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Alternative potential of endodontic regenerative strategies for mature teeth to conventional root canal obturation therapy and comparison of different strategies in practice: an integrative systematic review

Ryan Maher Gorayeb

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação do Prof. Doutor Paulo Miller

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Eu, Ryan Maher Gorayeb, declaro ter atuado com absoluta integridade na elaboração deste trabalho, confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

DEDICATION

It is with genuine gratitude and warm regard that I dedicate this work to my family, and many friends. A special feeling of gratitude to my mother whose words of encouragement and push for tenacity ring in my ears, and to my father who left too soon but who will always be present in my heart. My sisters, Sarah who helped me during my studies, and Lena. I also dedicate this dissertation to the people who are important to me and have supported me throughout the process. I will always appreciate all they have done, especially Caterina e Mathieu, Elodie, Kevin, Mai…

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I would like to express my thanks to the entire CESPU institution, for giving me the opportunity to learn in a new language, this profession that I am passionate about.

And this beautiful country, for giving me this chance to discover, and grow up.

RESUMO

INTRODUÇÃO : A vitalidade pulpar é essencial para um dente saudável. No caso de infecções irreversíveis, os tratamentos endodônticos convencionais são eficazes mas não curam, utilizam material inerte e não são isentos de complicações. As estratégias regenerativas ganharam interesse principalmente para os dentes imaturos. Uma vez que a maioria dos tratamentos endodônticos é realizada em dentes maduros, é legítimo questionar a eficácia dos mesmos nestes dentes.

MÉTODOS : Foi realizada uma pesquisa bibliográfica na base de dados PUBMED, utilizando a expressão de pesquisa avançada: ((regenerative endodontics[MeSH Terms]) OR (regeneration[MeSH Terms])) AND ((terapia de canal radicular[MeSH Terms]) OR (polpa dentária[MeSH Terms])). Os critérios de eligibilidade foram: publicações em língua inglesa e portuguesa, publicadas nos utimos 10 anos. Foram obtidos 18 artigos após a seleção final. Foi realizada uma análise com as seguintes variáveis : anestésico, irrigante, medicamento intracanal, «scaffold», selante, radiografia, sinais e sintomas, exame imuno-istoquímico, vitalidade da polpa, taxa de sucesso.

RESULTADOS : 1. Existem resultados radiográficos, clínicos (sinais e sintomas), e de vitalidade pulpar positivos para as estratégias regenerativas, sendo poucos os estudos que realizam o exame imuno-histoquímico. 2. Todos os protocolos apresentam semelhanças, mostrando um consenso.

CONCLUSÃO : 1. Estratégias regenerativas são uma alternativa terapêutica válida, em circunstâncias específicas, para dentes maduros. 2. É possível padronizar protocolos, sendo necessários mais estudos para consolidar os procedimentos neste campo.

PALAVRAS-CHAVE: endodontia regenerativa; regeneração; terapia de canais radiculares; polpa dentária

ABSTRACT

INTRODUCTION: Pulpal vitality is essential for a healthy tooth. In the case of irreversible infections, conventional endodontic treatments are effective but do not heal, use inert material and are not without complications. Regenerative strategies have gained interest mainly for immature teeth. Since most endodontic treatments are performed on mature teeth, it is legitimate to question their effectiveness on these teeth.

METHODS: A literature search was performed on the PUBMED database, using the advanced search expression: ((regenerative endodontics[MeSH Terms]) OR (regeneration[MeSH Terms])) AND ((root canal therapy[MeSH Terms]) OR (dental pulp[MeSH Terms])). The identification criteria were publications in English and Portuguese and published since 10 years, 18 articles remained after screening. An analysis was performed with the following variables: anaesthetic, irrigant, intracanal drug, scaffold, sealant, radiography, signs and symptoms, immunohistochemical examination, pulp vitality, success rate.

RESULTS: 1. There are positive radiographic, clinical (signs and symptoms), and pulp vitality results for regenerative strategies, with few studies performing immunohistochemical examination. 2. All protocols present similarities, showing a consensus.

CONCLUSION: 1. Regenerative strategies are a valid therapeutic alternative, under specific circumstances, for mature teeth. 2. It is possible to standardize protocols, and further studies are needed to consolidate procedures in this field.

KEYWORDS: regenerative endodontics; regeneration; root canal therapy; dental pulp

