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Revascularization: a review of clinical reports on a contemporary treatment modality for endodontics.

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Abstract

Objectives: The purpose of this review is to provide a general update on clinical studies of revascularization which is frequently pronounced in recent endodontic literature.
Materials and methods: A PubMED search was carried out to retrieve articles pertaining to clinical attempts on revascularization. Articles extending until 2014 were retrieved. Filtering was performed to make necessary exclusions and an overall number of 62 articles were included.
Results: Though a search of the available literature shows that there are variations among different authors in terms of treatment strategies in revascularization, the methodology appears to be a reliable and predictable treatment option.
Conclusion: The establishment of a well-standardized protocol will render revascularization to be more strongly accepted as a universal treatment modality.
Clinical relevance: Revascularization is a conservative methodology whereby proliferation of a new connective tissue into a previously infected and necrotic pulp space is expected. In case a correct strategy is followed, continuation of root development with concurrent thickening of root canal walls will provide many advantages both for the patient and the clinician. With more standardized treatment protocols awaited for the future, the methodology will be better established in routine clinical practice.

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Introduction

New developments on stem cell biology and regenerative endodontics in an attempt to highlight the regenerative mechanisms of pulp and surrounding tissues introduced researchers with the pulp tissue revitalization/revascularization concept which is frequently pronounced in recent endodontic literature. The keystone clinical reports by Iwaya et al. [1] and Banchs and Trope [2] and various case reports and clinical trials attempted thereafter rendered revascularization to be better implemented in procedures where preservation of tooth vitality as well as maintenance of physiological root development are the major targets. A review of the literature reveals that there is emerging body of cases supporting this conservative approach; therefore the aim of this review article was to make a general analysis of prominent clinical investigations performed on revascularization procedures which have gained specific attention in recent years. A PubMED, OVID and MedLINE search was conducted by inserting keywords "revascularization and endodontics" or "revascularization, endodontics and clinical cases" and after an initial filtration, a total number of 125 articles were retrieved. Another filtering was made and clinical case reports in English related with regenerative endodontic therapy which involved a well explanation of the methodology were included whereas animal, *in vitro* and *ex-vivo* studies and non-English articles were excluded. Some review articles and histological studies were used as references to make a clearer explanation of the topic, where necessary. An overall of 62 articles were included, 42 of which were case reports. Table 1 gives an overview of the prominent clinical reports on the topic of revascularization between years 2001 and 2014.

The history of regeneration of pulp-dentin complex dates back to 1961 with the first description of the topic by Ostby [3] followed by the documentation of root development and apical barrier formation in pulpal necrosis cases in children by Rule and Winter [4]. Nygaard-Ostby and Hjortdal) [5] performed studies that can be considered as the forerunner of pulpal regeneration. The clinical reports by Iwaya et al. [1] and Banchs and Trope [2] served as the first attempts that gave a start to a variety of clinical cases leading to a considerable accumulation of evidence. The case by Iwaya et al. [1] belonged to a 13 year old-girl with a history of swelling of the right mandibular buccal vestibule. Instead of standard root canal treatment protocol, administration of intracanal medicaments (metronidazole and ciprofloxacine) was selected, root canal was left empty and coronal seal was made. Follow-up period revealed thickening of the root canal wall and complete apical closure. Banchs and Trope [2] were the first to present a new technique to revascularize immature permanent teeth with apical periodontitis. This technique would serve as a reference for many clinical investigations and cases performed thereafter and were specifically derived by resembling circumstances where regeneration took place after the avulsion of a permanent immature tooth. Under such conditions, new tissue would likely proliferate into the pulp space through the wide apex, replacing the necrotic portion. Considering that soaking an avulsed tooth with doxycycline or minocycline prior to replantation would enhance the success of the procedure by eradication of bacteria on the root surface and apical foramen, they attempted to create a similar environment for the regeneration of pulp tissue in a necrotic infected tooth. The case they described belonged to an 11 year old boy whose mandibular right second premolar with an immature apex was associated with a lingual sinus tract formation due to the fracture of one cusp, leading to micro exposure and necrosis of the pulp. The method described by the authors was specifically based on the application of a tri-antibiotic paste containing ciprofloxacin, metronidazole, and minocycline as described by Hoshino et al. [6] to a depth of approximately 8 mm. into the canal following debridement without mechanical instrumentation. In the 26-day recall of the patient when the tooth was asymptomatic and there was no trace of sinus tract formation, an explorer was used to irritate the tissue gently to create some bleeding into the canal following which a mineral trioxide aggregate paste was placed on the blood clot formed 3 mm apical of the CEJ. The large radiolucency disappeared within 2 months, and at the 24- month recall, the root walls were thick and the development of the root apical to the restoration was similar to that of the adjacent and contralateral teeth. An interesting finding of the authors would be the positive response of the tooth to vitality testing. This methodology would serve as a reference for a variety of revascularization cases performed later in spite of some modifications preferred by different authors.

