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Review Article

Regenerative Endodontics: A Comprehensive Review

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Abstract

Immature teeth diagnosed with pulp necrosis have been traditionally treated with apexification or apexogenesis approaches. Unfortunately, these treatments provide little or no benefit in promoting continued root development. Regenerative endodontic therapy is a biologically based procedure that aims to replace previously damaged pulp-dentin complex by new vital tissue, resulting in partial restoration of the functional properties of the involved tooth. It has emerged as an important alternative in treating teeth with otherwise questionable long-term prognosis because of thin, fragile dentinal walls and a lack of immunocompetency. Most therapies use the host's own pulp or vascular cells for regeneration, but other types of dental stem cell therapies are under development. This article reviews the literature related to the various aspects of regenerative procedure including role of stem cells, growth factors and scaffolds, the clinical considerations, outcomes and drawbacks and how these procedures may increase the prognosis for immature teeth with necrotic pulp tissue.

Keywords: *Stem cells; Growth factors; Scaffolds; Clinical considerations; Drawbacks; Outcomes*

Introduction

The ultimate goal for tissue engineering and regenerative medicine is to develop therapies to restore lost, damaged, or aging tissues using engineered or regenerated products derived from either donor or autologous cells. It involves using a biodegradable scaffold in the shape of the new tissue that is seeded with either stem cells or autologous cells from biopsies of damaged tissues.

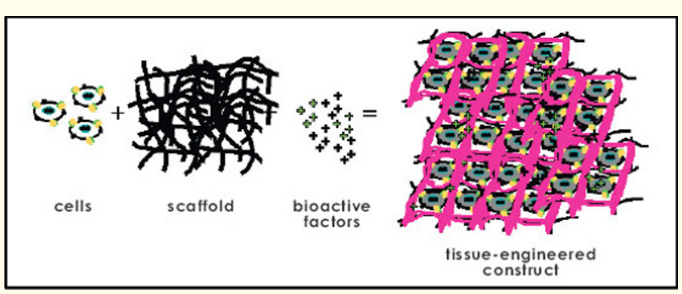

Figure 1: *Tissue engineering triad.*

Dental stem cells and their sources

Stem cell niches

Stem cell behavior is regulated by a local microenvironment referred to as ‘‘the stem cell niche,’’ which is characterized by 3 essential properties [8,9]:

1. A niche provides an environment where the stem cell number is regulated.
2. It is the place where a stem cell controls the maintenance, quiescence, self-renewal, and recruitment toward differentiation, fate determination, and long-term regenerative capacity.
3. The niche influences cell motility.

It is assumed that multiple niches exist in teeth.

Beginning in 2000, several human dental stem/progenitor cells have been isolated and characterized. These include:

Dental pulp stem cells (DPSCs)

Dental pulp stem cells were first isolated from human permanent third molars in 2000 [10] and were characterized as clonogenic and highly proliferative. Colony formation frequency was high and produced densely calcified, but sporadic, nodules [13]. Dentin and pulp-like tissues were generated following the transplantation of these cells in hydroxyapatite/tricalcium phosphate scaffolds into immunodeficient mice [11,13]. A follow-up study confirmed that these cells fulfilled the criteria needed to be stem cells: an ability to differentiate into adipocytes and neural cells and odontoblasts and self-renewal capabilities [12].

Stem cells from human exfoliated deciduous teeth (SHED)

Stem cells isolated from human exfoliated deciduous teeth are highly proliferative and capable of differentiating into a variety of cell types, including osteoblasts, neural cells, adipocytes, and odontoblasts, and inducing dentin and bone formation. Like dental pulp stem cells, these cells can generate dentin-pulp-like tissues with distinct odontoblast-like cells lining the mineralized dentin-matrix generated in HA/TCP scaffolds implanted in immunodeficient mice [13]. However, these cells have a higher proliferation rate than dental pulp stem cells and bone marrow mesenchymal stem cells, suggesting that they represent a more immature population of multipotent stem cells [13,14].

Stem cells from apical papilla (SCAP)

Dental papilla is derived from the ectomesenchyme induced by the overlaying dental lamina during tooth development. It evolves into dental pulp after being surrounded by the dentin tissue produced by odontoblasts. The physical and histologic characteristics of the dental papilla located at the apex of developing human permanent teeth was described and termed as apical papilla. This tissue is loosely attached to the apex of the developing root and can be easily detached with a pair of tweezers.

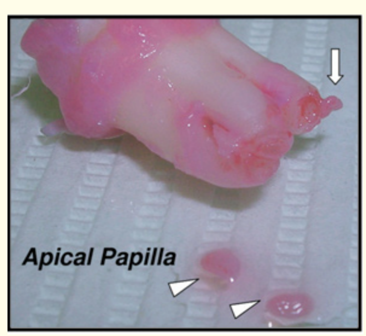

Figure 2: *Apical Papilla.*

Periodontal ligament stem cells (PDLSCs)

McCulloch [15] reported the presence of progenitor/stem cells in the periodontal ligament of mice in 1985. Subsequently, the isolation and identification of multipotent mesenchymal stem cells in human periodontal ligaments were first reported in 2004. Seo and colleagues [16] demonstrated the presence of clonogenic stem cells in enzymatically digested periodontal ligament and further showed that human periodontal ligament stem cells transplanted into immunodeficient rodents generated a cementum/periodontal ligament-like structure that contributed to periodontal tissue repair. Later work showed that periodontal ligament stem cells differentiation was promoted by Hertwig's epithelial root sheath cells *in vitro* [17].