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LIST OF ABBREVIATIONS, ACRONYMS, ABBREVIATIONS AND ACRONYMS

AAE: American Association of Endodontists

CBCT: Cone Beam Computed Tomography

CEBC: Calcium-enriched based cement

CH: Calcium hydroxide

CLG: Collagen

CRCT: Conventional root canal treatment

CS: Conventional strategy

CSBC: Calcium sulphate-based cement

DAP: Double antibiotic paste

DPSCs: Dental pulp stem cells

EDTA: ethylenediaminetetraacetic acid

G-CSF: Granulocyte colony-stimulating factor

HA: Hydroxyapatite

IRR: Internal root resorption

MRI: Magnetic resonance imaging

MSCs: Mesenchymal stem cells

MTA: Mineral trioxide aggregate

NaOCl: Sodium hypochlorite

NP: Pulp necrosis

PAI: Periapical index

PBB: Periapical bleeding and Biodentine ™

PBMTA: Periapical bleeding and MTA

PPP: Platelet Poor Plasma

PRF: Plasma Rich Fibrin

PRP: Platelet Rich Plasma

RCT: Randomized clinical trial

REP: Regenerative endodontics procedures

RS: Regenerative strategy

SDF-1 α: Stromal cell-derived factor 1

SI: Signal intensity

TAP: Triple antibiotic paste

XR: Radiography

TABLE OF MATERIALS

1. INTRODUCTION

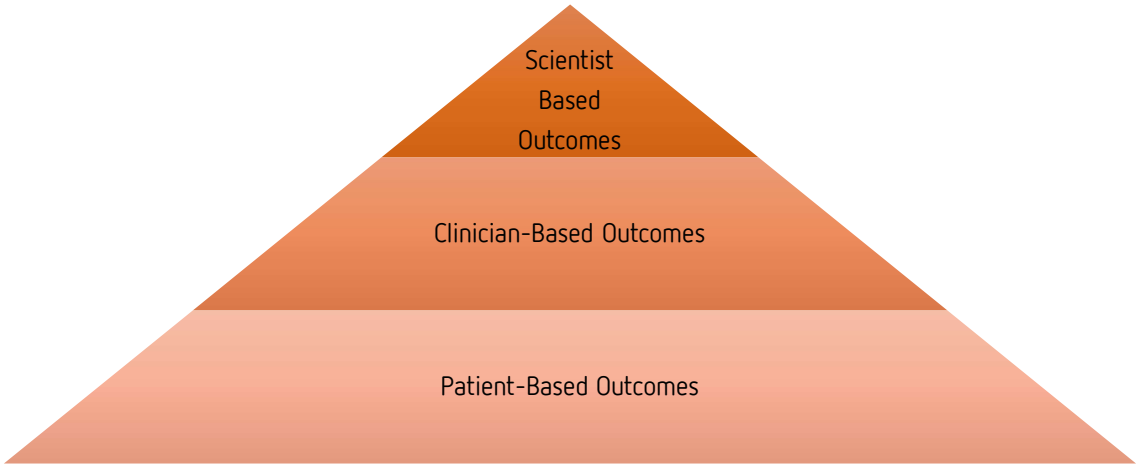
Tooth vitality is due to a structure located in the innermost part of the teeth, called the pulp. It is responsible for the blood supply to the teeth. When compromised, tooth vitality can be affected. When there is a loss of vitality, we can have irreversible pulpitis or necrosis (heavy infection). The conventional strategy (CS) for preserving the natural dentition is pulpectomy and root canal treatment. (1) These procedures are usually performed to prevent tooth extraction. Although the CS has a high success rate (1) : it is using biocompatible but inert materials which are foreign to the organism; it does not restore vitality, it causes proprioceptive and structure loss. (2) Unfortunately, many patients who would like to save their teeth but are not given the possibility which usually ends in an extraction, which can lead to the placement of an artificial implant for example. (1) Furthermore the failure is underestimate (1); it causes fracture, discoloration of the crown (3,4); there is immunity and hydration problems (5) and secondary infections and complications exist and thus require a new treatment response. (1)

Technological developments and scientific research have evolved towards a more conservative way : regenerative strategies (RSs) are increasingly attracting the interest of clinicians and researchers. (6) Due to the nature of the treatment, the AAE (American Association of Endodontists) has been prioritising this strategy as it recognises the enormous potential of regenerative endodontics. The term "regenerative endodontics" was used and confirmed by the AAE in 2007.(1,7) There is no consensus on the true meaning of clinical regenerative endodontics, and there is confusion over the concept and the term. The terms "revitalisation", "revascularisation" and "regeneration" could mean what we will call "regenerative strategies" (RS) in general. "Revascularization" is the establishment of the vascularity (density/network of blood flow). "Revitalization" refers to the process of regeneration of tissues that were lost in the canal (soft tissues, blood vessels, etc.) (8,9) "Regeneration" includes revascularization and can only be completed once it has included the presence of odontoblastic layer, and other supporting tissues such as parasympathetic nerve fibers, stem/progenitor cells, and interstitial fibroblasts. These tissues help regenerate the pulp cells and nourish the new tissue. (7) If the tissue is replaced with scar tissue, then regeneration is not possible. It could wound healing or reparation. (10) Histologic studies are required to confirm this. We consider that a RS is an interesting

technique that allows the pulp space to be filled with vital tissue, a "*biological obturation*". (11)

The regeneration of a tissue as complex as the dental pulp in an empty canal is a challenging process due to the hostile environment, infected and then devitalized. (12) The American Association of Endodontists (AAE) clinical considerations for regenerative endodontic procedures defines success as having a primary goal that involves the elimination of symptoms, such as pain, swelling, and radiographies evidence. The second goal is to repair and develop mineral deposits, while the tertiary goal is to stimulate the response of the nervous system in the objective of valid vitality testing.(13,14)

Figure 1. Trilevel of outcomes (15)



Tooth maturity is characterised by age, pulp volume, and the size of the apical foramen. A mature tooth has taken the time to complete its apexogenesis and apexification and has a smaller volume. (16)

Initially the treatment is designed for apex closure for immature teeth. Therefore, it is legitimate to question the benefit in mature teeth, which represent the large part of patients treated endodontically. Moreover, this has recently shown important development and has been very promising and instigating in the field of dentistry.

2. OBJECTIVE AND HYPOTHESIS

Regarding the objectives: The objective 1 is to show that the use of regenerative techniques leads to scientific (histological, molecular) and clinical evidence, to be an alternative to conventional treatments for mature teeth. The objective 2 is to answer the question of whether the data allow us to standardise a protocol or give a reference protocol.

Regarding the hypothesis: The hypothesis 1 is "RSs can probably be an alternative to CSs for mature teeth". The hypothesis 2 is "It is possible to establish guidelines and reference protocols for clinical practice."

