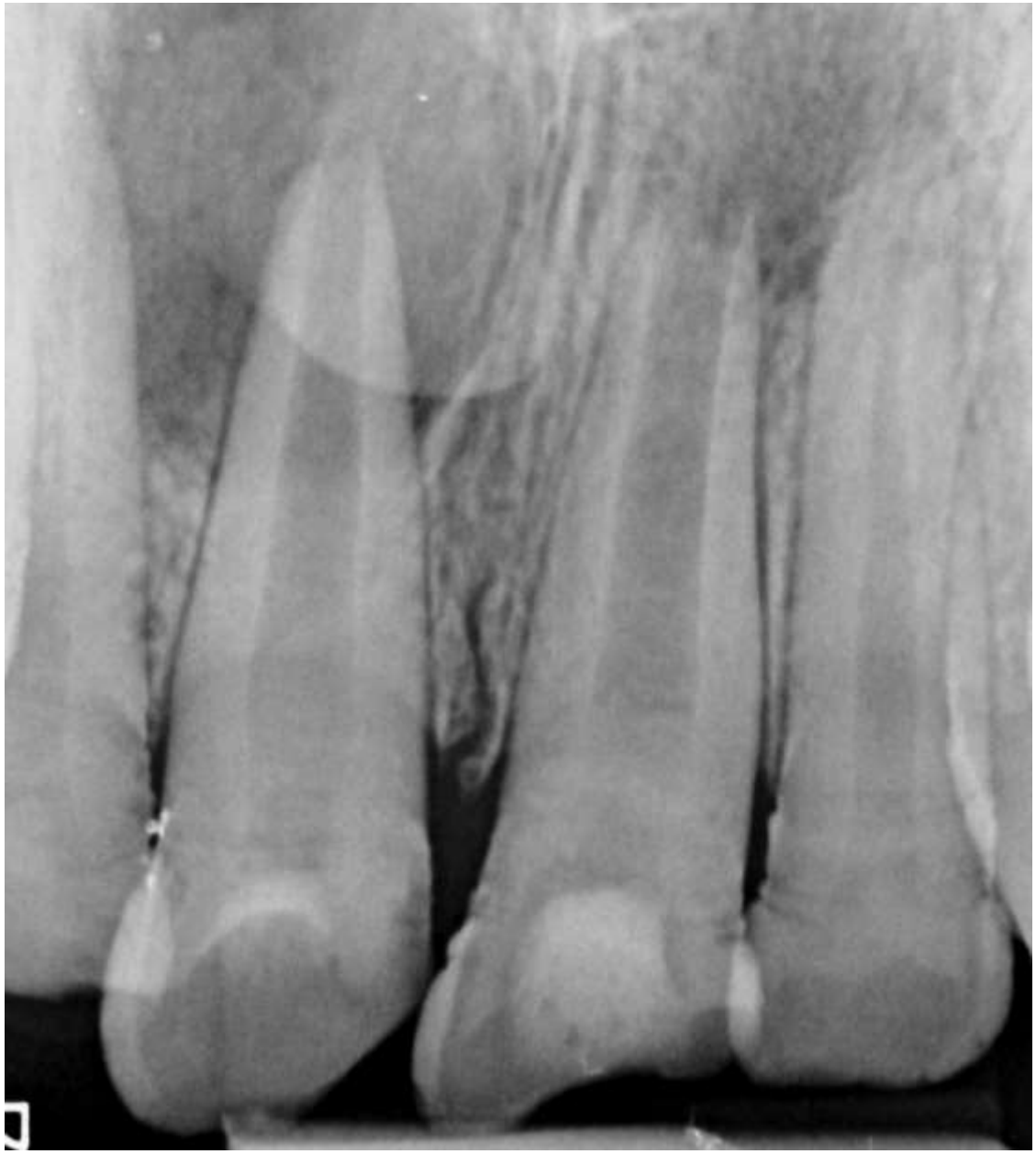

20000
15000
10000
5000
Luminescence (RLU)
*
***
n.s.
Control
TAP
DAP
Ca(OH)2
TAP
DAP
1,000 mg/ml
1 mg/ml