Table 1. *An overview of prominent clinical studies on revascularization.*

Study type	Author	Age and gender	Irrigation	Intracanal medication	Duration of medicament	Pulp space	Intracanal coronal barrier and restoration	Follow-up period	Results
Individual (One or few case reports)	Iwaya et al.	13-year-old female	5% NaOCl 3% H_2O_2	Metronidazole ciprofloxacin	6 weeks	Empty	$Ca(OH)_2$ Glass ionomer cement, adhesive composite resin	5-month, 15month, 30-month	30-month: Complete closure of the apex and thickening of the root wall.
	Banchs and Trope	11-year-old male	20 ml of 5.25% NaOCl 10 ml of Peridex.	TPA	26 days	Blood clot	MTA, bonded resin restoration	6-month, 1-year, 18month, 2-year	2-year: Complete root development. Closure of the apex and thickening of the dentinal walls. Positive response to the cold test.
	Chueh and Huang (n=4)	10-year old Female (n=2)	20 ml of 2.5% NaOCl	$Ca(OH)_2$	5 weeks-3 months	Empty	$Ca(OH)_2$ Caviton, IRM, Amalgam	7-month, 11-month, 20-month, 35-month	35/36-month: A marked reduction of the root canal space and maturation of the root apex (n=3)
	Cotti et al.	9-year old female	5.25% NaOCl	$Ca(OH)_2$	1 week	Blood clot	MTA, GIC, Adhesive composite resin	3, 6, 12, 18, 24, and 30 months	After 8 months: Progressive development of the root, formation of the coronal barrier, narrowing of the apical area,and the apical closure.
	Gelman and Park	8-year-old male	6% NAOCl	TPA	35-days	Blood clot	MTA, cavity liner, TPH composite	3-month, 6-month, 11-month	11-month: Grayish discoloration, Complete root development and dentinal wall thickening, particularly in the root's apical third.
	Miller et al.	9 year old male	2% CHX 17% EDTA	TPA	6 weeks	Blood Clot	MTA+resin ionomer	1-18 month	18-month: All teeth responded positively to CO2. Root development and apical closure was observed.
	Kottoor et al.	11-year-old male	20 mL of 5.25% NaOCl for 20 min	TPA	3-weeks	Blood clot	white MTA, glass ionomer cement, composite resin	3-month, 1-year, 3-year, 5-year	3 and 5- year: Apical closure and dentinal wall thickening was evident along with attainment of root length.
	Forghani et al.	11 year old female	20 mL of 5.25% NaOCl for 20 min	TPA	3 weeks	Blood Clot	MTA, composite	6, 12, 18 months	Both teeth showed increased root lengths, root wall thickness and apical closure. Left central incisor showed greater improvements.

	Lin et al.	6-year-old boy	2% CHX, 5.25% NaOCl	Calcium hydroxide, TPA	4-weeks	Blood clot	MTA, bonded resin	6-month, 16-month	16-month: No evidence of increased thickening of the canal walls and continued root development. The tooth is extracted.
	Becerra et al.	11 year old female	5.25% NaOCl	TPA	26 days	Blood clot	MTA, IRM-composite resin	1 year-18 months,2 years	18 months and 2 years: Normal periapical condition and narrowing of the root apex. In CBCT scans,,.the lingual portion of the root canal wall was thicker and longer than the buccal one. A calcified structure was present approximately at the center of the canal lumen, just before the wide foramen
Case series (n)	Shah et al. (n=14)	9-18-year old Female (n=5)	3%hydrogen peroxide and 2.5% NaOCl	Formocresol	Undefined	Blood clot	Glass ionomer cement	6-month, 3.5-years (n=14)	6-month, 3.5-years: Excellent periapical healing (n=7) Excellent dentinal wall thickness (n=8) Excellent increase in root length (n=10) Excellent periapical healing, dentinal wall thickness and increase in root length (n=7)
	Ding et al. (n=12)	8-11 years of age. Male (n=5)	20 mL of 5.25% NaOCl	TPA	7 days	Blood clot	Grey MTA	6-months, 15-months (n=3)	15-month: Complete root development with a closed apex. Responded positively to the electric pulp tester
	Petrino et al. (n=6)	6,11,13 year old male (n=2) female (n=2)	20 mL of 5.25% NaOCl, 10 mL of 0.12% CHX	TPA	3-weeks (n=2), 34-days (n=2), 2-weeks (n=2)	Blood clot (n=6)	Collaplug (n=4), MTA and composite (n=6)	6-month (n=2) 1-year (n=4)	1-year: Radiographic examination showed healing of the radiolucent lesions (n=4) Increased thickness of the apical area (n=1). Formation of an apical barrier (n=1) Increase in root wall thickness and length (n=2) The patient had a positive response to vitality testing (n=2)
	Kim et al. (n=3)	10, 12 years old	10 mL of 3% NaOCl for 2 min	Metronidazole ciprofloxacin cefaclor	2-weeks (n=2) 1-week (n=1)	Blood clot	MTA, gutta-percha (Obtura-II), composite resin restoration	6-week (n=1) 2-month (n=2) 24-month (n=1) 42/48-month (n=2)	6-week/2-month: Radiolucency had diminished 24-month: Completely closed root apex, no periapical pathosis, increase in root length and root wall thickness 42/48-month: Increase in root thickness
	Çehreli et al.	8,5 year old male	NaOCl	$Ca(OH)_2$	3 weeks	Blood clot	MTA composite	3 months	Complete periradicular healing was observed after 3 months, followed by progressive thickening of the root walls and apical closure.
	Aggarwal et al.	24 year old female	NaOCl	$Ca(OH)_2$	2 weeks	Blood clot	MTA+CIS +composite	24 months	24 months follow-up shows root elongation and apical closure in the tooth treated with revascularization induced maturation procedures.
	Jung et al. (n=9)	9 -14 years of age	10 mL of 5.25% NaOCl	TPA (n=6), erytromycin +$Ca(OH)_2$+TPA (n=1) $Ca(OH)_2$ +TPA (n=1) $Ca(OH)_2$ (n=1)	60 days (n=2) 11 and 70 days (n=2) 2and3 weeks (n=2) 1-week (n=3)	MTA (n=3) Blood clot (n=5) Empty (n=1)	Gutta-percha filling with obtura (n=2), MTA+ IRM/ caviton+ bonded resin restoration (n=5)	6-month and 5-year (n=2), 10-month (n=1), 6 and 12 and 24 month (n=5),	6-month/5-year: Closure of the apex and thickening of the dentinal walls were obvious (n=2) 10-month: Continued development of the apex 12-24-month: Closure of the apex and thickening of the dentinal walls (n=3). Excellent periapical healing (n=2) Calcification of the canal