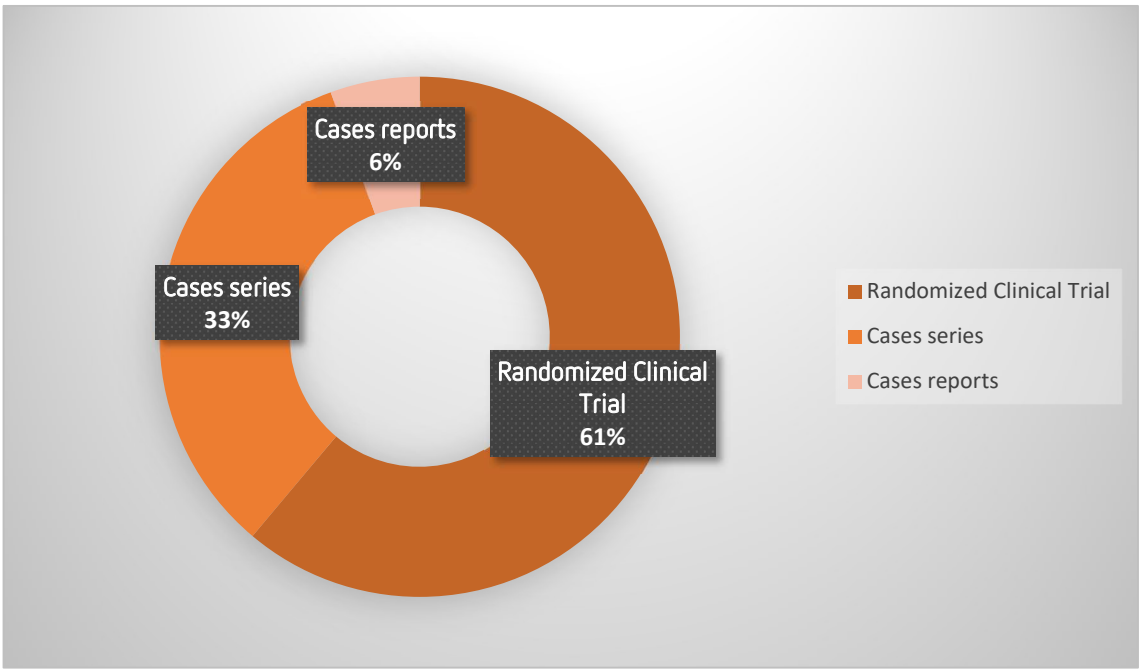
3. METHODS

To select the articles of the study a literature search was performed in the PUBMED database, using the advanced search expression: ((regenerative endodontics[MeSH Terms]) OR (regeneration[MeSH Terms])) AND ((root canal therapy[MeSH Terms]) OR (dental pulp[MeSH Terms])). The identification criteria were: publications in English and Portuguese and published since 10 years (until 18/05/2022) which resulted in 926 articles for the screening step.

During the screening, after remove duplicates and title/abstract without interest, 916 were excluded because they did not meet the study objectives, leaving about 10 eligible articles.

After through full text reading, 10 articles were included, and then 8 articles were manually added, thus leaving 18 articles.

Figure 2. Proportion of the articles



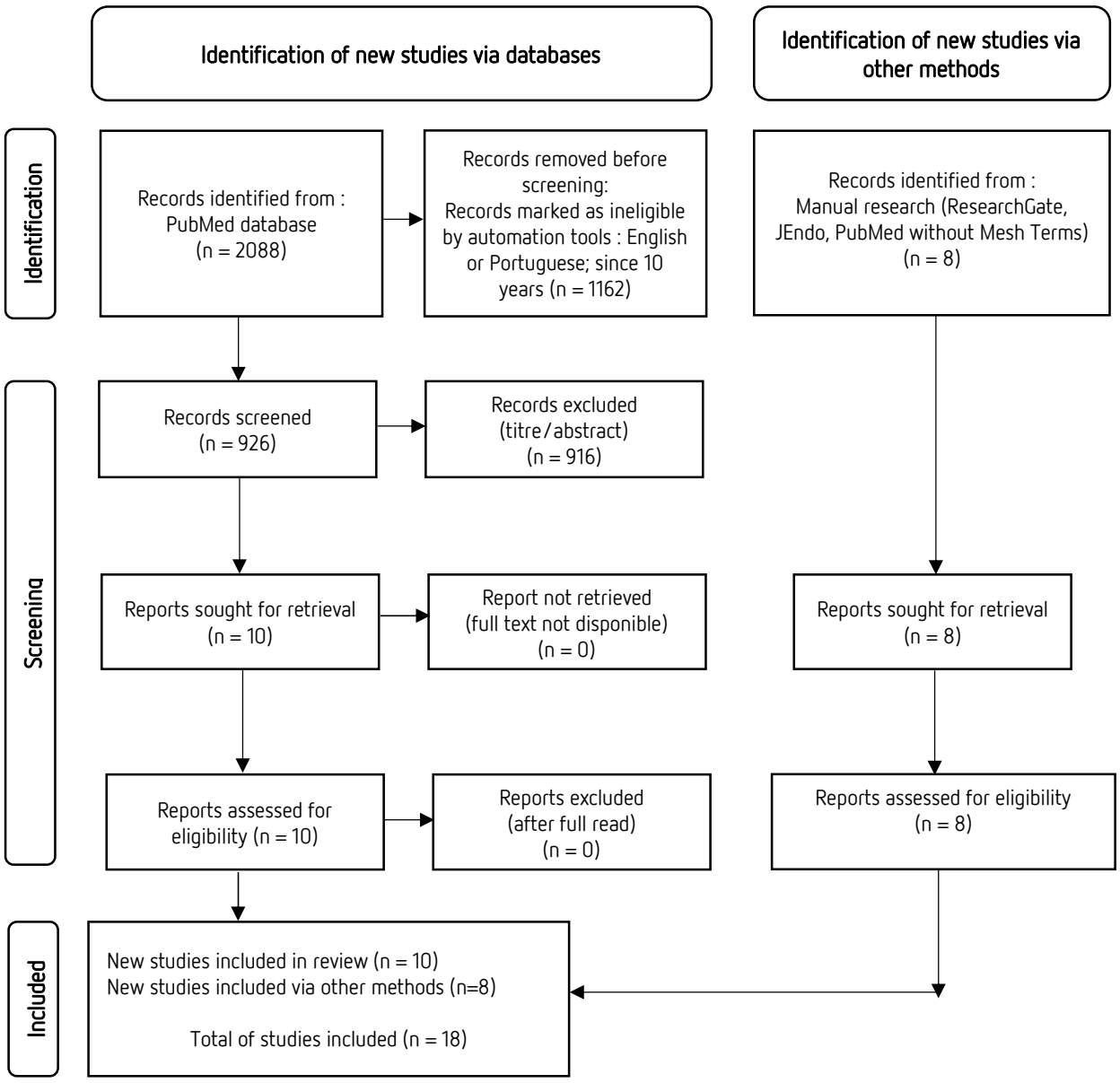


Figure 3. PRISMA 2020 Flow Diagram

The selected articles were catalogued, considering the following sections: authors and year of publication; population; intervention; comparison; outcomes; and with theses following variables: anaesthetic; irrigant; intracanal drug; scaffold; sealant; radiography; signs and symptoms; immunohistochemical examination; pulp vitality (sensitivity test or perfusion unit); success rate.

In addition, the discussion was supported by other 82 relevant articles, obtained from the PUBMED database, addressing the topic, to broaden the theoretical basis and which are also included in the bibliography references.