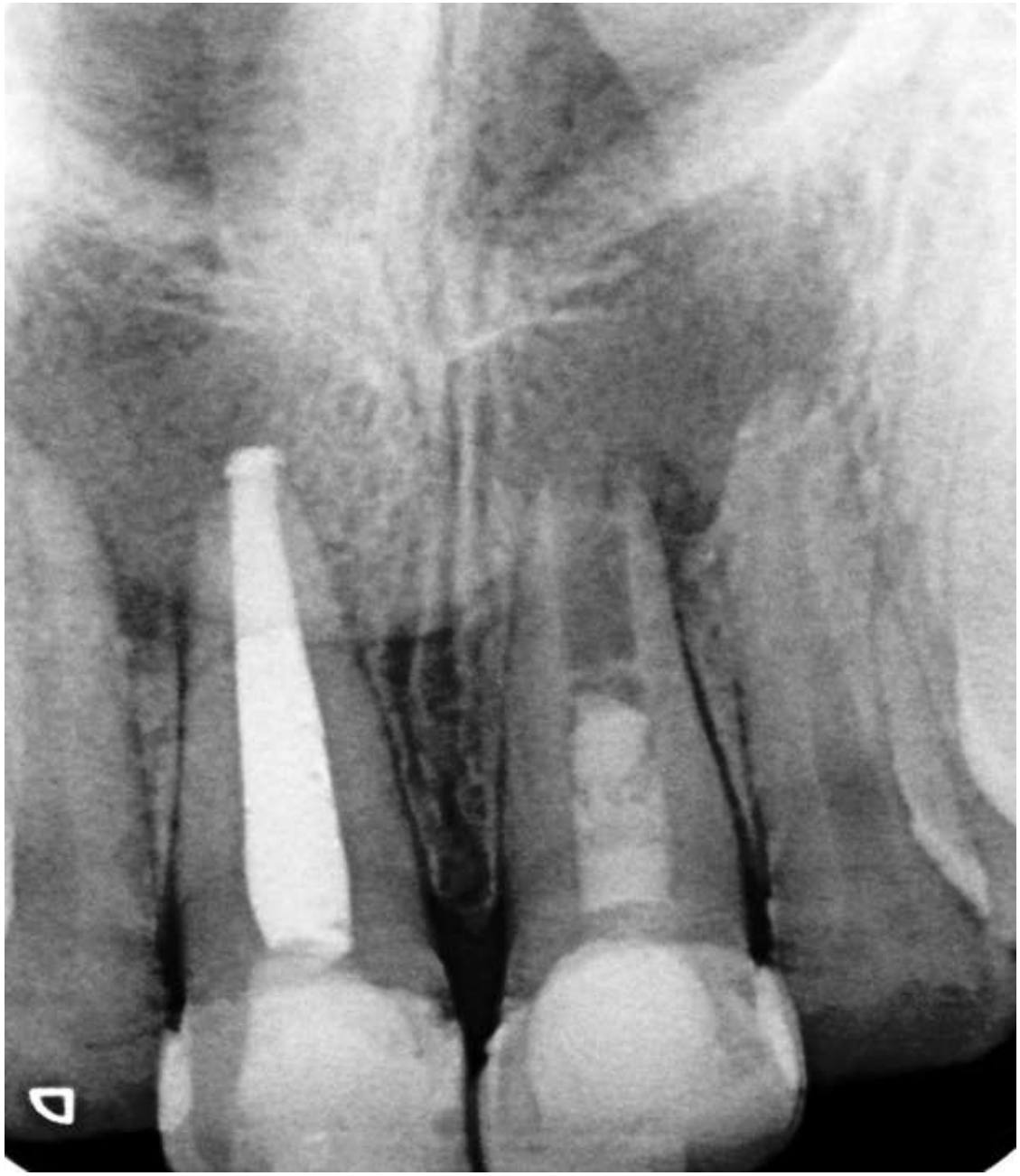

***
*
*
125
100
75
50
25
0
−25
Percent change in root width
MTA control
NSRCT control
Triple antibiotic
Ca(OH)2
Formocresol

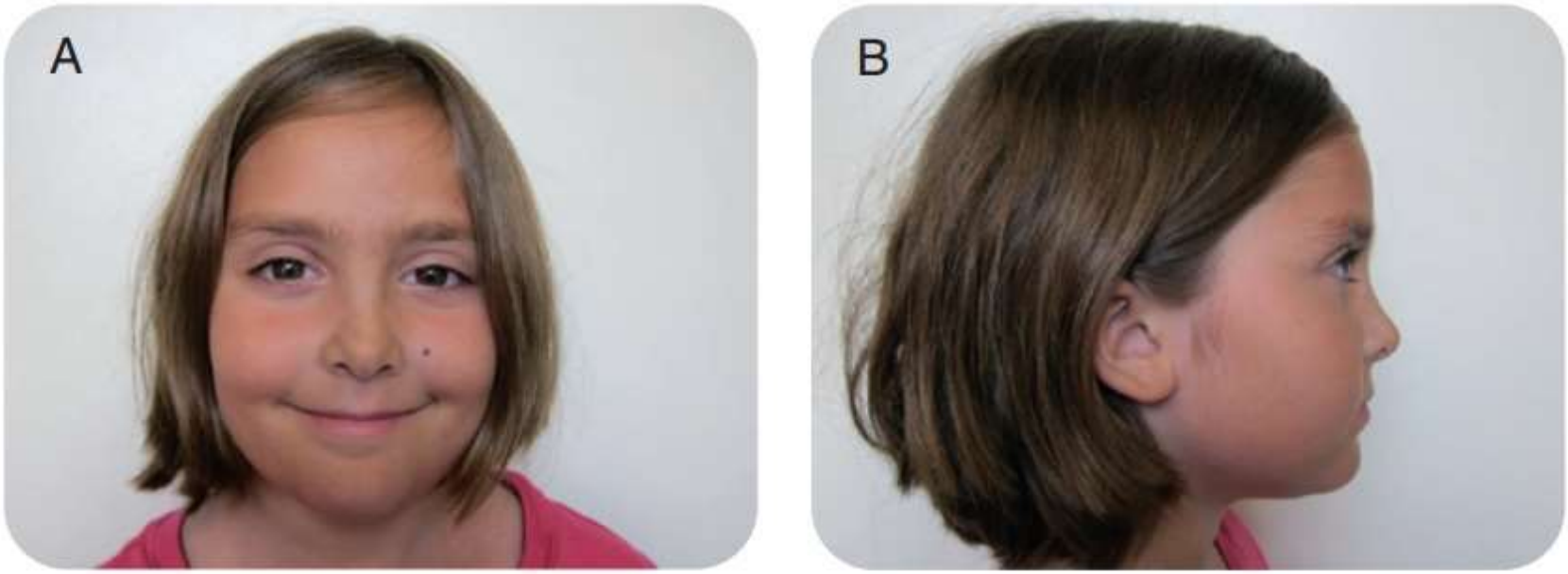

***
***
80
70
60
50
40
30
20
10
0
−10
Percent change in root length
MTA control
NSRCT control
Triple antibiotic
Ca(OH)2
Formocresol