Dental follicle precursor cells (DFPCs)

The dental follicle forms at the cap stage by ectomesenchymal progenitor cells. It contains the developing tooth germ, and progenitors for periodontal ligament cells, cementoblasts, and osteoblasts. Dental follicle precursor cells were first isolated from the dental follicle of human third molars.

Induced pluripotent stem cells and dental pulp pluripotent stem cells (iPSCs)

Recent reports have described successful attempts to develop pluripotent stem cells from pulps recovered from deciduous teeth and third molar [18].

Constructs and scaffolds

The purpose of a scaffold is to provide a physiochemical and biological three-dimensional microenvironment for cell growth and differentiation, promoting cell adhesion, and migration. Scaffold should be effective for transport of nutrients, oxygen and waste, should be biocompatible, nontoxic, and have proper physical and mechanical strength [19].

transport of nutrients, oxygen and waste, should be biocompatible, nontoxic, and have proper physical and mechanical strength [19]. Scaffolds can be classified into:

1. Casted (fairly rigid and custom made for specific purposes)
2. Injectable (low viscosity gels that can be delivered and molded at the site that requires tissue regeneration) [20]

They can also be classified as:

a. Biological/Natural
b. Artificial/Synthetic

Physical properties of tissue engineering scaffolds and common types of scaffolds for dental regeneration are given in Table 1 and Table 2 respectively.

Scaffold Type	Average Dimensions	Hydration Capacity	Pores/Linear Inch	Average Pore Size	Weight/Wet Weight
Polymer	5 × 4 mm 0.039 cm^3	30 ml	120-/+20	100-200 mm	5.2 mg/32 mg
Collagen	4.5 × 4.2 mm 0.039 cm^3	25 ml	120-/+20	100-200 mm	3.5 mg/45 mg
Calcium Phosphate	5 × 3 mm 0.058 cm^3	30 ml	60-/+10	200-400 mm	45 mg/99 mg

Table 1: *Physical Properties Of 3-dimensional tissue engineering scaffolds.*

Scaffold Type	Properties	Advantages	Limitations
Hydrogel	A colloid jelly-like scaffold Self-assembling peptides often mixed with hydrogel	Injectable, biocompatible and absorbed by body	Low stem cell survival, lacks functional strength, variable outcomes
Collagen Polymer Calcium Phosphate Silk Fibrin	Sponge Brittle Fibers Centrifuged from peripheral blood	Easy to handle, clinically effective absorbed by body Easily made scaffold from host peripheral blood	Lacks functional strength to support tissues or muscle movement
Bone	Sourced from donor and freeze-dried into a powder	Bone grafts are clinically very effective	Expensive and requires a donor, risk of contamination
Synthetic bone	Bone defect filling material	Clinically effective and safe	Not as effective as natural bone
Skin	Sourced from a cadaver or host	Clinically effective	Need cadavers or donor skin, risk of contamination
Synthetic skin	Wound dressing material	Clinically useful and safe	Temporary fix, patient will still need skin grafts

Table 2: *Common types of scaffolds for dental regeneration.*

The development of self-assembling peptides in the past decade has provided a new class of tissue engineering scaffolds, which have good biocompatibility, excellent handling properties, and strong potential as a carrier material for anti-inflammatory and bioactive molecules. For example, multidomain peptides are synthetic peptides that self-assemble into multisubunit nanofibers [21].

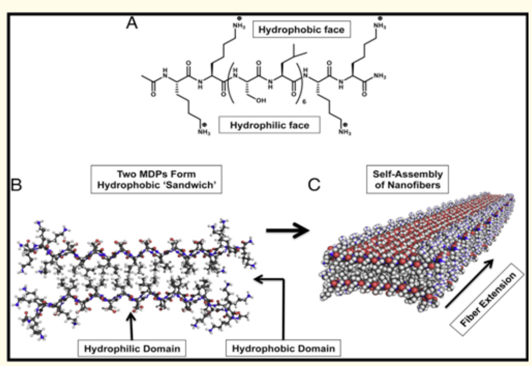

Figure 3: *Multidomain peptide.*

A commercially available self-assembling injectable hydrogel, that is, Puramatrix (BD Biosciences, Franklin Lakes, NJ) presents favorable viscosity for use as an injectable scaffold. When mixed with a sucrose-based solution and/or cell culture medium, it triggers fast self-assembling, leading to its gelification and generation of a tridimensional environment that provides cell adhesion and enables cell proliferation.

Morphogenic Factors In Pulp Regeneration

Some common morphogenic factors and their function in dental development, regeneration, and tissue engineering are given in Table 3.