4. RESULTS

Table 1 A. Relevant data from the studies selected for the integrative review (CS/RS)

Author (Year) (Type) Diagnosis	Follow up MAX	Popu-lation	Intervention : RS	Comparison	Findings					Outcome
					Radiographic evidence Magnetic resonance imaging (MRI), Cone Beam Computed Tomography (CBCT), XR, Periapical Index (PAI), aspect of the canal, alignment of the tooth)	Clinical signs and symptoms (level of pain, swelling, sinus tract)	Immunohistochemical examination (histologic/ Markers and proteins)	Pulp vitality (Perfusion units/ Sensibility test)	Success Rate (based on XR)	
			Irrigant (sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA). Drugs (antibiotic, calcium hydroxide (CH)). Scaffold : blood clot (BC); Plasma Rich Fibrin (PRF); Platelet Rich Plasma (PRP); Mesenchymal stem cells (MSCs); Platelet Poor Plasma (PPP), Collagen (CLG), Hydroxyapatite (HA). Sealant (MTA; calcium sulphate–based cement (CSBC), calcium enriched mixture cement (CEMC); Biodentine™, gelatin sponge) Anesthetic (lidocaine, isocaine, mepivacaine, mepecaine).						
BRIZUELA (2020)(17) RCT	12 Months	36 teeth	[Platelet Poor Plasma (PPP) + Encapsulated Umbilical Cord Mesenchymal Stem Cells (UC-MSC)]	[CRCT]	-	12 months : no adverse effects, and 100% clinical efficacy for both groups.	-	12-months : From 6% to 56% on the cold test.	CS - 18/18 (100%) REP - 18/18 (100%)	Possible endodontic use. Promotion of dentin-pulp regeneration. Promising alternative.

NP (pulp necrosis) + Periapical lesion (PAL)			1st Appointment : Canal conventional cleaning, 2,5% sodium hypochlorite, calcium hydroxide 2d Appointment : 17% EDTA, K8 bleeding, PPP-UC-MSCs, Biodentine™ Anesthetic NOT REPORTED	1st Appointment : Canal conventional cleaning, 1 % sodium hypochlorite, Calcium hydroxide 2d Appointment : 17% EDTA, gutta-percha cones, Topseal, wave condensation technique				From 0% to 28% on the hot test, and from 17% to 50% on the electrical test. Perfusion unit (laser Doppler : 60.6% → 78.1% (12months).		
ARSLAN (2019)(18) Randomi-zed clinical trial (RCT) (NP)	12 MONTHS	46 teeth	[BC + Mineral trioxide aggregate (MTA) (PBMTA)] 1st Appointment : Canal conventional cleaning, 1 % sodium hypochlorite, (triple antibiotic paste) TAP	[CRCT] 1st Appointment : Canal conventional cleaning, 1 % sodium hypochlorite, calcium hydroxide CH)	12 months : favourable clinical and radiographic outcomes were found in 92.3% (REPs) and 80% (CRCT). (follow-up rate of about 73.4%) The difference was not statistically significant.	12 months : favourable clinical outcomes. (follow-up rate of about 73.4%)	-	Half of the teeth treated with REPs responded to the electric pulp test.	CRCT - 16/20 (80%) REP - 24/26 (92.31%)	Regenerative endodontic procedures have the potential to be used as a treatment option for mature teeth with large periapical radiolucency.

			2d Appointment : Sodium hypochlorite (NaOCl), 5% of ethylenediaminetetraacetic acid (EDTA) Bleeding (over instrumenting with a K25 file) for bleeding induction for blood clot formation MTA Isocaine 3%	2d Appointment : NaOCl, 5% EDTA gutta-percha cones, epoxy resin-based sealer cold lateral condensation						
JHA (2019)(19) RCT NP / PAL	18 MONTHS	30 teeth	[BC + calcium sulphate–based cement (CSBC) (Seal Bio)] 1st Appointment : Canal conventional cleaning, 2,5 % sodium hypochlorite, 3 antibiotics 2d Appointment : Seal Bio technique : 17% EDTA, bleeding with K 20 file, CSBC 3% mepivacaine	[CRCT] 1st Appointment : Canal conventional cleaning, 2,5 % sodium hypochlorite, triple antibiotic 2d Appointment : cold lateral condensation, gutta-percha cones	18 Months : Group 1 : PAI 3.6 →1.1 Group 2 : PAI 3.4 →1.2 Difference not significant.	13/15 teeth completely healed in group I (Seal Bio), whereas 12/15 teeth completely "healed" in group II (obturation), 2/15 teeth in group I and 3 in group II were considered as "healing." None showed any evidence of persisting disease. Difference not significant.	-	-	CS – 12/15 (80%) REP – 13/15 (86.67%)	Similar result Seal Bio/Gutta Percha. Less time, less sensitive, less cost effective.

NAGEH (2017)(20) RCT NP / PAL	12 MONTHS	30 teeth	[Plasma Rich Fibrin (PRF)] 1st Appointment : 1,5% hypochlorite, (double antibiotic paste) DAP 2d Appointment : 17% EDTA, K20-40 bleeding, PRF + MTA Anesthetic NOT REPORTED	[CRCT] 1st Appointment : 1,5% hypochlorite, DAP 2d Appointment : 17% EDTA, gutta cones, top seal, cold lateral condensation technique	12 months : No fracture (crown – root).	-	-	Root canal disinfection is essential. The PRF technique is an effective endodontic technique.	CRCT - 15/15 (100%) REP - 15/15 (100%)	Primordial root canal disinfection. The PRF technique is an effective endodontic technique.
SHAH (2012)(21) RCT NON-VITAL / PAL	2 years	220 teeth	[BC + CSBC (Seal Bio)] 1st Appointment : Canal conventional cleaning, 2,5 % sodium hypochlorite, TAP (metrogyl, ciprofloxacin, tetracycline) 2d Appointment : "Antimicrobial solution", 17% EDTA, bleeding with K 20 file, CSBC Anesthetic NOT REPORTED	[CRCT] After control of intra canal infection by chemo-mechanical means, the root canals will be obturated with gutta percha and sealer cement.	XRay + CBCT (Disappearance or reduction in size) Formation of a natural, biological seal of mineralized tissue at the root apices.	Complete control and resolution of clinical signs and symptoms of periapical disease, such as pain, swelling, inflammation and restoration of function.	-	-	CRCT - 100/110 (90.91%) REP - 102/110 (92.72%)	Not Recorded.