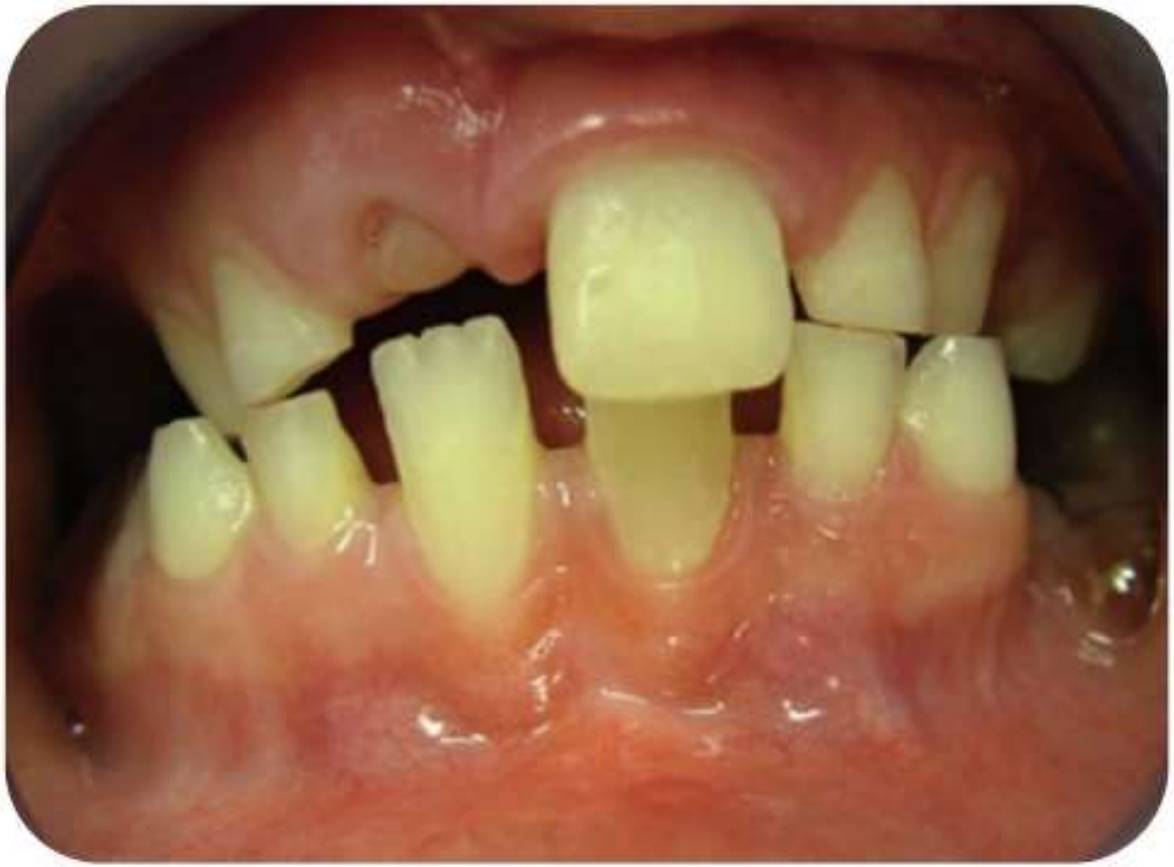
- This provides an internal test that the mathematic analysis was appropriate. The results indicate that these two negative control groups had minimal measured changes in root width or root length, with the anticipated finding that instrumentation with files of greater taper resulted in a slight but detectable loss of apical root wall width.
- The results indicated that revascularization treatment with either the TAP or Ca(OH)2 medicament produced significantly greater increases in root length compared with either the MTA or NSRCT control groups.