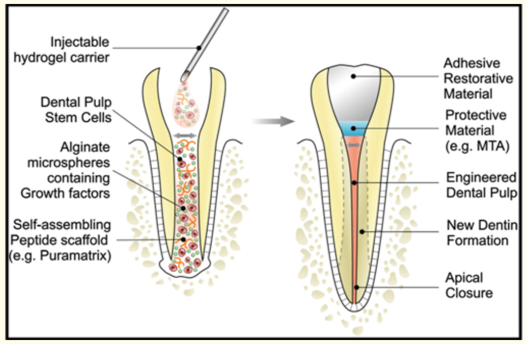

Figure 4: *Puramatrix.*

Signaling Molecules	Target cells	Primary effects	Interactions
PDGF	Dental pulp cells	Cell proliferation Dentin matrix synthesis Odontoblastic differentiation Dentinogenesis	Combined with IGF-1 or dexamethasone, increased cell proliferation Combined with PDGF, increased cell proliferation
TGF β1	Dental pulp cells Dental pulp stem cells	Cell proliferation Extracellular matrix synthesis Odontoblastic differentiation Dentinogenesis Chemotaxis	Combined with FGF2, increased odontoblastic differentiation
BMP 2	Dental pulp cells	Odontoblastic differentiation Dentinogenesis	
BMP 4	Dental pulp cells	Odontoblastic differentiation Dentinogenesis	
BMP 7 (OP-1)	Dental pulp cells	Dentinogenesis	
BMP 11 (GDF 11)	Dental pulp stem cells	Odontoblastic differentiation Dentinogenesis	
VEGF	Dental pulp stem cells	Odontoblastic differentiation Cell proliferation	Under osteogenic conditions, increased osteogenic differentiation
FGF 2 (bFGF)	Dental pulp stem cells Dental pulp cells	Chemotaxis Cell proliferation Cell proliferation Dentinogenesis	Combined with TGFβ 1, increased odontoblastic differentiation
IGF	Dental pulp cells	Cell proliferation Odontoblastic differentiation	Combined with PDGF, increased cell proliferation
NGF	Dental papilla cells	Odontoblastic differentiation	
Cytokine			
SDF-1	Dental pulp cells	Chemotaxis Cell proliferation	

Table 3: *Morphological factors.*

Clinical considerations for regenerative endodontic procedures

Two case reports can be credited with sparking recent interest in regenerative endodontics. The first, which was by Iwaya., *et al*. [22] showed that sodium hypochlorite and hydrogen peroxide disinfection of necrotic tissue in an immature premolar followed by canal medication with metronidazole and ciprofloxacin resulted in continued root formation and clinical evidence of renervation. The second, by Banchs and Trope [23] showed a similar outcome of continued root development and renervation after canal disinfection with sodium hypochlorite and chlorhexidine and the placement of triple antibiotic paste (TAP; i.e., ciprofloxacin, metronidazole, and minocycline). Both of these case reports supported 3 important principles of regenerative endodontic procedures:

1. Bacterial elimination from the canal system.
2. Scaffold creation for the in growth of new tissue.
3. Creation of a bacteria-tight seal to prevent reinfection.

Following the case reports in the early 2000's, [23,24] there have been several case studies showing the successful regeneration of tissue in the necrotic canal space of permanent teeth with immature apices. A summary of these case studies is shown in Table 4.

Study	Sample (*n*)	Instrumentation	Irrigation	Intracanal medicament	Duration of medicament	Final irrigation	Evoked blood clot	PRP/PRF	Collagen sponge
Iwaya et al, 2001 (9)	Case report (1)	No	5% NaOCl+ 3% H_2O_2	Antibiotics: Metronidazole Ciprofloxacin	5 weeks	Not clearly reported	No	No	No
Banchs & Trope, 2004 (2)	Case report (1)	No	5.25% NaOCl+ 0.12% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	26 days	5.25% NaOCl	Yes	No	No
Cheung & Huang, 2006 (11)	Case series (4)	No	2.5% NaOCl	$Ca(OH)_2$	5 weeks–3 months	2.5% NaOCl	No	No	No
Thibodeau & Trope, 2007 (12)	Case report (1)	No	1.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Cefaclor	11 weeks	1.25% NaOCl	Yes	No	No
Petrino, 2007 (13)	Case report (1)	No	5.25% NaOCl+ 0.12% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	Not clearly reported	5.25% NaOCl	Yes	No	No
Jung et al, 2008 (14)	Case series (9)	No	2.5% NaOCl (*n* = 4)	Antibiotics: Metronidazole Ciprofloxacin Minocycline (n = 6)	7–67 days	2.5% NaOCl (*n* = 4)	Yes (*n* = 4)	No	No (*n* = 8)
				3mix antibiotics+ $Ca(OH)_2$ (n = 2)	70–88 days				
			5.25% NaOCl (*n* = 5)	$Ca(OH)_2$ (*n* = 1)	1 week	5.25% NaOCl (*n* = 5)	No (*n* = 5)		Collatape (Zimmer Dental, Carlsbad, CA) (*n* = 1)
Shah et al, 2008 (15)	Case series (14)	Minimal	3% H_2O_2 +2.5% NaOCl	Formocresol	Not clearly reported	Not clearly reported	Yes	No	No
Cotti et al, 2008 (16)	Case report (1)	Minimal	3% H_2O_2+ 5.25% NaOCl	$Ca(OH)_2$	2 weeks	5.25% NaOCl	Yes	No	No
Reynolds et al, 2009 (17)	Case report (2)	No	6% NaOCl + 2.0% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	6% NaOCl	Yes	No	No
Shin et al, 2009 (18)	Case report (1)	No	6% NaOCl+ 2.0% CHX	1-step treatment	—	6% NaOCl +2.0% CHX	No	No	No
Thibodeau, 2009 (19)	Case report (1)	No	1.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Cefaclor	11 weeks	1.25% NaOCl	Yes	No	No
Ding et al, 2009 (7)	Case series (3)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	1 week	5.25% NaOCl	Yes	No	No
Chueh et al, 2009 (20)	Case series (23)	No	2.5% NaOCl	$Ca(OH)_2$	1–25 months	Not reported	No	No	No
Thomson & Kahler, 2010 (21)	Case report (1)	No	1% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Amoxicillin	6 weeks	1% NaOCl	Yes	No	No
Kim et al, 2010 (22)	Case report (1)	No	3% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	6 weeks	3% NaOCl	Yes	No	No