ASGARY (2011)(22) RCT IRREVER -SIBLE PULPITIS	12 MONTHS	407 pa-tients	[Pulpotomy + calcium-enriched mixture cement] 1st Appointment : CEM 2d Appointment: CSBC Anesthetic, irriguant, drug, scaffold NOT REPORTED	[CRCT] Canal preparation was conducted using step-back technique	Favorable clinical success rates in the two study arms did not show statistical difference; however, the radiographic success rate in the VPT/CEM was significantly greater than CRCT arm at the two follow-ups.	The outcome of clinical success or failure was determined by subjective symptoms and objective observation of inflammation and/or infection. Objective signs including abscess, swelling, sinus tract, redness, and tenderness were recorded by the general dentists at each follow-up.	-	-	CRCT = 172/175 RS = 163/167	A pulp with irreversible pulpitis contains dental pulp stem cells (DPSCs-IPs) with immense tissue regenerative potential DPSCs-IPs will allow the pulp to heal after appropriate treatment.

Table 1 B. Relevant data from the studies selected for the integrative review (RS/RS)

Author (Year) (Type) Diagno-sis	Follow up MAX	Popu-lation	:RS	Comparison	Findings					Outcome
			Irrigant : NaOCl, EDTA. Drugs antibiotic, CH. Scaffold : PRF, PRP, MSCs, PPP, BC, CLG, HA. Sealant (MTA; CSBC, CEMC, Biodentine™, gelatin sponge). Anesthetic (lidocaine, isocaine, mepivacaine, mepecaine).		Radiograph-ic evidence (MRI, CBCT, XR, Periapical Index PAI, aspect of the canal, alignment of the tooth)	Clinical signs and symp-toms (level of pain, swelling, sinus tract)	Immunohisto-chemical examination (histologic/Markers and proteins)	Pulp vitality (Perfusion units/Sensibility test)	Success Rate (XR)	
YOUSEF (2022A) (23) RCT NP / PAL	12 month	20 teeth	[BC + MTA] 1st Appointment : 1,5% sodium hypochlorite, NaOCl, 17% EDTA, CH	[PRF] 1st Appointment : 1,5% sodium hypochlorite, NaOCl, 17% EDTA, CH	There was a significant increase in peri radicular healing in both groups at 6 and 12 months, compared to that at baseline.	-	-	The results showed a decrease in the mean readings of the electrical pulp tester during the follow-up period.	PBMTA = 10/10 = 100% PRF = 10/10 = 100%	The results of this study suggest that the use of regenerative procedures like PRF or regenerative techniques could be beneficial for the treatment of mature teeth with necrotic pulps.

			2d Appointment : k25 bleeding , PRF membrane was fragmented and placed incrementally, MTA Mepecaine 3% with vaso-constrictor.	2d Appointment : 17% EDTA k25 bleeding, MTA Mepecaine 3% with vasoconstrictor.	No significant difference between the studied groups.			At the 12-month follow-up period, there was no statistically significant difference between the groups.		However, more evidence is needed to confirm the various outcomes of these procedures.

WAGIH TAREK ALI (2021) (24) RCT NP + PAL	12 months	28 teeth	[PRF] 1st Appointment : 2% hypochlorite, 17% EDTA, DAP 2d Appointment : same irrigation, K15 bleeding, PRP upon a collagen plug + Biodentine Anesthetic not reported	[PRP] 1st Appointment : 2% hypochlorite, 17% EDTA, DAP 2d Appointment : same irrigation, K15 bleeding, PRP upon a collagen plug + Biodentine Anesthetic not reported	12 months : PRF group had no significant differences in the survival rate and in the lesion healing with PRP.	-	-	Statistically significant difference (P < .0001) between baseline and the 12-months follow-up, with not statistically significant difference between the two groups.	PRF = 13/14 = 92,8% PRP = 13/14 = 92,8%	The combination of PRF or PRP and revascularization protocols was successful in treating patients with periapical lesions and preserving their mature teeth. The regenerative procedure is a success. Platelet concentrates were also useful in regaining the perception of stimuli that cause pain for necrotic teeth.

MITTAL (2020) (25) RCT NP	12 MONTHS	36 teeth	[BC + Biodentine] 1st Appointment Canal conventional cleaning, 1,5% sodium hypochlorite, NaOCl, DAP 2nd Appointment NaOCl, 17%EDTA Bleeding (over instrumenting K15-25) for bleeding induction for blood clot formation Biodentine 1mm Anesthetic not recorded	[CLG] = 2nd Appointment: Bleeding (plugger) + Sterile granule of collagen in the canal. + Biodentine	[HA] = 2nd Appointment: NaOCl, 17% EDTA, Bleeding (K15-25) + Biodentine	[PRF] = 2nd Appointment: Bleeding (plugger) + PRF n in the canal. + Biodentine	All group showed good PH in all groups at the end of 12 months.	-	-	All groups did not show a positive response to heat and electric pulp testing. Pulp sensibility : non-significant results in all intervals. 12 months : 66.6% in PRF, 44.4% in the collagen group, 33.3% in the hydroxyapatite group and 11.1% in the periapical bleeding group showed a positive response to the cold test.		
EL-KATEB (2020) (26) RCT NP/PAL	12 MONTHS	18 teeth	[BC+ Biodentine (PBB)]	[BC + Biodentine]			All 18 teeth were symptom free with healing of the periapical lesions.	(12 months) 2 groups: minimum 60% regain sensi-bility.	-	-	X3=9/9=100 % X5=9/9=100 %	The use of REPs was not affected by the apical diameter for regeneration of vital pulplike tissue.

			1st Appointment : Canal conventional cleaning, 1,5% sodium hypochlorite, CH PTN files X3 (canal preparation) 2d Appointment : 1,5% sodium hypochlorite, 17% EDTA Bleeding (over instrumenting K25) Biodentine 3% mepivacaine without vaso-constrictor	1st Appointment Canal conventional cleaning, 1,5% sodium hypochlorite, CH PTN files X5 (canal preparation) 2d Appointment : 1,5% sodium hypochlorite, 17% EDTA Bleeding (over instrumenting K25) Biodentine 3% mepivacaine without vasoconstrictor	No significant difference between (signal intensity) SI normal contralateral teeth and treated teeth.					This tissue can be assessed using MRI in a quantitative and not invasive manner.