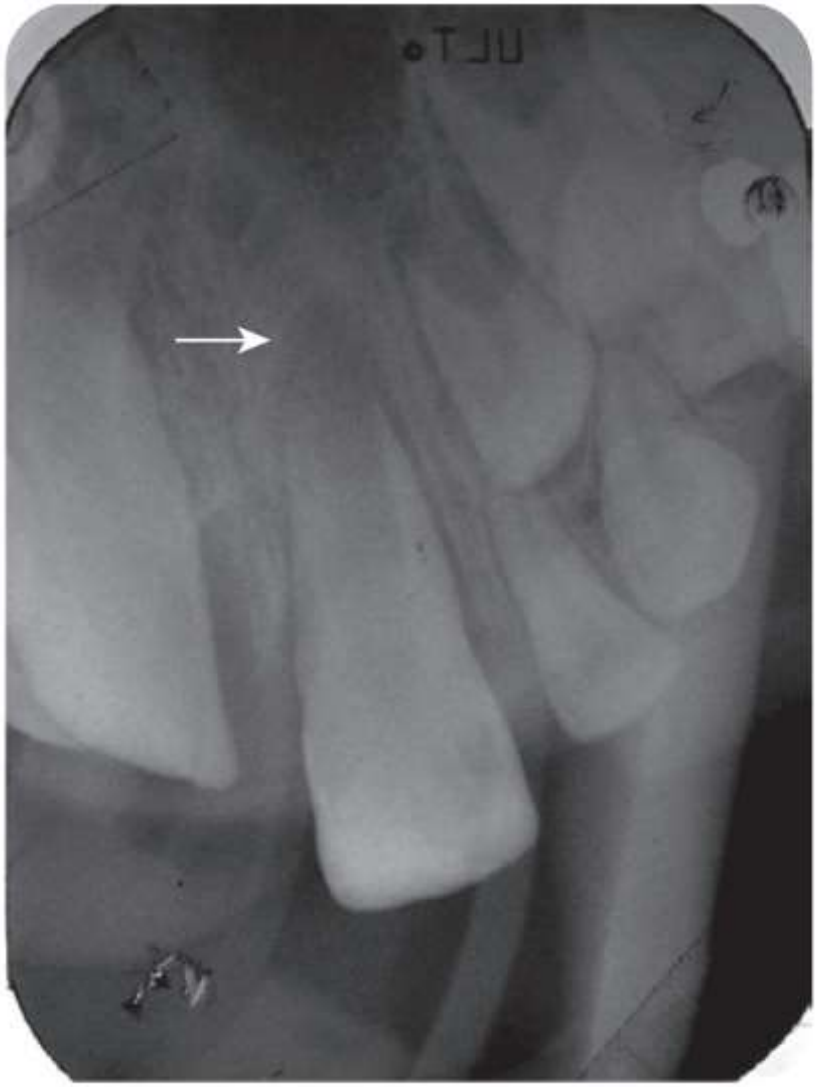

500 µm
A
B
C
D
50 µm
E
100 µm

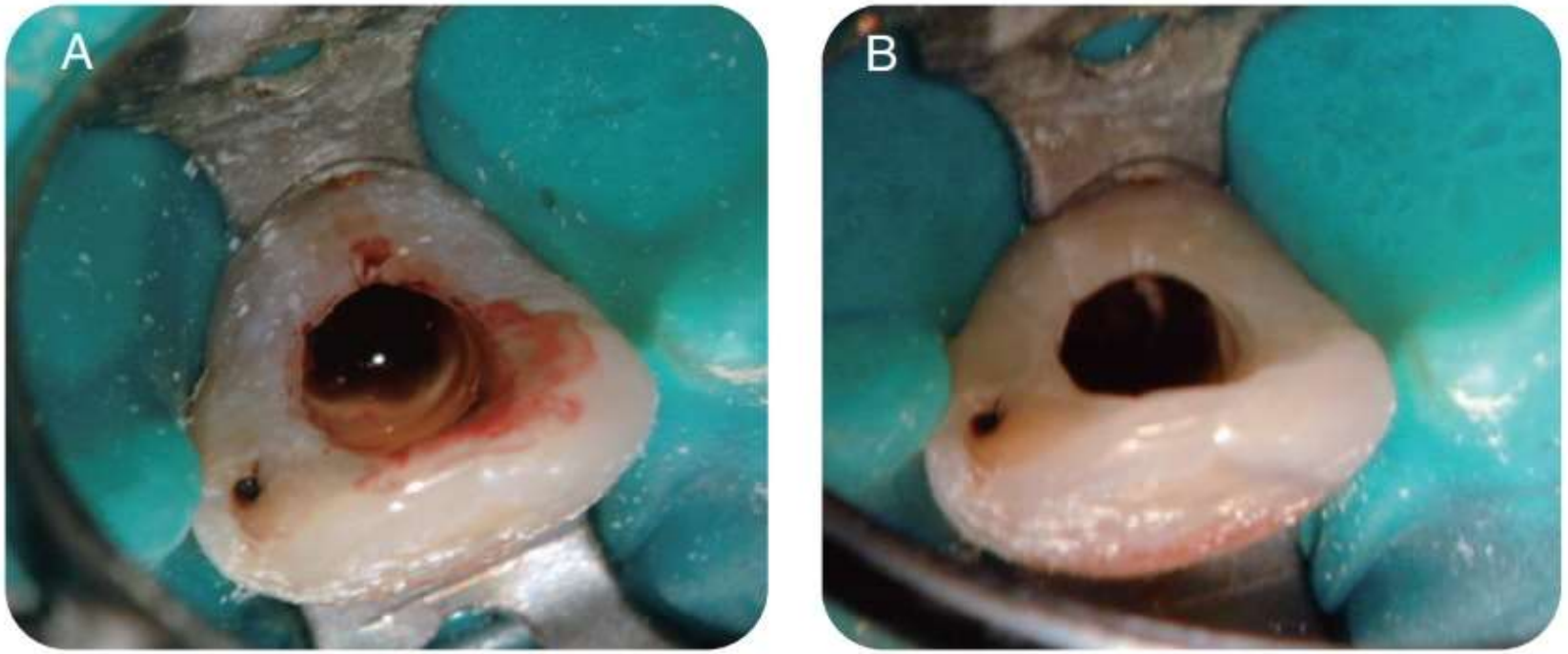




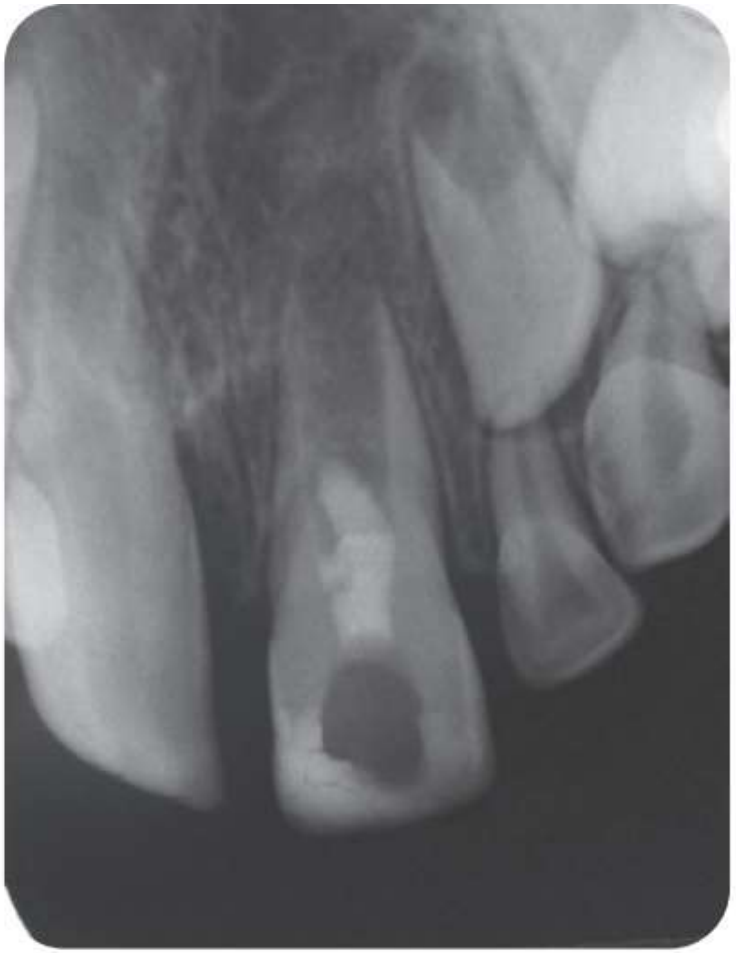

A
B



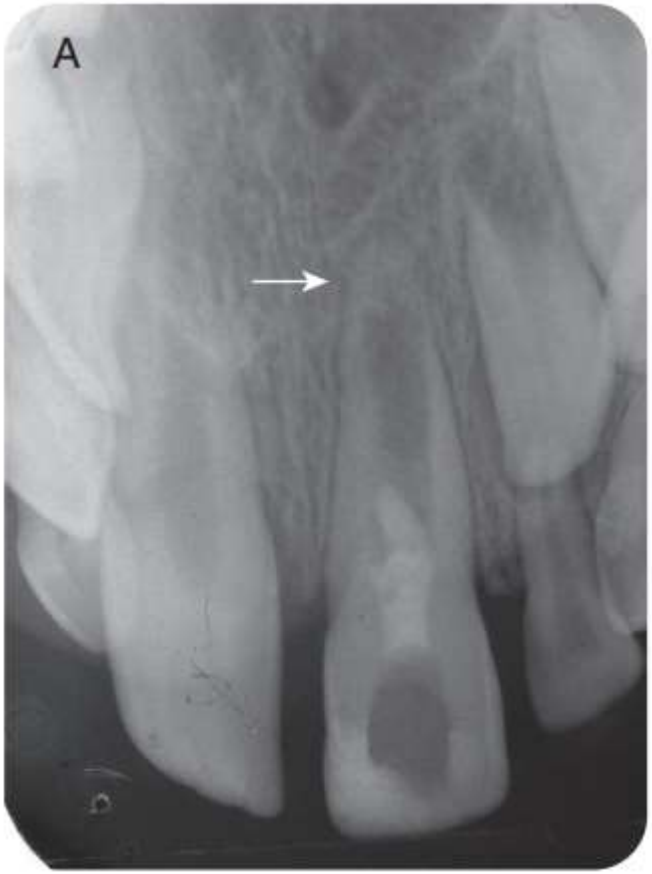