Table 4a

Study	Sample (n)	Instrumentation	Irrigation	Intracanal medicament	Duration of medicament	Final irrigation	Evoked blood clot	PRP/PRF	Collagen sponge	Intra cor bar
Petrino et al, 2010 (23)	Case series (6)	No	5.25% NaOCl+ 0.12% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	2–5 weeks	5.25% NaOCl	Yes	No	CollaPlug (Zimmer Dental) (n = 4)	M
Iwaya et al, 2011 (24)	Case report (1)	No	5% NaOCl + 3% H_2O_2	$Ca(OH)_2$	5 weeks	Not clearly reported	No	No	No	Ca(
Cehreli et al, 2011 (25)	Case series(6)	No	2.5% NaOCl	$Ca(OH)_2$	3 weeks	2.5% NaOCl+ sterile saline	Yes	No	No	M
Jung et al, 2011 (26)	Case report (1)	No	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	2 weeks	2.5% NaOCl	Yes	No	No	M
Torabinejad & Turman, 2011 (27)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	22 days	Sterile saline	No	PRP	No	M
Nosrat et al, 2011 (28)	Case report (2)	Minimal	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	3 weeks	5.25% NaOCl	Yes	No	No	CEM c
Chen et al, 2012 (29)	Case series (20)	Minimal	5.25% NaOCl	$Ca(OH)_2$	Not clearly reported	5.25% NaOCl	Yes	No	No	MT
Jadhav et al, 2012 (30)	Case series (20)	Minimal	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	Not clearly reported	Not clearly reported	Yes	No (n = 10) PRP (n = 10)	No (n = 10) Collagen Sponge (n = 10)	Glass ic cem
Dabbagh et al, 2012 (31)	Case series (18)	No	5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	2–6 weeks	5% NaOCl+ Sterile saline	Yes	No	No	MT
Nosrat et al, 2012 (32)	Case report (2)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	5.25% NaOCl	No (efforts for intracanal bleeding)	No	No	MT
Shimizu et al, 2012 (33)	Case report (1)	Minimal	5.25% NaOCl	$Ca(OH)_2$	2 weeks	Not clearly reported	Yes	No	No	MT
Lenzi & Trope, 2012 (34)	Case report (2)	No	2.5% NaOCl +Solution of antibiotics	Antibiotics: Metronidazole Ciprofloxacin Minocycline	35 days	Sterile saline	Yes	No	No	MT
Torabinejad & Faras, 2012 (35)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	22 days	Sterile saline	No	PRP	No	MT
Aggarwal et al, 2012 (36)	Case report (1)	Minimal	5.25% NaOCl+ 2% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	9 weeks	5.25% NaOCl	Yes	No	No	MT
Shivashankar et al, 2012 (37)	Case report (1)	No	5.25% NaOCl+ 0.2% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	21 days	Sterile saline	No	PRF	No	MT
Kim et al, 2012 (38)	Case report (3)	No	3% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Cefaclor	1–2 weeks	3% NaOCl+ Sterile saline	Yes	No	No	MT