							IRM, bonded resin restoration (n=2)	17-month (n=1)	space (n=1). A greatly reduced periradicular radiolucency (n=1) 17-month: Complete resolution of the radiolucency with continued apical closure
	Nagy et al. (n=36)	9-13 years of age	10 mL 2.6% NaOCl	Metronidazole ciprofloxacin, doxycycline	3-weeks	MTA Plug (n=12) REG (n=12) FGF (n=12)	MTA Plug+ thermoplasticize d gutta-percha (n=12) MTA+ adhesive composite resin (n=24)	3, 6, 12, 18-month (n=26)	18-month: The success rates for the MTA, REG, and FGF groups according to the following radiographic assesment criterias,were 100%, 90%, and 80%, respectively: An increase in root length, an increase in root thickness, a decrease in apical diameter, a change in periapical bone density
	Kahler et al. (n=16)	*10 years and 5 months	1% NaOCl	Metronidazole ciprofloxacin, amoxicillin	4-weeks	Blood clot	MTA, glass ionomer cement	18-month	18-month: Incomplete apical closure: 47.2% Complete apical closure: 19.4% (n=16).Change in root length varying in a range from 2.7% to 25.3% and for root dentin thickness from 1.9% to 72.6% (n=9)
	Jeruphan et al. (n=61)	8-24 years of age Female (n=25) Male (n=36)	2.5% NaOCl (n=20)	TPA (n=20)	Aproximately 3-months (n=20), 17± 12.6 months (n=22),	Ca(OH) (n=22) MTA (n=19) REG (n=20)	Glass-ionomer and resin composite (n=19), Gutta-percha (n=22)	6-months	6-month: The root width percentage in the revascularization group (28.2%), MTA (0.0%) and calcium hydroxide apexification groups (1.5%). The percentage increase of root length in the revascularization group (14.9%), MTA (6.1%) and calcium hydroxide apexification groups (0.4%).
	Alobaid et al. (n=31)	6-16 years, male (n=17)	NaOCl, CHX, and/or EDTA	TPA, DA, and/or $Ca[OH]_2$	Not clear	REG (n=not defined) $Ca(OH)_2$ (n=7) MTA (n=5)	MTA and resin-bondedrestoratio n (n=19), gutta-percha/AH Plus sealer, resin-bonded restoration (n=7), thermoplasticize d gutta-percha and AH Plus Sealer (n=5)	#REG:15.4±9 months (n=8), Apexification groups: 15.5±10.4 months (n=15)	Revascularization treatment has comparable, but not superior, clinical and radiographic outcomes with traditional apexification-$Ca(OH)_2$andMTA apexification procedures.
	Nagata et al. (n=23)	7-17 years old	20 mL 6% NaOCl, and 10 mL 2% CHX	TAP (n=12) Ca(OH)2and 2% chlorhexidine gel-CHP (n=11)	21 days	Blood clot	CollaCote fibers, white MTA, coltosol, composite resin	1, 3, 6, 9, 12, 15, and 19 months	9-19-months: Apical closure was significantly observed in both groups. Increase in root length was demonstrated in 5 teeth (41.7%) and 3 teeth (27.3%) of groups TAP and CHP, respectively. Thickening of lateral dentinal walls was observed in 5 teeth of each group.
	McTigue et al. (n=39)	6-17 years old	3% NaOCl or 0.12% CHX.	TPA (n=10), Ciprofloxacin, metronidazole , clindamycin (n=29)	3-4 weeks	Blood clot	Grey and white MTA	1-4 year (n=29), Up to 1-year (n=3)	Apical healing (n=31), closure of the root apex (n=23), the root walls thickened (n=22), and root length increased (n=21).
	Saoud et al. (n=20)	Not defined	2.5% NaOCl	TPA	2-weeks	Blood clot	MTA, light-cured composite resin	1, 3, 6, 9, and 12 months	55% (11/20) showing complete apical closure. The within-case percent change in root length averaged less than 1% at 3 months and increased to 5% at 12 months. The within-case percent change in root thickness averaged 3% at 3 months and 21% at 12 months.
Single visit revascul	Shin et al.	12-year old female	3 ml 0.12% CHX	Nothing	6 weeks	Empty	MTA, GP Calamus Composite	2, 6,-weeks, 7, 13 and 19 months	13- and 19-months: The diameter of the open apex had decreased and thickening of the radicular walls were evident.The