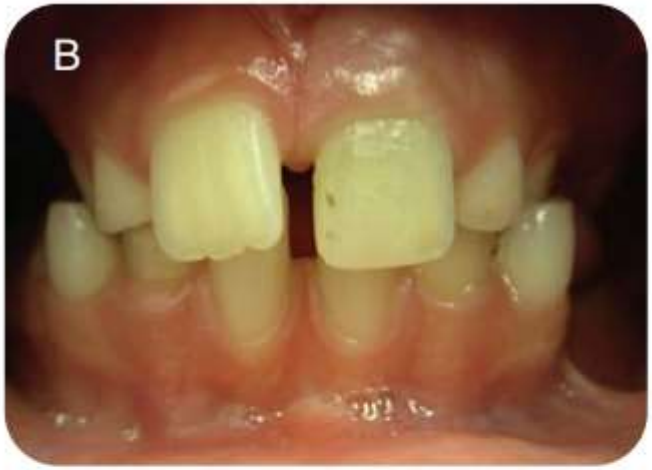

A
B

Three-month post-op radiograph

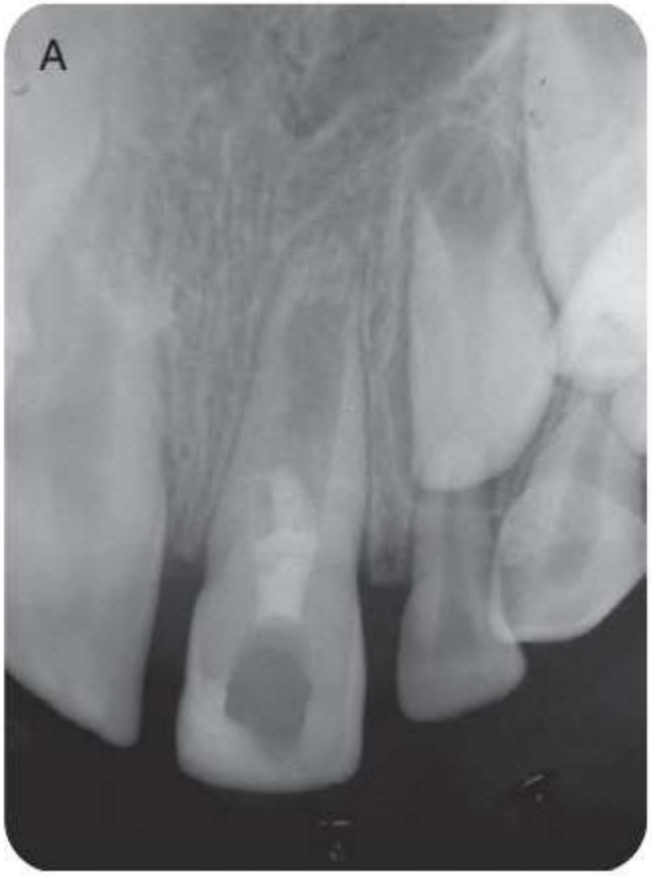


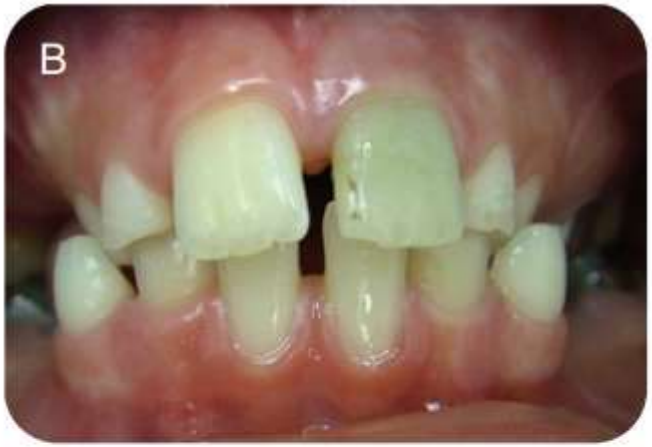
Six-month post-op radiograph