Table 4b

Miller et al, 2012 (39)	Case report (1)	No	2.0% CHX+ 17% EDTA	Antibiotics: Metronidazole Ciprofloxacin Minocycline	6 weeks	2.0% CHX+ 17% EDTA	Yes	No	No	MTA
Gelman & Park, 2012 (40)	Case report (1)	No	6% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	35 days	6% NaOCl	Yes	No	No	MTA
Jeeruphan et al, 2012 (4)	Retro-spective cohort (20)	No	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	29 days	2.5% NaOCl	Yes	No	CollaPlug	MTA
Narayana et al, 2012 (41)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	2 weeks	5.25% NaOCl	Yes	No	No	MTA
McTigue et al, 2013 (42)	Case series (39)	Minimal	3% NaOCl or 0.12% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline (n = 10) Antibiotics: Metronidazole Ciprofloxacin Clindamycin (n = 29)	3–4 weeks	3% NaOCl	Yes	No	No	MTA
Paryani & Kim, 2013 (43)	Case report (2)	Complete	5.25% NaOCl	$Ca(OH)_2$	1 week	5.25% NaOCl+ 17% EDTA	Yes	No	Collacote (Zimmer Dental)	MTA
			5.25% NaOCl+ 17% EDTA	Antibiotics: Ciprofloxacin	22 days	5.25% NaOCl+ 17% EDTA	Yes	No	Collacote	
Nosrat et al, 2013 (44)	Case report (1)	Minimal	2.5% NaOCl	$Ca(OH)_2$+ Antibiotics: Amoxicillin	54 days	2.5% NaOCl	Yes	No	Collacote	MTA
Martin et al, 2013 (45)	Case report (1)	Complete	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	5 months (next session had been scheduled after 4 weeks)	5.25% NaOCl	Yes	PRP	No	MTA
Soares et al, 2013 (46)	Case report (1)	Minimal	2.0% CHX	$Ca(OH)_2$ (mixed with 2% CHX gel)	21 days	17% EDTA+ Sterile saline	Yes	No	No	MTA
Shimizu et al, 2013 (47)	Case report (1)	No	2.6% NaOCl	$Ca(OH)_2$	11 days	2.6% NaOCl	Yes	No	No	MTA
Keswani & Pandey, 2013 (48)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	3 weeks	Sterile saline	No	PRF	No	MTA
Cehreli et al, 2013 (49)	Case report (2)	No	2.5% NaOCl	$Ca(OH)_2$	3 weeks	2.5% NaOCl+ Sterile saline	Yes	No	No	MTA
Mishra et al, 2013 (50)	Case report (1)	Minimal	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	Sterile saline	No	PRF	No	MTA
Forghani et al, 2013 (51)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	3 weeks	5.25% NaOCl + sterile saline	Yes	No	No	MTA
Sonmez et al, 2013 (52)	Case report (3)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	2 weeks	5.25% NaOCl	Yes	No	No	MTA
Kottoor et al, 2013 (53)	Case report (1)	Minimal	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	3 weeks	5.25% NaOCl	Yes	No	No	MTA

(Continued)

Table 4c

Study	Sample (*n*)	Instrumentation	Irrigation	Intracanal medicament	Duration of medicament	Final irrigation	Evoked blood clot	PRP/PRF	Collagen sponge	Intracanal coronal barrier
Yang et al, 2013 (54)	Case report (1)	No	5.25% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	2.5% NaOCl+ Sterile saline	No (efforts for intracanal bleeding)	No	No	Glass ionomer cement
Noy et al, 2013 (55)	Case report (1)	No	2.5% NaOCl + 2.0% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	21 days	2.5% NaOCl+ Sterile saline	Yes	No	No	MTA
Chen et al, 2013 (56)	Case report (1)	No	3% NaOCl+ CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	3% NaOCl+ Sterile saline	Yes	No	No	MTA
Hargreaves et al, 2013 (6)	Case report (1)	Minimal	1.5% NaOCl+ 17% EDTA	$Ca(OH)_2$+ Antibiotics: Metronidazole Ciprofloxacin	44 days	17% EDTA	Yes	No	Collatape	MTA
Jadhav et al, 2013 (57)	Case series (6)	Minimal	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	4-8 weeks	Not clearly reported	Yes	PRP (*n* = 3) No (*n* = 3)	No	Glass ionomer cement
Bezgin et al, 2014 (58)	Case report (2)	No	2.5% NaOCl+ 0.12% CHX	Antibiotics: Metronidazole Ciprofloxacin Cefaclor	3 weeks	EDTA+ Sterile saline	No	PRP	No	MTA
Becerra et al, 2014 (59)	Case report (1)	No	5.25% NaOCl+ 2.0% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline	26 days	Sterile saline+ 5.25% NaOCl	Yes	No	No	MTA
Lin et al, 2014 (60)	Case report (1)	No	5.25% NaOCl	$Ca(OH)_2$+ Antibiotics: Metronidazole Ciprofloxacin Minocycline	4 weeks	5.25% NaOCl	Yes	No	No	MTA
Nagy et al, 2014 (61)	Prospective study (24)	Minimal	2.6% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Doxycycline	3 weeks	2.6% NaOCl+ Sterile saline	Yes	No	No (*n* = 12) Gelatin with bFGF (*n* = 12)	MTA
Kahler et al, 2014 (62)	Case series (16)	No	1 % NaOCl	Antibiotics: Metronidazole Ciprofloxacin Amoxicillin	4 weeks	1 % NaOCl	Yes	No	No	MTA
Jadhav et al, 2014 (63)	Case report (1)	Minimal	2.5% NaOCl	Antibiotics: Metronidazole Ciprofloxacin Minocycline	3 weeks	17% EDTA	Yes	PRP	No	Glass-ionomer cement
Amit et al, 2014 (64)	Case report (1)	Minimal	5.25% NaOCl+ 17% EDTA	Antibiotics: Metronidazole Ciprofloxacin Cefaclor	3 weeks	Sterile saline	Yes	No	No	MTA
Nagata et al, 2014 (65)	Prospective study (23)	No	6% NaOCl+ 2.0% CHX	Antibiotics: Metronidazole Ciprofloxacin Minocycline (*n* = 12) $Ca(OH)_2$ (mixed with 2% CHX gel) (*n* = 11)	21 days	17% EDTA+ Sterile saline	Yes	No	Collacote	MTA