arization									radiographs demonstrated evidence of complete periradicular bone healing and root maturation. Complete resolution of condensing osteitis.
Alternative disinfectants	Soares et al.	9-year-old female	2%CHX gel,	Ca(OH)2 and 2% CHX gel	21 days	Blood clot	MTA, coltosol, composite resin	1, 3, 6, 9, 12, 15,and 24 months	24-months: Continuing root development, apical closure, and thickness of the root canal space without complete calcification.
PRP supplement	Torabinejad and Turman	11-year-old male	10 mL of 5.25% NaOCl	TPA	22 days	PRP	Grey MTA, Cavit and amalgam	5 1/2 half months	5 1/2 half months: Further root development, and continued apical closure. Positive response to cold and an electric pulp test.
	Martin and Ricucci	9-year-old male	10 mL 5.25% NaOCl	TPA	5-months	Blood clot and PRP	MTA, bonded resin restoration	7, 14-months, 2 years 1 month	2 years 1 month: Oblique fracture of the lingual cusps extending to the alveolar crest bone level. The distal canal appeared to be obliterated by the hard tissue, and the mesial canal space was reduced in size.
	Jadhav et al. (n=20)	15-28 years	20 mL 2.5% NaOCl	TPA	Not stated	Blood clot (n=10) Blood clot +PRP (n=10)	Resin-modified glass ionomer cement	6-month, 12-month	12-month: Excellent periapical healing (n=5) Excellent apical closure (n=9) Excellent root lengthening (n=4) Excellent dentinal wall thickening (n=3)
	Jadhav et al. (n=6)	10, 13 and 23-years old (n=3),	20 ml of 2.5% NaOCl	TPA	4 weeks	Blood clot (n=3) Blood clot+ PRP (n=3)	Resin modified glass ionomer cement	6-month, 12-month	12-month: Excellent periapical healing (n=2) Excellent apical closure (n=1) Excellent root lenghtening (n=1)
	Jadhav et al.	40-year-old male	20 mL of 2.5% NaOCl	TPA	3-weeks	Blood clot +PRP	Resin modified glass ionomer cement	6-month, 12-month	6-12-month: Periapical healing, apical closure, root lengthening, and dentinal wall thickening.
	Keswani and Pandey,	7-year-old male	10 mL of 5.25% NaOCl	TPA	3-weeks	PRF clot	White MTA and bonded resin restoration	7-months 12month, 15-month	12- 15-month: Positive response to cold and an electric pulp test.. Continued thickening of root canal walls, root lengthening and apical closure.
Dens invaginatus	Yang et al.	11-year-old male	30 mL of 5.25% NaOCl	TPA	4-weeks	Empty	Fuji IX glass ionomer cement and composite resin	24-month	24-month: Open root apex was closed, and the wall of the root canal was thickened.
	Narayana,	11-year-old male	5 mL of 5.25% NaOCl	TPA	2 weeks	Blood clot	White MTA, glass ionomer cement and light-cured composite resin	5-months, 12months	12-month: Periapical area appeared to have normal trabeculation with appearance of bridge formation. There was no radiographic evidence of an increase in the root canal wall thickness.
	Kumar et al.	10-year-old male	1% NaOCl	Odontopaste (5% clindamycin hydrochloride and 1% triamcinolone acetonide	3-weeks	$Ca(OH)_2$, White MTA and Thermoplasticized gutta-percha with AH26 sealer	Polycarboxylate cement base, glass ionomer cement, resin composite restoration	5-months, 28-months	28-months: Apical barrier with a ‘‘soap-bubble’’ appearance. Apical bone healing.
Discoloration	Thibodeau and Trope,	9-year-old male	1.25% NaOCl	Metronidazol	11 weeks	Blood clot	MTA, acid- etch	3-month,	12.5-month: Continued radicular wall thickening,

				cefaclor			Composite restoration	6-month, 9.5month 12.5month	apical closure, and narrowing of the canal space.
	Reynolds et al.	11-year-old female	20 mL of 6% NaOCl, 10 mL of 2.0% CHX	TPA	4-weeks (n=1), 34-days (n=1)	Blood clot	Grey MTA, resin-bonded composite	18-month	Clinically, both mandibular second premolars responded within normal limits to cold test. Periradicular bone healing and significant root development with maturation of the dentine
	Thibodeau et al.	9-year-old male	1.25% NaOCl	Metronidazole ciprofloxacin, cefaclor	11 weeks	Blood clot	White MTA, bonded composite restoration	3-month, 6-month, 12-month, 16-month	16-month: Further calcification of the canal space due to root wall thickening
	Kim et al.	7-year-old female	3% NaOCl	Metronidazole ciprofloxacin, cefaclor	6-weeks	Blood clot	MTA, resinmodified glass ionomer base, bonded composite resin restoration	8-months	Resolution of the radiolucency, with the evidence of continued apical closure.
Molars	Nosrat et al. (n=2)	9-year-old male, 8-year-old female	5.25% NaOCl for 20 min	TPA	3 weeks	Blood clot	Calcium enriched mixture, glass ionomer, amalgam	3-month, 6-month, 12-month, 18-month	18-months: Periapical radiolucencies were healed. Distal root was fully developed, but mesial root showed only thickening of the root walls
	Çehreli et al. (n=6)	8-11 years of age	10 mL 2.5% NaOCl	Calcium hydroxide	3-weeks	Blood clot	MTA, glass ionomer cement, acid-etch resin composite (n=5), amalgam (n=1)	3-months (n=6) 9-months (n=3), 10-months (n=3)	9/10-months: A visible increase in the root dimensions. All roots showed complete or advanced apical closure. Positive response to cold testing (n=2)
	Sönmez et al. (n=3)	9-year old	10 ml of 5.25% NaOCl	TPA	2 weeks	Blood clot	Grey MTA, composite resin	24-month (n=3)	24 month: Continued thickening of the dentinal walls with apical closure. The radiographs showed complete resolution of periapical radiolucencies

The main reason for attempting this new approach was to enhance root development and strengthening root canal walls which is not the case with the traditional calcium hydroxide methodology in which hard tissue formation occurs in the apical portion only. Meanwhile, calcium hydroxide application carries the risk of weakening the tooth and predisposing it to fracture when used for a prolonged period. Even in the single visit apexification technique by MTA which aims to create apical barrier formation in the short term, root thickening is not expected, resulting in potential susceptibility to fracture. As a result, Banchs and Trope [2] gave a start to a promising methodology where an artificially created blood clot acted as a matrix for the proliferation of a new tissue into the pulp space. Banchs and Trope [2] presumed the origin of the proliferating tissue as some remaining vital tissue and Hertwig's epithelial root sheath in the apical portion despite the presence of an apical lesion. Clinical studies performed after the pioneering case by Banchs and Trope [2] were initially limited to one or few cases; however it appears that as years progressed, researchers attempted to perform more advanced clinical series, prospective and retrospective studies comprising a higher number of patient populations as well as treatments performed on specific cases. Therefore; this review article will be divided into some major headings to make a better classification of the existing literature on the topic. Prior to such a division, it is important to summarize the rationale behind revascularization procedures.

What is expected in a revascularization procedure?