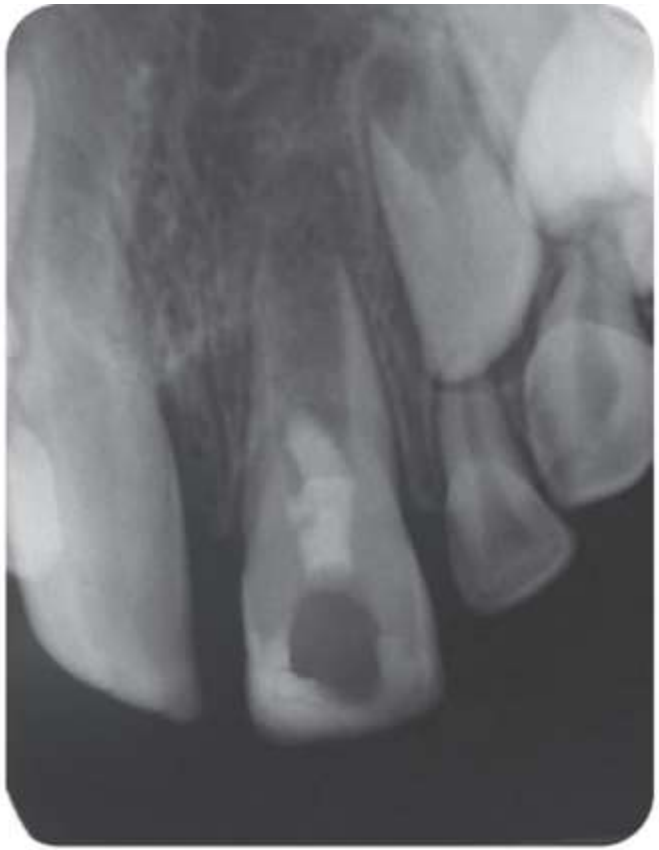


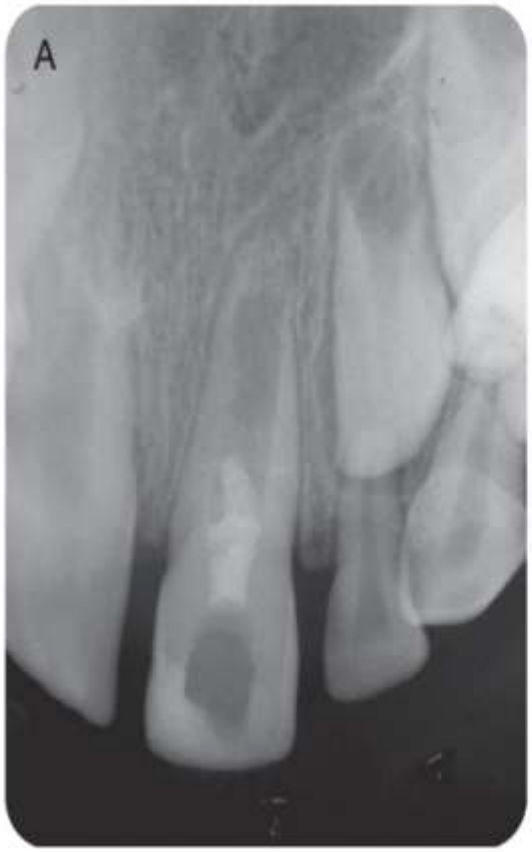


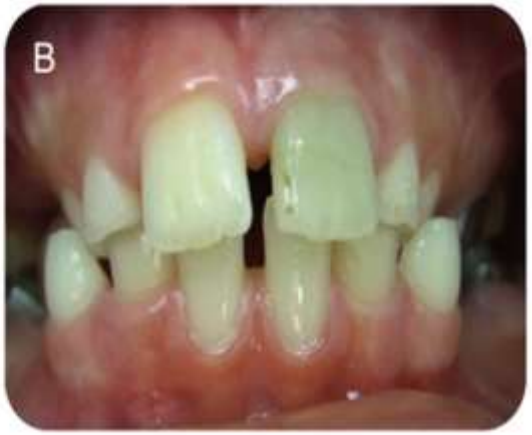
12-month post-op radiograph

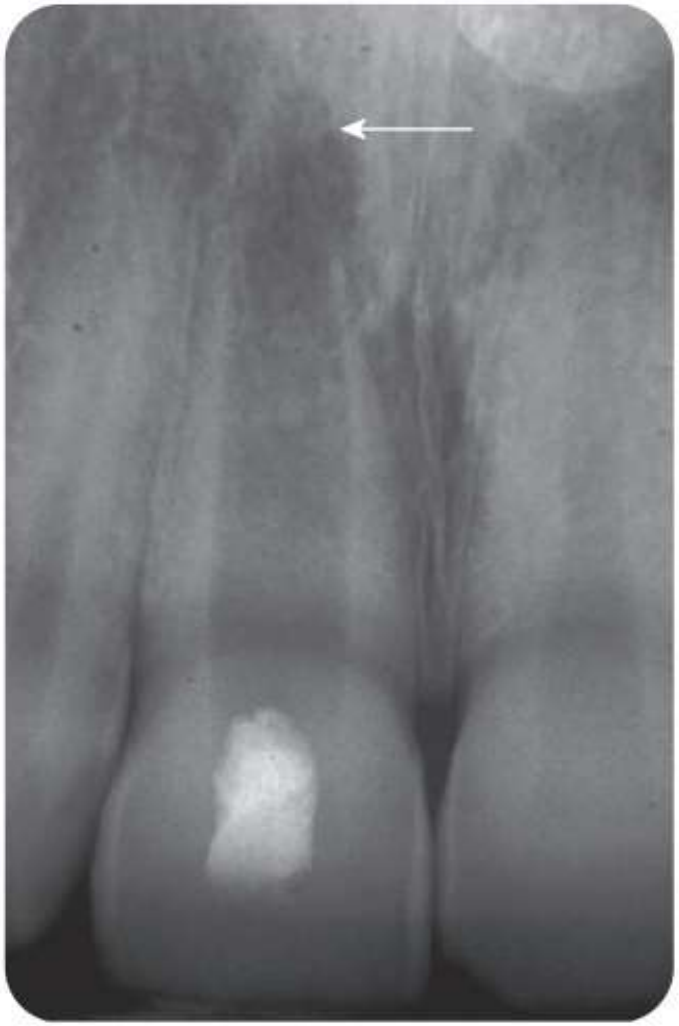