bFGF, basic fibroblast growth factor; CEM, calcium-enriched mixture; PRF, platelet-rich fibrin; PRP, platelet-rich plasma.

Table 4d

Regenerative Endodontics: Regeneration or Repair

Regenerative endodontics should be considered as 2 entities. One is dentin-pulp complex regeneration which relates to preservation of pulp vitality and pulp capping. The second is dental pulp regeneration that relates to regeneration of a vital tissue into an empty but infected root canal space.

Remodeling of dentin does not occur. Histologically, tertiary dentin can appear to be similar to secondary dentin; however, it is never truly the same and does not form a continuum with preexisting dentin. The method currently used to determine the origin of the tissue secreted by the processes of repair/regeneration is generally based on cellular markers (usually proteins). Whereas the synthesis of dentin-like tissue by odontoblast-like cells can be considered as a regenerative process, an ectopic biomineralization process would be considered more as a reparative one. The in vitro and in vivo experiments are usually aimed at investigating a reparative process rather than a true process of regeneration (progenitor migration/recruitment/differentiation). The histologic analysis of teeth treated by simple revascularization [24] or by filling with platelet-rich plasma [25] shows that a mineralized layer was deposited on the radicular walls. This newly formed tissue appeared to be of periodontal origin rather than pulpal origin implying that the progenitor cells migrating from the apical papilla or from the surrounding periradicular tissues would have differentiated into cells of periodontal origin. Radiographic analysis of these cases may have been deceptive and may have led us to think that the mineralized tissue was dentin. Current considerations for regenerative endodontic procedures are summarized in Table 5.

Current considerations for REPs

Case selection:

- Tooth with necrotic pulp and an immature apex
- Pulp space not needed for post/core, final restoration
- Compliant patient

Informed consent:

- Two (or more) appointments
- Use of antimicrobial(s)
- Possible adverse effects: staining of crown/root, lack of response to treatment, pain/infection
- Alternatives: MTA apexification, no treatment, extraction (when deemed nonsalvageable)
- Permission to enter information into AAE database (optional)

First appointment:

- Local anesthesia, rubber dam isolation, access
- Copious, gentle irrigation with 20 mL NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (eg, needle with closed end and side vents, or EndoVac). To minimize potential precipitate in the canal, use sterile water or saline between NaOCl; lower concentrations of NaOCl are advised, to minimize cytotoxicity to stem cells in the apical tissues.
- Dry canals
- Place antibiotic paste or calcium hydroxide. If the triple antibiotic paste is used: (1) consider sealing pulp chamber with a dentin bonding agent to minimize risk of staining, and (2) mix 1:1:1 ciprofloxacin/metronidazole/minocycline (or, if esthetics are crucial, then consider a 1:1 mixture of ciprofloxacin/metronidazole).
- Deliver into canal system via lentulo spiral, MAP system, or Centrix syringe
- If triple antibiotic paste is used, ensure that it remains below the CEJ (to minimize crown staining)
- Seal with 3 to 4 mm of Cavit, followed by immediate restorative material, glass ionomer cement, or another temporary material
- Dismiss patient for 3 to 4 weeks

Second appointment:

- Assess response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment time with antimicrobial, or alternative antimicrobial.
- Anesthesia with 3% mepivacaine without vasoconstrictor, rubber dam, isolation
- Copious, gentle irrigation with 20 mL of ethylenediamine tetraacetic acid, followed by normal saline, using a similar closed-end needle
- Dry with paper points
- Create bleeding into canal system by overinstrumenting (endo file, endo explorer)
- Stop bleeding 3 mm from CEJ
- Place CollaPlug/CollaCote at the orifice, if necessary
- Place 3 to 4 mm of white MTA and reinforced glass ionomer and place permanent restoration

Follow-up:

Clinical and radiographic examination:

- No pain or soft tissue swelling (often observed between first and second appointments)
- Resolution of apical radiolucency (often observed 6–12 months after treatment)
- Increased width of root walls (this is generally observed before apparent increase in root length and often occurs 12–24 months after treatment)
- Increased root length

Data from Available at: www.aae.org/Dental_Professionals/Considerations_for_Regenerative_Procedures.aspx.