In case revascularization and continued root development is targeted in a necrotic tooth with an immature apex, it is essential that a minimally invasive methodology should be preferred with adequate disinfection to eradicate the microorganisms that might prevent the regenerative process. The rationale of revascularization is that if a sterile tissue matrix is provided in which new cells can grow, pulp vitality can be reestablished. The traditional approach is to create a bleeding into the root canal space in order to allow the formation of a blood-clot that will serve as a scaffold for regenerative process [2,7]. The procedure encompasses the incorporation of copious irrigation, preferably by NaOCL and EDTA and medication by calcium hydroxide or an antibiotic paste. [2,7]. Other factors which might affect the outcome are the width of the apical opening which determines the degree of blood supply, the characteristics and regenerative potential of the remaining tissue and the provision of an adequate coronal sealing [2,7]. Sonoyama et al. [8] drew attention to the presence of stem cells in the apical papilla (SCAP) with the potential to proliferate into odontoblast-like cells and that the apical papilla serves as a very important resource for the revascularization process to occur.

The blood clot formed might enhance the migration of cells from this rich stem-cell source. Lovelace et al. [9] further indicated that the provoked bleeding step in revascularization procedures triggers considerable accumulation of undifferentiated stem cells into the pulpal space which serve as important contributors to the regeneration of pulpal tissues after the disinfection by tri antibiotic paste. Furthermore, the blood clot itself, being a rich source of growth factors has been indicated to play an important role in regeneration [10]. Platelet-derived growth factor, vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor, and tissue growth factor are the major mechanisms described that have the potential to stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, cementoblasts, etc. from the immature, undifferentiated mesenchymal cells in the newly formed tissue matrix [10].

Beccerra et al. [11] studied the tissue formed in the canal of a successful human revascularized/revitalized tooth and concluded that the newly formed soft connective tissue showed similarity to that in the periodontal ligament and cementum-like or bonelike hard tissue, which is comparable with the histology observed in the canals of teeth from animal models of revascularization/revitalization. On the other hand, the histologic processing of a failed case of maxillary central incisor which was replanted after avulsion and extracted after 16 months due to abscess formation revealed complete destruction of the tissue in the canal and formation of biofilm in the apical portion also penetrating into the dentinal tubules. Srisuwan et al. [12] further demonstrated the significant role of direct vascular supply in tissue regeneration and the promotion of connective tissue production with the addition of cells and growth factors.

In general, the following radiographic results are likely to be expected in revascularization procedures:

1. Increased thickness of the canal walls and continued root maturation
2. No significant continuation of root development with the root apex becoming blunt and closed
3. Continued root development with the apical foramen remaining open
4. Severe calcification (obliteration) of the canal space
5. A hard-tissue barrier formed in the canal between the coronal restoration and the root apex [13].

Clinical Studies on Revascularization

Clinical studies on individual one or few case reports

After the initial introduction of revascularization by tri-antibiotic paste by Banchs and Trope [2], further reports started to be published by researchers on single or few cases. Chueh and Huang [14] reported 4 clinical cases of immature teeth that developed periradicular periodontitis or abscess. The treatment protocol included no instrumentation but only copious amount of 2.5% NaOCl and use of calcium hydroxide as medication. Successful results with mature apex development were observed during a period of 7 months to 5 years without complications. An important difference between their study and the one by Banchs and Trope [2] was the calcium hydroxide medication. Banchs and Trope [2] did not advocate the use of calcium hydroxide due to its possible detrimental effects on viable tissue with the potential to differentiate into a new pulp as well as Hertwig epithelial root sheath. Despite their preference to use calcium hydroxide in all cases, they supported the statement by Banchs and Trope [2] by drawing attention to the prevention of hard-tissue formation in the coronal half of the canals where $Ca(OH)_2$ was placed far down into canals. Another case example supporting the triantibiotic paste treatment modality was reported 3 years after the onset by Banchs and Trope [2] where an immature maxillary central incisor with apical swelling was subjected to revascularization procedure with a similar methodology [15]. A successful result was achieved after disinfection of the canal space with a topical antibiotic paste followed by a blood clot scaffold induced from the periapical tissues. As in the report by Banchs and Trope [2], follow-up of the case showed normal responses to percussion, palpation, pocket probing depths, and mobility, however lack of response to vitality testing.

The authors emphasized the significance of case selection and encouraged the clinicians to attempt the revascularization procedure for clinical conditions where the width of the immature apex is greater than 1 mm in a mesio-distal dimension for allowing the successful ingrowth of the vital tissue. Similar to the report by Banchs and Trope [2], the characteristics of the ingrowing vital tissue was questionable and whether the tissue was in fact pulp or pulp-like tissue remained unclear. On the other hand, it was certain that the created blood-clot not only served as a pathway for the migration of connective tissue cells such as macrophages and fibroblasts as well as osteogenic cells from the periapical area but also contained growth and differentiation factors that played an essential role in wound healing. Another advantage of the technique would be the opportunity to try traditional calcium hydroxide and MTA apexification techniques even if the vital tissue underwent necrosis and a periapical lesion developed, as stated by the authors. Since the root would be strengthened by the development of the root canal walls, obturation of the root canal space would be facilitated allowing a more predictable root canal treatment.

Cotti et al. [16] reported the successful treatment and revascularization of a traumatized maxillary central incisor of a

9-year old girl. A sinus tract formation was also present and the authors used a similar methodology as those described by the previous reports; however, this time, no antibiotic paste was applied and a calcium hydroxide medication was preferred due to the unavailability of the tri-antibiotic paste. Good condition of the periapical tissues, development of the apex and hard tissue barrier formation against the MTA in the coronal portion were the favorable findings during the follow-up of up to 30 months. Following the pioneering cases reported by Iwaya et al. [1] and by Banchs and Trope [2], there was a trend to perform series of cases to further validate the applicability and predictability of the revascularization procedure. Meanwhile, single and few cases continued to be reported [11,17-21] with successful outcomes showing continued root development and apical closure. On the other hand, Çehreli et al. [22] reported the successful results of the revascularization procedure on a severe extrusive luxated maxillary incisor where dislodgement of the cortical dislodgement of the cortical bone was also observed. Two cases which are worth comparison are the ones by Davidovich et al. [23] and Lin et al. [24] in which avulsed maxillary central incisors were replanted. In the first report, whilst no intervention was made, root development continued despite evident calcifications within the root canal space. However; in the latter case in which revascularization was attempted using the tri-antibiotic paste and calcium hydroxide, the tooth had to be extracted due to lack of further root development and thickening of canal walls. These 2 cases are good examples of possible different outcomes using different treatment approaches.