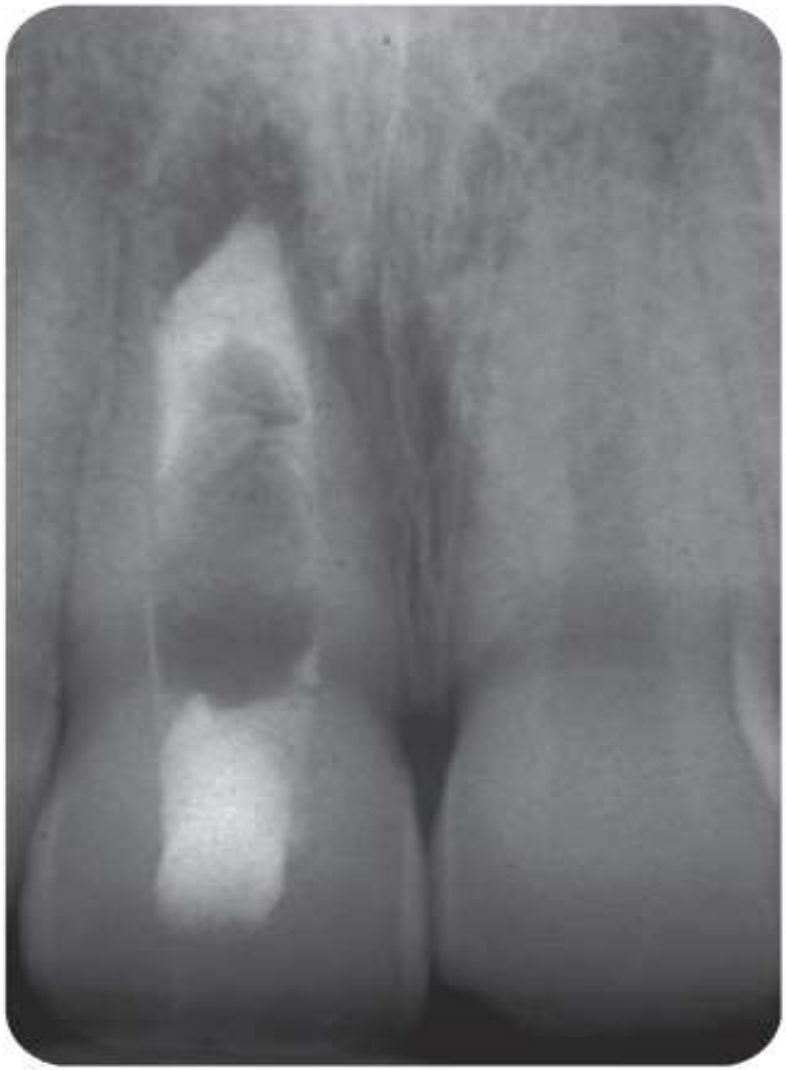


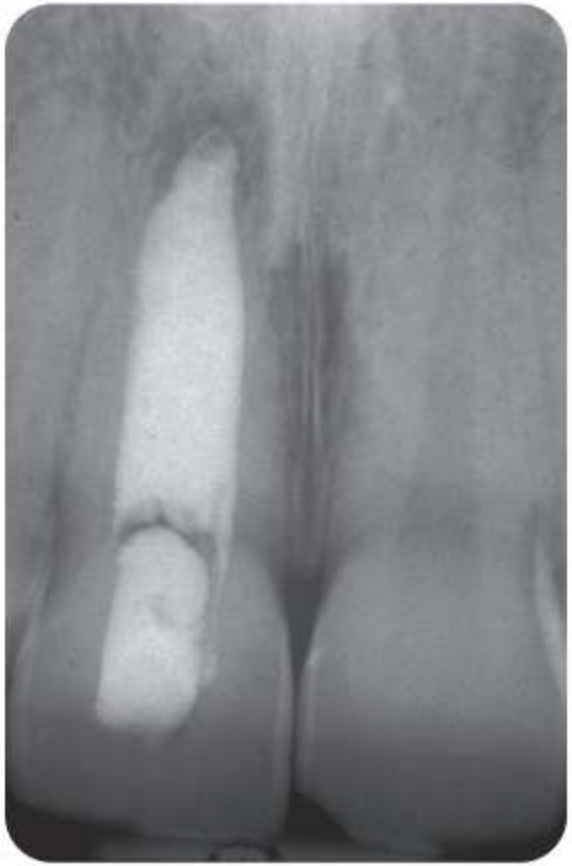



A

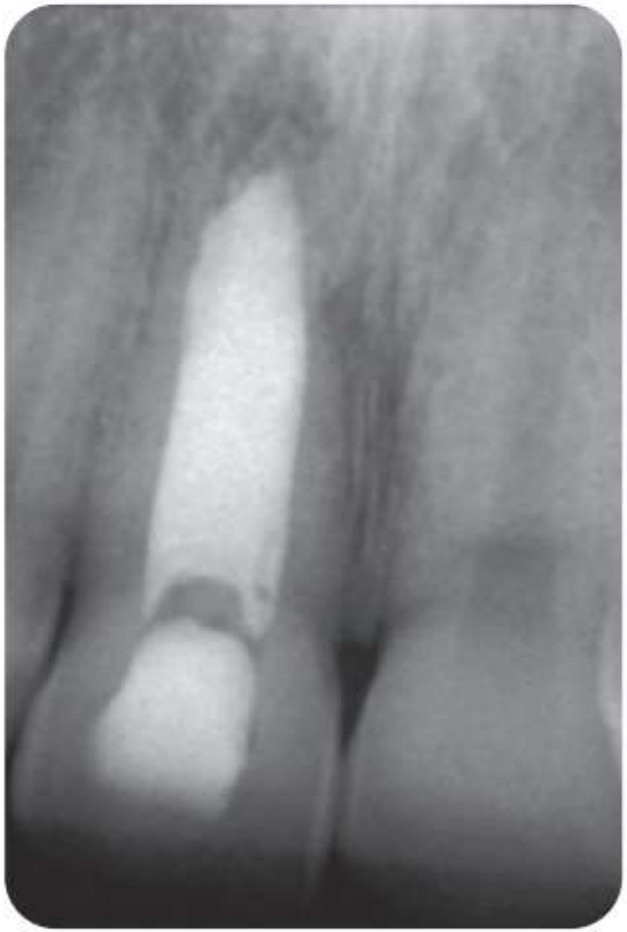

B

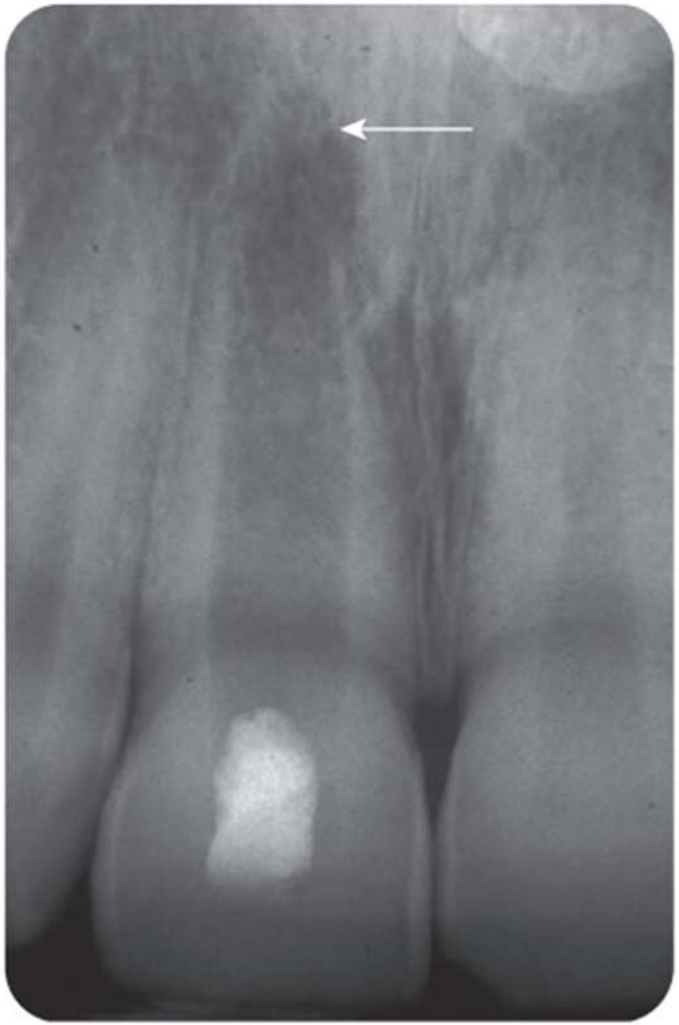