Table 5: *Current Considerations for Regenerative Endodontic Procedures.*

Various outcomes of regenerative procedures are summarized in Table 6.

TABLE 4. Summary of Radiographic Outcomes of Current Published Clinical Studies

Study	Study design/sample (n)	Patients' age (y)	Diagnosis	Radiolucency resolution	Apical closure	Root lengthening	Root thickening	Recall time
Iwaya et al 2001 (11)	Case report (1)	13	PN + PP	1/1	1/1	Not clearly reported	1/1	30 mo
Banchs and Trope 2004 (21)	Case report (1)	11	PN + PP	1/1	1/1	1/1	1/1	24 mo
Cheung and Huang 2006 (22)	Case reports (4)	9–10	PN + PP	4/4	4/4	(1/1) In the other cases, not clearly reported	4/4	7–60 mo
Thibodeau and Trope 2007 (23)	Case report (1)	9	PN + PP	1/1	1/1	Not clearly reported	1/1	12.5 mo
Petrino 2007 (24)	Case report (1)	8	PN + PP	1/1	1/1	Not clearly reported	1/1	8 mo
Jung et al 2008 (25)	Case series (9)	9–14	PN + PP	9/9	8/9	(2/3) In the other cases, not clearly reported	8/9	10–60 mo
Shah et al 2008 (26)	Case series (14)	8–17	PN + PP	13/14	Not clearly reported	10/14	8/14	6–42 mo
Cotti et al 2008 (27)	Case report (1)	9	PN + PP	1/1	1/1	Not clearly reported	1/1	30 mo
Reynolds et al 2009 (28)	Case report (2)	11	PN + PP	2/2	2/2	Not clearly reported	2/2	18 mo
Bose et al 2009* (29)	Retrospective cohort (48)	Not clearly reported	PN + PP	Not clearly reported	Not clearly reported	% Root wall lengthening, 14.72	% Root wall thickening, 25.1	>6 mo
Ding et al 2009 (6)	Case report (3)	8–9.5	PN + PP	3/3	3/3	Not clearly reported	3/3	15–18 mo
Chueh et al 2009 (30)	Case series (23)	7–14	PN + PP	23/23	21/23	Not clearly reported	Not clearly reported	6–108 mo
Shin et al 2009 (31)	Case report (1)	12	PN + PP	1/1	1/1	1/1	1/1	19 mo
Thibodeau 2009 (32)	Case report (1)	9	PN + PP	1/1	1/1	1/1	1/1	41 mo
Thomson and Kahler 2010 (33)	Case report (1)	12	PN + PP	1/1	1/1	Not clearly reported	1/1	18 mo
Petrino et al 2010 (34)	Case series (6)	6–13	PN + PP	6/6	0/6	3/6	4/6	9–12 mo
Kim et al 2010 (35)	Case report (1)	7	PN + PP	1/1	1/1	1/1	1/1	8 mo
Cehreli et al 2011 (36)	Case series (6)	8–11	PN + PP	6/6	6/6	6/6 % Root wall lengthening, 7.71	6/6 % Root wall thickening, 26.50	9–10 mo
Iwaya et al 2011 (37)	Case report (1)	7	PN + PP	1/1	1/1	Not clearly reported	1/1	13 y
Jung et al (2011) (38)	Case report (1)	8	PN + PP	1/1	1/1	0/1	0/1	31 mo
Torabinejad and Turman 2011 (7)	Case report (1)	11	PN + PP	1/1	1/1	1/1	1/1	6 mo
Nosrat et al 2011 (39)	Case report (2)	8–9	PN + PP	2/2	2/2	1/2	2/2	15–18 mo
Chen et al 2012 (40)	Case series (20)	7–13	PN + PP	20/20	Not clearly reported	15/20	20/20	6–26 mo
Jadhav et al 2012 (41)	Case series (20)	15–28	PN + PP	20/20 5/20 excellent 11/20 good 4/20 satisfactory	20/20 9/20 excellent 6/20 good 5/20 satisfactory	20/20 4/20 excellent 11/20 good 5/20 satisfactory	20/20 3/20 excellent 8/20 good 9/20 satisfactory	12 mo
Jeeruphan et al 2012* (42)	Retrospective cohort (20)	8–24	PN + PP	20/20 16/20 healed 4/20 healing	Not clearly reported	19/20 % Root wall lengthening, 14.9	20/20 % Root wall thickening, 28.2	>6 mo
Nosrat et al 2012 (43)	Case report (2)	14	PN + PP	2/2	2/2	0/2	0/2	6 y
Lenzi and Trope 2012 (44)	Case report (2)	8	PN + PP	2/2	1/2	0/2	1/2	21 mo
Torabinejad and Faras 2012 (45)	Case report (1)	12	PN + PP	1/1	1/1	1/1	1/1	14 mo
Aggarwal et al 2012 (46)	Case report (1)	24	PN + PP	1/1	1/1	0/1	1/1	24 mo
Shivashankar et al 2012 (47)	Case report (1)	9	PN + PP	1/1	1/1	1/1	1/1	12 mo
Kim et al 2012 (48)	Case report (3)	10–12	PN + PP	3/3	3/3	(1/1) In other cases, not clearly reported	3/3	24–48 mo
Miller et al 2012 (49)	Case report (1)	9	IRP + PP	1/1	1/1	1/1	1/1	18 mo
Gelman and Park 2012 (50)	Case report (1)	8	PN + PP	1/1	1/1	1/1	1/1	11 mo
Narayana et al 2012 (51)	Case report (1)	11	PN + PP	1/1	0/1	0/1	0/1	12 mo
McTigue et al 2013 (52)	Case series (32)	6–17	PN + PP	31/32	23/32	21/32	22/32	7–51 mo
Paryani and Kim 2013[†] (53)	Case report (2)	11–14	PN + PP	2/2	Complete root formation	Complete root formation	Not clearly reported	18–22 mo
Nosrat et al 2013 (54)	Case report (1)	8	PN + PP	1/1	1/1	0/1	0/1	31 mo
Martin et al 2013 (55)	Case report (1)	9	PN + PP	1/1	1/1	Almost complete root formation	1/1	25 mo
Soares et al 2013 (56)	Case report (1)	9	PN	1/1	1/1	1/1	1/1	24 mo
Shimizu et al 2013 (57)	Case report (1)	9	PN + PP	1/1	1/1	Not clearly reported	1/1	26 mo
Keswani and Pandey 2013 (58)	Case report (1)	7	PN + PP	1/1	1/1	1/1	1/1	15 mo
Cehreli et al 2013 (59)	Case report (2)	8.5	PN + PP	2/2	2/2	2/2 % Root wall lengthening, 17.65	2/2 % Root wall thickening, 58.09	18 mo
Mishra et al 2013 (60)	Case report (1)	11	PN + PP	1/1	1/1	Not clearly reported	1/1	12 mo
Forghani et al 2013 (61)	Case report (1)	9	PN + PP	1/1	1/1	1/1	1/1	18 mo
Sönmez et al 2013 (62)	Case report (3)	9	PN + PP	3/3	(1/1) In other cases, not clearly reported	Not clearly reported	(1/1) In other cases, not clearly reported	24 mo
Kottoor et al 2013 (63)	Case report (1)	11	PN + PP	1/1	1/1	1/1	1/1	5 y
Yang et al 2013 (64)	Case report (1)	11	PN + PP	1/1	1/1	Not clearly reported	1/1	24 mo
Noy et al 2013 (65)	Case report (1)	12.5	PN + PP	1/1	0/1	0/1	0/1	48 mo
Chen et al 2013 (66)	Case report (1)	8	PN + PP	1/1	1/1	1/1	1/1	12 mo
Hargreaves et al 2013 (67)	Case report (1)	9	PN + PP	1/1	1/1	1/1	1/1	12 mo
Bezgin et al 2014 (8)	Case report (2)	12	PN/PN + PP	2/2	2/2	Not clearly reported	2/2	12 mo