Although multiple visits were preferred in the majority of the cases in the literature, an alternative single visit case presentation was made by Shin et al. [25]. The authors described a case in which a mandibular right second premolar with a necrotic pulp, sinus tract, periradicular radiolucency and an immature apex underwent revascularization via a single treatment approach. The treatment protocol included coronal irrigation using 6% NaOCL and 2% chlorhexidine without any instrumentation and a final coronal packing with MTA. A successful result was obtained in 19 months. They commented that this treatment modality was a conservative means to preserve the vitality of the pulp stem cells to allow the regenerative process to ensue. The authors added that although the single visit technique offered advantages such as elimination of contamination between appointments and prevention of detrimental results due to poor patient compliance, it still should be limited to cases where the pulp is not completely necrotic and there is still some viable tissue in the root canal. Although this approach appears to be applicable to clinical situations, there still remains the problem of detecting the actual characteristics of the remaining vital tissue. Despite the fact that the already remaining viable tissue can serve as an efficient means of enhancing cell proliferation, a definite conclusion cannot be drawn with respect to its quality and ability to promote the migration of new connective tissue. As this is rather a subjective means of evaluation, the selection of single visit revascularization still needs to be performed with caution.

Some of the reports focused on more specific issues such as revascularization in invagination cases, multi-rooted teeth or strategies to eliminate discoloration due to tri-antibiotic paste. Although tri-antibiotic paste treatment appeared to have multiple positive properties, disadvantages of this novel approach were also indicated by some, such as discoloration [26], development of resistant bacterial strains [27,28] and allergic reaction [29].

Discoloration

Discoloration was emphasized as a major drawback of the tri-antibiotic paste, basically related to the use of minocycline. It has been advocated that discoloration issue can be addressed either by leaving minocycline out thus using a bi-antibiotic paste or by using Cefaclor as a substitute for the minocycline [7,15]. Reynolds et al. [30], described a novel approach which seals the dentinal tubules of the chamber, avoiding any contact between the tri-antibiotic paste and the dentinal walls. The use of Cefaclor instead of minocycline and a successful outcome in an immature permanent maxillary central incisor was also reported by Thibodeau [31]. Kim et al. [32] used the triple antibiotic mixture of ciprofloxacin, metronidazole, and minocycline to disinfect the root canal system for revascularization of a tooth with a necrotic pulp.

Dark discoloration occurred 6 weeks after the application of the paste. The authors, under *in vitro* conditions showed minocycline as the major cause of discoloration and a dentine bonding agent used to isolate the antibiotic mixture was partially successful in preventing discoloration. Based on this result, they performed revascularization in 3 cases which they followed for up to 42 months, this time, using Cefaclor instead of minocycline, and obtained successful results. It appears that substituting minocycline with another antibiotic or complete exclusion of the minocycline component have positive impacts in cases where esthetics is the major concern, though there is yet no accumulated evidence about the clinical implications of this approach.

Management of dens invaginatus

Considering the treatment challenges the dens invaginatus anomaly poses for the practitioner, attempts have also been made to incorporate revascularization treatment when confronted with dens invaginatus in combination with an open apex. The use of CBCT to detect the characteristics and delineation of the anomaly has been expressed by Narayana et al. [33] who was the first to report the revascularization in a tooth with invaginatus as well as an open apex. While describing the case, the authors approached the treatment critically by drawing attention to possible detrimental effects of using 5.25% NaOCl as an irrigant and the lower cytotoxic effects of EDTA on apical stem cells. The use of epinephrine-free local anesthetic was also emphasized. Validation of the preference of this unique technique in dens invaginatus accompanied by immature apex was an issue that was also raised. The issue of patient preference in such circumstances is especially important and from a clinical point of view, the patient should be clearly informed about the pros and cons of the treatment option before commencing revascularization, as concluded by the authors.

Another case where Type II invagination with periapical lesion was conservatively treated was by Yang et al. [34]. Root filling was performed for the invagination because of the risk of infection. This was a good demonstration of a conservative approach for the treatment of dens invaginatus without directly attempting a surgical intervention. A note made by the authors on this issue was that the anatomic complexity of dens invaginatus increased the difficulty of MTA placement; thus glass ionomer was used on the root-filled invagination, instead. They emphasized that since the invagination did not extend until the apex in Type I and II invagination case, the invagination should be filled for the prevention of infection, unlike the case by Narayana et al. [33] where the invagination was Type III which extended until the apical portion. In the latter case, removal of the invagination followed by bleeding and placement of MTA was satisfactory because of the connection of the invagination space with the periapical tissues.

Kumar et al. [35] used different treatment modalities in 2 teeth diagnosed with dens invaginatus in the same patient. Whilst maxillary left lateral incisor was treated by apexification and endodontic treatment with mineral trioxide aggregate, the necrotic pulp of the permanent maxillary right lateral incisor was treated with regenerative endodontics. Both teeth showed periapical healing with evident root growth, thickening of root walls and completed apex formation as well as positive response to pulp testing in the revascularization case. A difference in their disinfection protocol was the utilization of Odontopaste (5% clindamycin hydrochloride and 1% triamcinolone acetonide; ADM, Brisbane, Queensland, Australia). Based on the assumption that dexamethasone, a corticosteroid encourages the differentiation of odontoblast-like cells and dentinogenesis, they presumed that triamcinolone included in Odontopaste may have similar effect.