IOHARA (2016) (27) RCT PULPECTOMIA	180 DAYS	28 teeth	[Transplantation of mobilized dental pulp stem cells (MDPSCs) and 7.5 ng/mL of granulocyte colony-stimulating factor (G-CSF= with an atelocollagen scaffold] Others variables not reported : animal study.	[Pulpectomy] : Not recorded [Collagen only] : Not recorded [Normal teeth] : Not recorded	The stem cell-based group showed a decrease in the SI,similar to that seen in the normal teeth, until 180. Compared to the group that received collagen, the stem cell group showed a significantly higher SI.	-	Pulp tissue was fully regenerated 90 days after cell transplantation	-	-	This asserts that pulp-like tissue has been created and corroborated by histological analysis, and that MRI is a non-invasive and effective method to quantify this neo-formation.

Table 1 C. Relevant data from the studies selected for the integrative review (RS)

Author (Year) (Type) (Diagnostic)	Follow up MAX	Pop.	RS	Compare	Findings					Outcome
			Irrigant : NaOCl, EDTA. Drugs antibiotic, CH. Scaffold : PRF, PRP, MSCs, PPP, BC, CLG, HA. Sealant (MTA; CSBC, CEMC, Biodentine™, gelatin sponge). Anesthetic (lidocaine, isocaine, mepivacaine, mepecaine).		Radiographic evidence (MRI, CBCT, XR, Periapical Index PAI, aspect of the canal, alignment of the tooth)	Clinical signs and symptoms (level of pain, swelling, sinus tract)	Immunohistochemical examination (histologic/Markers and proteins)	Pulp vitality (Perfusion units/Sensibility test)	Success Rate (XR)	
NAGEH (2022)(28) Case series Internal root resorption (IRR) + PAL	12 MONTHS	13 teeth	[PRF] 1st Appointment : 1,5% NaOCl, CH 2d Appointment : 17%% EDTA i-PRF+ PRF membrane + MTA 3% mepivacaine	-	12 months : CBCT verifications show lesions and periapical with a reduced volume Significant difference.	Resolution of signs and symptoms through the follow-up period in all the cases.	-	-	13/13=100%	i-PRF can help stop the development of the IRR and help the periapical healing process. It can reduce the number of appointments and increase patient compliance.

EL-KATEB (2021)(29) Case series NP+PAL	12 MONTHS	10 Teeth	[BC + Biodentine] 1st Appointment : Instrumentation was done using ProTaper Next system till size X3. Irrigation with 1.5 % NaOCl Calcium Hydroxide 2d Appointment : 17% EDTA, K25 bleeding + Biodentine	-	12 months : 9 teeth were symptom-free with a statistically significant decrease in the volume of the periapical.	-	-	-	9/10=90%	The study revealed that the use of REPs could be a successful treatment option for patients with periapical lesions. However, the size of the lesions could affect the healing process.
TURK (2020)(30) Case report previously treated	3 YEARS	1 Tooth	Apical bleeding was induced, and concentrated growth factors (CGFs) were placed inside the root canal. Anesthetic not recorded	-	Healing of apical lesion and hard tissue deposition.	Functional and asymptomatic tooth at all the recall visits.	-	The tooth responded to the electric pulp test and the thermal test since the first year.	-	REPs compared with CRCT are announced advantageous. This is done through a predictable and user-friendly procedure.

NAGEH (2018)(31) Case series NP + PAL	12 MONTHS	15 patients	[PRF] 1st Appointment : 1,5% NaOCl, DAP 2d Appointment : 17%% EDTA, K20-40 bleeding, PRF + MTA 3% mepivacaine	-	-	-	-	12 months : Highly significant difference (increase) in sensibility.	15/15=100%	The return of sensitivity would be evidence of pulp-like tissue formation. More conclusive and larger studies are needed.
NAKASHIMA (2017)(32) Case series Irreversible pulpitis	24 WEEKS	5 patients	[The MDPSCs transplant with (G-CSF) in atelocollagen] Isolation and in vitro expansion of MDPSCs	-	MRI signal of the regenerated tissue like normal dental pulp.	Positive outcome, without adverse events or toxicity.	-	The electric pulp test (EPT) of the pulp at 4 weeks demonstrated a positive response.	5/5=100%	

			Pulpectomy, 3%EDTA, Minocycline or 0.5% levofloxacin, gelatine sponge. Anesthetic not recorded		CBCT analysis shows dentin formation in 3 patients.					This study explains that MDPSCs can be used in a secure manner for efficiency in the complete pulp regeneration in humans.
SAOUD (2016)(33) Case series NP+AL	26 MONTS	7 teeth	[BC + MTA] 1st & 2d Appointment : 2,5% sodium hypochlorite CH 2d Appointment : 17% EDTA, K25 bleeding, MTA	-	The periapical lesions of 2 teeth were considered healed, and 5 teeth revealed healing.	Clinical signs/symptoms were absent in all teeth at follow-up visits at different time points.	-	Cold and electric test : without response.	7/7=100%	This treatment is a potential alternative for the treatment of necrotic teeth or teeth with apical periodontitis.

CHREPA (2015)(34) Case series PAL	Not recorded	20 patients	[BC + Gutta Percha] 1st Appointment : 6% hypochlorite, 17% EDTA, CaOH 2d Appointment : 6% hypochlorite, 17% EDTA, K20 bleeding 2% Lidocaine with epinephrine	-	-	-	MSCs were found within vasculature structures. MSC markers CD73, CD90, CD105, CD146 were significantly upregulated contrary to the nnegative MSCs, CD45.	-	Not recorded	The formation of a blood clot after apical bleeding allows the release of pro-regenerative stem cells.

5. DISCUSSION

5.1. Limitations of the study

The results should be interpreted with caution.

Although the subject is recent with a few existing studies, it was possible to find interesting studies. We quote each of the authors and bring the ideas and sources of each study, by cross-referencing the data, to bring a different type of study, a different approach, and a different point of view of the study. Future studies should be designed, accounting for RSs, with more prolonged evaluation periods and follow-up timing for the teeth under investigation, and higher population of study, by randomly comparing conventional and regenerative treatments, but also regenerative treatments with each other.

Although many PubMed articles, 1) do not have Mesh-Terms because they are not on MEDLINE or they are too recent, and 2) use different and/or not specific Mesh-Terms : we have tried to solve this problem by limiting unused Mesh-Terms (such as "mature"), and by manual searching and adding.

Even though the comparisons are different, it was necessary to update and explain it in a different way by comparing the regenerative methods with each other and with the conventional methods.