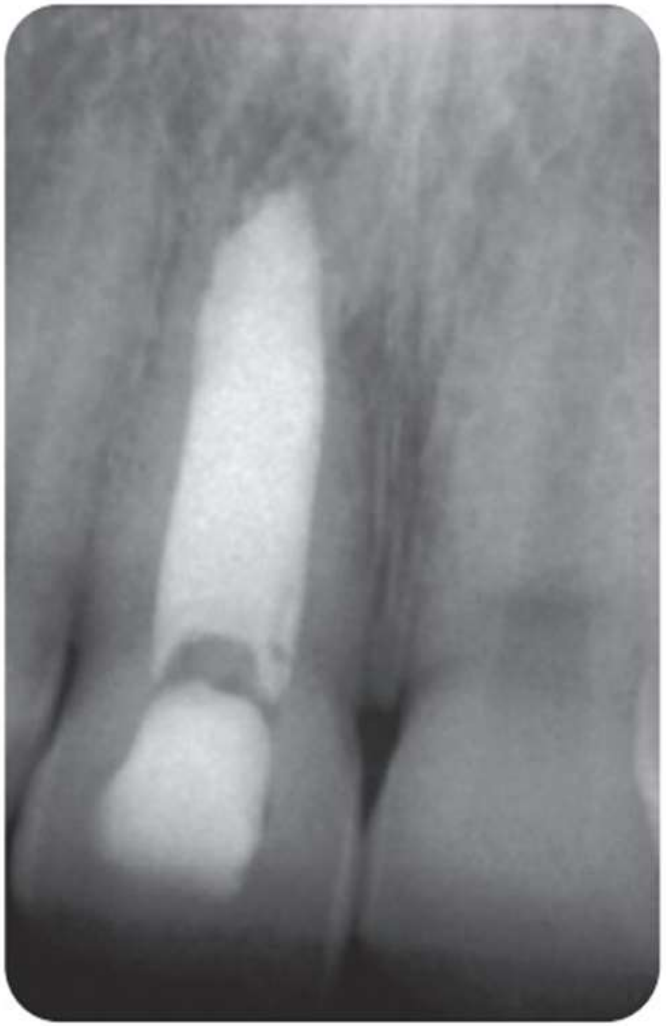


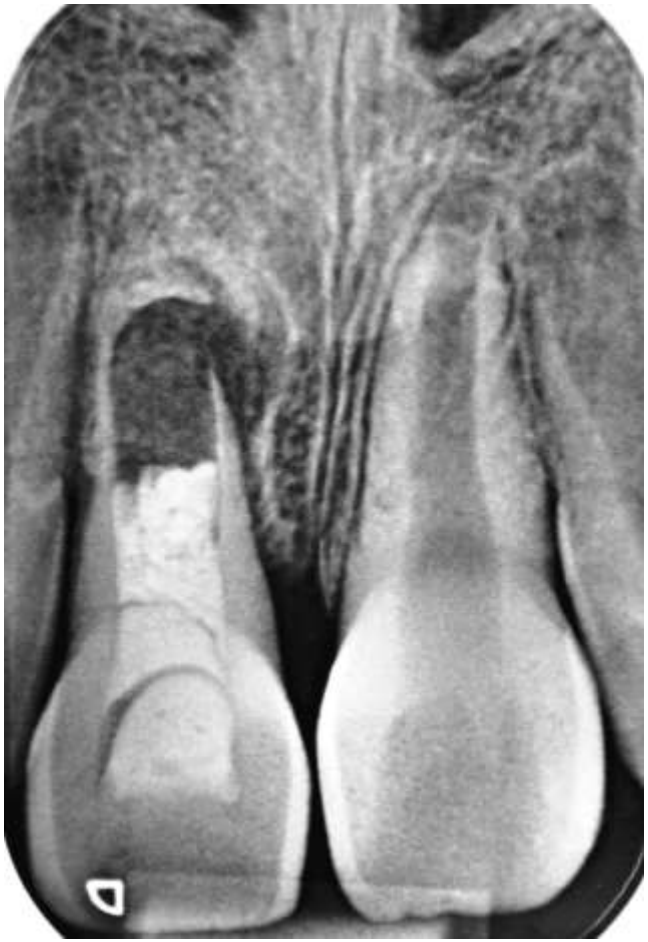


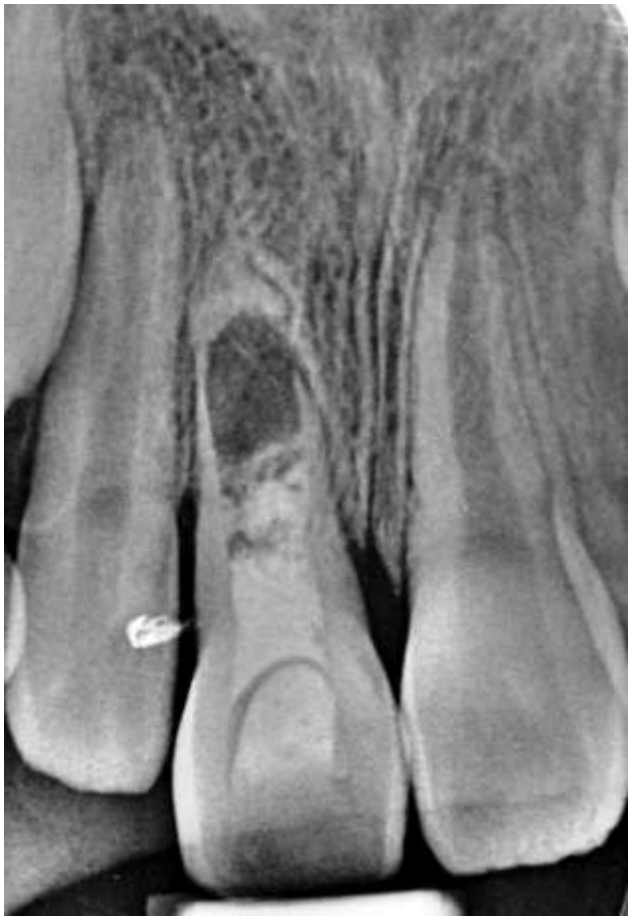
12-month post-op radiograph












Thank you for your attention