(Continued)

IRP, irreversible pulpitis; PN, pulp necrosis; PP, periapical pathology.
The studies are presented in chronological order.
*The clinical study was also presented in Table 3.
[†]The regenerative procedures were executed in permanent teeth with closed apex.

Table 6: *Outcomes of Regenerative Endodontic Procedures.*

Drawbacks and Unfavorable Outcomes

Discoloration: Discoloration of the tooth after regenerative endodontic treatments is a problem mostly related to the use of minocycline in the triple antibiotic paste. The main reason for tooth discoloration after treatment was the contact of minocycline in the triple antibiotic paste with coronal dentinal walls during treatment procedure. A recent study suggested sealing dentinal walls of the access cavity by using dentin bonding agent and composite resin before placement of triple antibiotic paste inside the canal.

Treatment Period: The required time for disinfection of the root canal space with triple antibiotic paste or calcium hydroxide and increased number of clinical sessions (compared with one-visit mineral trioxide aggregate apical barrier technique) are other drawbacks of regenerative endodontic treatment.

Challenging Histologic Outcomes of Animal Studies: Histologic and immmunohistologic studies have been performed on the outcome of regenerative endodontic treatments in dogs' teeth [26]. Thibodeau., *et al*. demonstrated histologic evidence of hard-tissue deposition on the root canal walls (43.9%), apical closure (54.9%), and formation of vital tissue in root canal space (29.3%) in a dog model. Histologic findings of animal studies showed that the tissue formed inside the canal was not pulp. However, the histologic outcomes of treatment of necrotic immature teeth might differ in humans from that of dogs' teeth.

Poor Root Development: Ideal regenerative procedure should result in increased root length, increased root wall thickness, and formation of the root apex. In some studies, the outcome of regenerative endodontic treatments of necrotic immature teeth was lower than ideal, including absence of increase in root length, absence of increase in root wall thickness [27], or lack of formation of tooth apex. To test the overall benefit of pulp regeneration, outcome criteria need to go beyond the mere radiographic demonstration of hard tissue deposition.

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