Revascularization of molars

The application of the revascularization methodology basically focused on single-rooted teeth since its introduction. Later, reports started to be published in which revascularization procedures were carried out by tri-antibiotic paste or calcium hydroxide medication. Nosrat et al. [36] described 2 cases in which mandibular 1st molars were successfully treated. An innovative method used in their report was the placement of CEM (calcium enriched mixture) instead of MTA. The reason for selecting calcium enriched mixture was the similarity of the surface characteristics of this material to human dentin unlike MTA and the ability to promote the differentiation of stem cells. They concluded that favorable outcomes were reached even when confronted with molars with complex root anatomy. The authors obtained unsatisfactory radiographic outcome in the mesial root of one of the cases and attributed this to the insufficient bleeding induced in the mesial canals with smaller diameters and openings. The authors suggested that CEM is an appropriate sealing biomaterial over blood clot in revascularization. Another important note made by the authors was that the efficient coronal seal incorporating the placement of additional glass ionomer over the CEM was a significant factor that accounted for the successful outcome.

Cehreli et al. [37] did not use the tri-antibiotic paste but preferred calcium hydroxide medication for 3 weeks in the coronal thirds for the disinfection procedure of 6 molar teeth and demonstrated an interesting resolution to the insufficient bleeding problem encountered by Nosrat et al. [36] by transferring some blood volume from other root canals. The authors also determined a tendency for root canal obliteration in some teeth and commented that the pattern of root development induced by regenerative endodontic treatment might be different from that of physiologic maturogenesis. Further evidence on successful revascularization of immature permanent molars using the triantibiotic paste 3Mix-MP paste (including ciprofloxacin, metronidazole, minocycline) was reported by Sönmez et al. [38]. In general, these cases support the promising result that regenerative endodontic procedures should not be limited to single-rooted teeth and multi-rooted teeth may also respond well in case correct strategies are followed. Caution still needs to be exercised in dealing with narrower mesial or buccal root canals and additional measures may need to be taken to facilitate scaffold formation. PRP might serve as a good alternative in these specific situations.

Chlorhexidine as intracanal medication

An alternative methodology for disinfection was described where chlorhexidine was used as medication due to its antimicrobial properties and low cytotoxicity [39]. The maxillary incisor of a 9 year old girl was revascularized successfully. Mechanical instrumentation of the cervical and middle thirds of the root canal in association with intracanal dressing composed of calcium hydroxide and 2% chlorhexidine gel provided satisfactory root development in necrotic immature central incisor of a 9 year-old girl. The authors commented that favorable outcomes were obtained with the use of this treatment modality and increase in root canal thickness as well as apical closure were noted. Another study also used chlorhexidine as intracanal medication with favorable results [40]. However; there are few studies where chlorhexidine is used in the revascularization procedure. In case the success of chlorhexidine gel can be validated by further clinical trials, it may serve as a good option to sodium hypochlorite with its antimicrobial properties, low cytotoxicity and prolonged effect. Corticosteroids as described above [35] are other substitutes to traditional disinfection methodologies; however they need to be investigated by future research by considering drawbacks, as well.

Platelet-rich-plasma (PRP) supplement in revascularization

Supplementary scaffolds such as platelet-rich plasma and platelet-rich fibrin have helped to change the scope of the revascularization procedure and enabled this treatment modality to be applied in adults as well. Platelet-rich-plasma (PRP) has been described as an ideal scaffold because of its autologous characteristics, easy preparation, presence of growth factors and the ability to entrap growth factors by the formation of a 3-dimensional matrix. The increase in platelet content has been described as directly proportional with the number of growth factors secreted by them that help in the proliferation of stem cells to induce healing and regeneration of tissues. Furthermore, the lower concentration and unpredictability of growth factors in the induced blood clot, the possibility of necrosis that erythrocytes may undergo and the deleterious effects on the matrix have been indicated as factors that support the use of a supplementary scaffold such as PRP [41].

The chairside preparation and application of the technique has been described as follows [42]. After drawing blood by venipuncture, it is collected in a sterile tube containing anticoagulant. Later, it is centrifuged for separating platelet rich plasma (PRP) from the red blood cell infraction. Another centrifuging is made by transfer of the supernatant layer to another tube and finally the precipitated PRP at the bottom of the tube is mixed with calcium chloride for the activation of platelets and neutralization of pH. The introduction of the PRP into the root canal is made with sterile cotton pliers and pluggers. The access cavity is sealed using glass ionomer. The initial case using PRP as a revascularization method was reported by Torabinejad and Turman [43] where the accidentally extracted and replanted maxillary second premolar of an 11-year-old boy was subjected to revascularization by using PRP obtained from the patient's blood following the application of triantibiotic case. Resolution of the periapical lesion, continuation of root development, and apical closure as well as positive response to sensibility tests led the authors to conclude that PRP is potentially an ideal scaffold for the revascularization procedure.

PRP which hastens the effect of revascularization has been used clinically in some case series, as well. A case series was presented by Jadhav et al. [41] where twenty patients with non-vital, immature anterior teeth were randomly categorized into 2 groups and subjected to revascularization with and without PRP. Clinical and radiographic follow-up showed marked improvement in healing and apical closure in cases treated using PRP: Jadhav et al. [44] compared apexogenesis induced by revascularization, with and without PRP in non-vital, immature anterior teeth and observed a marked difference in periapical healing, apical closure and dentinal wall thickening of teeth treated by revascularization with PRP.

The histological characteristics of the tissue formed after revascularization by PRP was elucidated by Martin et al. [45] and by examining the fractured and extracted mandibular first molar of a 9-year-old boy previously subjected to revascularization using either a mixture of PRP and a blood clot or a blood clot alone on the same tooth. Although the tissues formed in the canals were mineralized tissue and some fibrous connective tissue without any characterization of pulp-like tissue with odontoblast-like cells, the authors commented that the presence of any vital and mineralized tissue was much more favorable than any artificial material used and can be considered as success. Keswani and Pandey [46] introduced and used PRF (platelet rich fibrin), a second generation platelet-concentrate for the revascularization of a maxillary first incisor of a 7-year-old boy. They supported this approach by stating that PRF provides continuous release of growth factors contrary to PRP in which there is a dramatic decrease after an initial sudden release and indicated that it is a promising methodology.