Even though all the variables are not always used in all studies, and even if they do not provide the same conclusive capacity as the histological approach, they have been used to give an overall idea of the potential of the strategies used with further insights. Several variables have not been considered, we assume that they are not relevant to the subject of our research, such as the gender of the person, as well as the type of material used for the external restoration. Finally, cross-referencing all these data allows us to conjecture the outcome of RSs.

5.2. Findings in context

5.2.1. The potential alternative of RSs to conventional treatments

5.2.1.1. Immuno-histo-chemical evidence

5.2.1.1.1. Immuno-chemical evidence

Biochemical markers show the establishment of growth factors and proteins related to cell activity and signalling : a study observed an increase in MSC markers CD73, CD90, CD105, and CD146 with a pro-mineralizing potential, unlike markers CD45. (34) This proves an increase in cell activity and signalling. Always demonstrating pro-regenerative markers, an animal study, explains that the use of Stromal cell-derived factor 1 (SDF-1α) allowed the growth of pulp-like tissue as evidenced by the increase in LC3 and Atg5. (35) These elements can be the cause of new tissue growth, but histological studies should in a more direct way allow the analysis of the neo formed tissue.

5.2.1.1.2. Histological evidence

Several studies have conducted an analysis showing that the neo-formed tissue is not pulp-like. Mittal et al. (2021) and El-Kateb et al. (2020) speak of a living regenerated pulp-like tissue. (25,29) Iohara et al. (2016 speaks about a "*fully regenerated pulp tissue*" (27) These data are confirmed by several studies. (36–39) These studies have made it possible to establish that there is the presence of nerves, vasculature, or odontoblast like cells in the dentinal wall, like Arslan et al (2019) explains in a case report. (40)

However, this is not the case for all authors. Shah et al. (2012) explains that there is a creation of "remineralised tissue" at the root apices. (21) In an animal study, the researchers follow this idea of non-pulp-like tissue by referring to tissue resembling connective tissue, cementum, or bone for example. (41) This suggests wound repair, but not true dental pulp regeneration. Nevertheless, this issue has been studied differently by few authors, through sensitivity or blood flow, to see if the response of the regenerated tissue is similar.

5.2.1.2. The return to sensitivity and blood circulation

5.2.1.2.1. Thermic and electric test

Several studies show outcomes showing vitality and found a relationship between the use of a RS and improved sensitivity : Arslan et al. (2019a) shows that teeth respond to the electrical test, which confirms the Nakashima et al. (2017) study. (18,32) This improvement is also found in the study of Brizulela et al. (2019) which also shows an increase in thermal sensitivity to heat and cold, as in the Saoud et al. (2016) study. (17,33) Nageh et al. (2018) and Turk et al. (2020) also confirms this sensitivity of the treated teeth. (30,31)

However, it is easy to understand that these tests are subjective because we do not all have the same approach to pain. This is a disadvantage, and the protocols should be more detailed to make the tests more objective. This is why other methods of pulp sensitivity and vitality are important. (42) This is the case of one author who objectively demonstrated a relationship between the use of a RS and increased blood flow. (17)

5.2.1.2.2. Blood flow

Brizulela et al. (2019) explains that the percentage of unit perfusion measured by laser Doppler flowmetry increases. (17) This variable directly analyses the blood flow which can be an advantage. However, few studies have used this variable, which should be recommended for use in future studies.

5.2.1.3. Radiographic lesions: an overall decrease

These immunohistochemical and pulp sensitivity and vitality improvements previously discussed are the factors of the observable improvements in the patient : several authors have found a relationship between the use of RS and improvement of radiographic lesions, when compared to conventional therapy. Arslan et al. (2019a) states that the periapical lesions decreased after measuring the lesion area by software. In the same way, the volume of lesions has been revised downwards for Nageh et al. (2022) , Kateb et al. (2021), Turk et al. (2020). (18,26,28,30)

Shah et al. (2012) uses a more reserved approach by explaining positive radiographic controls ranging from “no increase to reduction” the lesion through CBCT analysis. (21) Another author talks about stopping of lesion progress. (43) Jha. et al. (2019) corroborates

these results by measuring a decrease in PAI at the lesion. (19) The same goes for the work of Nakashima et al. (2017), which explains the MRI (magnetic resonance imaging) is a good solution to analyse the creation of tissue. (32) Asgary S. et al. (2011) and Nageh et al. (2017) also observed a positive radiographic condition, with no coronal or root fracture at term. (20,22)

5.2.1.4. Symptoms and clinical conditions: a broad improvement

The radiographic features previously discussed are clinically visible : several authors have found a relationship between the use of RS and the improvement of patients clinical and symptomatologic conditions, when compared to conventional therapy. Overall, the authors using this variable as an analysis, all explained the symptoms improve or not worsen (17-34).

Nakashima et al. (2017) explains that no side effects or toxicity were observed. (32) Arslan et al. (2019a) states that the improvement of the patient's condition after RS via checking for oedema, sinus tract, and pain as well as percussion did not differ significantly from the CRCT group. (18)

Jha. et al. (2019) finds a similar result, however he distinguishes between "healed" teeth with combined clinical and radiographic normality, and "healing" teeth with clinical improvement but reduced radiographic transparency. (19) This desire to use a more objective analysis is observed in the work of Saoud et al. (2016) which makes a distinction between “healing” teeth and “healed” teeth too. (33) Brizuela et al. (2019) also goes in this direction. (17) This distinction makes the results more objective. For example, Asgary S. et al. (2011), who also observes an improvement, explains that the symptoms are subjective. (22) Shah et al. (2012) corroborates these results with measuring inflammation and restoration of function. (21)

All articles agree on the outcomes, saying that regenerative techniques present comparable results to conventional techniques, and could be potential alternatives. It would be interesting to know which protocol to use. (17–22)

5.2.2. The establishment of a standardised or reference protocol

5.2.2.1. RSs : similarities in the phases of the protocols

5.2.2.1.1. Establishment of a bacteria-free environment

The use of an endodontic treatment that does not include obturation or rapid tissue growth reinforces the importance of a bacteria-free canal. (44) This increases the survival and cell cycle regenerative properties of stem cells. (45) Disinfection starts with a root canal preparation and removal of infected tissue.