It has been stated that the presence of inflamed periapical progenitor cells (iPAPCs), stem cells from apical papilla that survived from inflammation (SCAP) may widen the horizon of the PRP technique [41]. An important attempt to accomplish this goal was a case performed by Jadhav et al. [42] where platelet-rich plasma was used as a supplementary scaffold for successful revascularization of a maxillary lateral incisor associated with large periapical lesion in a forty-year-old Asian male. This was a pioneering study as it was a contrary attempt to the perceived notion that the proliferation and regenerative capacity as well as the mobility of stem cells decrease by aging. The rationale behind the case performed by Jadhav et al. [42] was based on the growing evidence on the fact that perennial MSCs (bone morrow mesenchymal stem cells) recruited in regenerative endodontic procedures are less susceptible to aging and can be stimulated by signals generated through tissue damage and growth factors released from the scaffold [47,48]. In summary, although PRP appears to be patients as well, long-term clinical trials are mandatory to routinely implement its use in clinical practice.

Case series and clinical trials of revascularization

After some individual case reports including one or few cases at the initiation of the treatment modality, clinical series comprising a higher number of cases started to be published. A general statement that can be made regarding these studies is the lack of standardization in terms of irrigation and the medication modality. In terms of irrigants, all case series used NaOCl of various concentrations ranging from 1% to 6%. Additional irrigants selected included hydrogen peroxide [10], chlorhexidine and EDTA [40,49-51]. One study where both revascularization and apexification were performed in 2 groups also used EDTA as an irrigant [50]. Variations were also observed in terms of intracanal medications used for disinfection. In one study, medication with only calcium hydroxide was preferred for the treatment of immature necrotic permanent first molars of patients between 8-11 years old [37]. In others, triantibiotic paste was the medication of choice [38,49,52,53]. In some cases, minocycline was replaced with doxycilline [54] or cefaclor [31], whereas amoxycilline mixed with sterile water was the preferred antibiotic instead of minocycline in one study [55]. On the other hand, Shah et al. [10] selected formocresol as the disinfecting agent. In addition, Nagata et al. [40] preferred chlorhexidine gel for intracanal disinfection with successful apical closure observed in all cases.

In all case series, a blood clot was created in case revascularization was attempted. Some studies divided the patients into groups and used different protocols to make a comparative evaluation over time. In some, the prognosis of revascularization was compared with apexification using MTA or calcium hydroxide [50,53,56]. In one study, MTA apexification, revascularization with blood clot and a gelatin hydrogel incorporating FGF (fibroblast growth factor) was evaluated [54]. The follow-up period for the cases ranged from 1 month to 5 years and the general consensus of the findings at the end of the observation periods were the continuing apical development, thickening of root canal walls and resolution of the existing periapical lesions. Saoud et al. [57] who followed-up 20 cases treated with the traditional revascularization procedure commented on this issue and stated that apical closure is the most consistent finding whereas clinically meaningful radiographic root thickening and lengthening are less predictable after 1-year of follow-up.

In terms of vitality testing, one series determined positive response to sensibility tests for all the 3 cases they were able to trace following revascularization [52]. Petrino et al. [49] also reported a positive response to vitality testing for the 2 cases they examined over a period of 1 year. As can be observed from the table and the results indicated above, there is lack of standardization and harmony between case series from a variety of aspects which makes it difficult to draw definite conclusions as well as the establishment of a well-standardized protocol. The accumulation of the results of series using the same standardized protocol over time will certainly help the endodontic community to generate a universal and evidence-based treatment strategy for revascularization procedures.

New approaches for revascularization

Shiehzadeh et al. [58] brought into attention a novel approach and protocol for the clinical management of teeth requiring regenerative endodontics. The protocol they regarded as a possible paradigm shift included injection of mesenchymal stem cells by specific hydrogel scaffold filled in the entire tooth canal space and continuation of apical morphology. They described 3 cases where necrotic and immature teeth with periradicular periodontitis not treated with conventional apexification techniques were treated using this methodology. The authors regarded this as a shifting paradigm toward a biologic approach by providing a favorable environment for tissue regeneration. Thus, further support of this innovative methodology by accumulation of clinical evidence may provide us with a more feasible option that can enable faster regeneration to ensue.

Taken together, evidence of regenerative endodontics is growing rapidly and there is already a statement made by American Association of Endodontists regarding clinical considerations of regenerative procedures [59] for this conservative means of preserving tooth vitality. On the other hand, it is agreed that high levels of evidence for the outcome of regenerative endodontic procedures is still lacking, thereby preventing the establishment of a well-standardized protocol [60,61]. Furthermore, on a speculative basis, there might be a tendency to report successful cases in the literature. Despite the fact that the favorable outcomes of the revascularization modality cannot be denied, a careful focus on failed cases might enhance our knowledge on issues that need particular attention to facilitate the level of success of this conservative approach.

A final commentary by Nair [62] on the concept of regenerative endodontics is also worth noting and opens a brand new horizon for future endeavors. The author emphasized the fact that rapid development in ethically and legally applicable stem cells enabled the resolution of the problems inherent with the technique, that creation of a new tooth rather than one compromised by biofilm infection is no longer a dream and predicted a bright future for the science of endodontology in which intact new teeth could be grown in the patient's mouth by the stem cell technology.

Conclusions

This review article attempted to summarize the available prominent literature on clinical cases published regarding regenerative endodontics. It appears that there is a general consensus in terms of the reliability, predictability and success of regenerative endodontics despite the multiple variations among authors regarding various stages of the treatment protocols. Meanwhile, the indications for revascularization should be thoroughly evaluated before initiation of the treatment, and the necessity of root canal treatment rather than revascularization should always be kept in mind in specific cases such as mature permanent teeth with apical closure. The future awaits well-standardized protocol establishment as well strong expectations for the development of completely new teeth using the well-grounded improvement in stem cell technology.

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