5.2.2.1.1.1. Root canal preparation

In addition to the enlargement of the canal, the preparation involves the induction of bleeding to obtain a blood clot, which is usually done by instrumentation with a sterile ISO # K/#H file beyond the apex as explained. (46) Over instrumentation : 1) improves the blood flow capacity, with a radius between 0.25 and 0.5mm (47,48) 2) Allows the passage of cells, and scaffold. (34,49) 3) Allows tissue growth. (11,47,50) By the way, the stem cells in the blood clot can originate from different sources other than the apical papilla and might not differentiate into odontoblasts. (51) That canal enlargement can cause an increase in growth factor, for example TGF- β1.

However, El-Kateb et al. (2020) nuances this approach by showing positive effects on periapical radiographic lesions as well as a return to sensitivity with both X3 and X5 enlargement without significant difference. (29) In addition, studies have shown that apical enlargement of a mature tooth can cause dentine cracks, the incidence of apical cracks was estimated at 40%. (52)

In our studies, we observed instruments ranging from 10 to 40. (17-34) There is not consensus about the diameter of the file, but this variation is understandable as the teeth do not have the same initial diameter of the apical foramina nor the same fragility.

Asgary S. et al. (2011) uses a protocol that uses a regenerative strategy but has a slightly different protocol. (22) It is assumed that there are remnants of the pulp in the case of irreversible pulpitis, which can be used for treatment because they would contain stem cells. Thus, the root canal preparation is not complete because it is not made along the

entire length of the canal. The pulp can be healing if the blood flow is sufficient. But there is a condition : if the right treatment is conducted.

5.2.2.1.1.2. Abundant irrigation

This preparation is done under abundant irrigation. Sodium hypochlorite is a disinfectant, which is regularly used in pulp treatment procedures. (53) Several studies use HS at concentrations of 1 (18), 1,5 (31), 2,5 (54) (17,19–21,26,31,55) and 6% (32).

Efficacy and adverse effects appear to be related to concentration (56). A dose of 1.5% was considered satisfactory. (57) A dose > 5% showed cytotoxic effects on the dentin, which play an important role in the regenerative process. (58) A concentration range is therefore present.

5.2.2.1.1.3. Intracanal medication

Disinfection is then followed using an intra-canal medication. Overall, the authors use calcium hydroxide (17,21,29,55) or an antibiotic or a double (31) or triple (18,19,21) antibiotic paste (DAP/TAP). Asgary S. et al. (2011) uses calcium-enriched mixture cement (CEM). (22)

We may ask why there is so much difference. The use of ciprofloxacin or DAP in comparison to TAP, allows for better tissue growth, cytoskeleton with vimentin, as well as better vascularity and inflammation control. (44) In addition, TAP can cause crown staining (tetracycline). (59) The use of HC in addition to simple DAP showed no difference. (60) The use of HC allows the proliferation of stem cells from the apical papilla (SCAPs) and the release of growth factors. (61) However, antibiotics, like any other drug, are not free of adverse effects. Effects are dose-dependent, as the antibiotic may be pro- or anti-regenerative. (62) Scaffolds can counteract theses adverse effects. (11,63) Among these effects, we can mention the influence on SCAPs. (64) The data are different, but AAE gives, with immatures teeth, the idea of using both tri-antibiotic pastes or calcium hydroxide. (5,65) In the face of all this, it is possible to extrapolate the results for mature teeth, or simply to use calcium hydroxide, which has few side effects while remaining effective.

5.2.2.1.2. Induction of the regenerative process

5.2.2.1.2.1. Introduction of stem cells

A difference between SR and CS is the internal materials used after root canal preparation: It is a "bio" and tissue-inducing material (blood clot, scaffolds) inf the first, but it is foreign to the body and inert in the last, and therefore the authors agree that the regenerative approach starts with the simulation of stem cells (17-34).

This induction can be done using EDTA, which is the final irrigant generally used before bleeding : overall, the majority of the authors use EDTA at a concentration between 3 and 17%, with 17% being the recommendation of the European and American endodontic associations. (66,67) A first possible reason is that EDTA is shown to liberate interesting molecules & growth factor-beta from dentin (66,68,69) This release could include TFG-b, bone morphogenic prtein-2, platelet-derived growth factor, vascular endothelial growth factor (70–72), which probably controls cell metabolism (73), the cycle of pluripotent cells into odontoblast (46,65), like migration, differentiation, and adhesion. (66) Another reason is this would therefore increase stem cell survival and expression, by increasing the disinfectant capacity of sodium hypochlorite. (36) One more reason is that it also neutralises the negative properties of sodium hypochlorite. (11,74)

The authors agree that intracanal bleeding also helps to achieve this goal. Induced intracanal bleeding provides MSCs in root canals of mature teeth with periapical lesions. (34) The majority the authors perform voluntary bleeding. (17-34) In the study of Asgary et al. (2011), researchers directly use the pulp remnants left over from the root canal treatment. (22) (it is a kind of pulpotomy that has been forced). It is important to note that vasoconstrictor used in anaesthetics can influence blood flow, which is directly proportional to the amount of cells or growth factors. Using anaesthetics without vasoconstrictor in the second session is important. (18,19,29,31,65)

5.2.2.1.2.2. Supporting scaffolds

Further steps are needed to start the regenerative process : the authors agree with the establishment of a scaffold : they are materials that have been used to cause desirable cellular interactions in the regenerative process (17-34,75) Because they contribute to the formation of new functional tissues for medical purposes (cell cycle, neovascularisation,

etc) the appropriate application of scaffolds could improve DSCs proliferation, differentiation, adhesion, and migration, which may promote their ability to repair the injured tissues and regenerate functional tissues. (75) But the choice of scaffolds must be relevant. (76,77) This scaffold can be used by two types of strategy.

5.2.2.1.2.2.1. Cell based strategy

The stem cells are not used in this case via cell homing but by external addition. These cells can come from the umbilical cord mesenchymal (UCM-SCs + PPP Brizuela et al. (2020) (17) or dental pulp (DP-SCs + G-CSF in Nakashima et al. (2017) (32) and other studies confirm this (27,78) for pulp regeneration. The clinical efficacy and safety of these cell-based strategies has been well investigated in the published literature. (17,32,46,79,80) In our opinion, even if the investigation is present in the literature, the effectiveness and safety of these procedures is not guaranteed, and deserves more studies on patients, especially randomized studies. Moreover, this method requires technical conditions (storage, transplantation), ethical and consent approvals (27,32) for the patient, and therefore time and money.

An alternative is possible. Pre-erythrocytes have potential for odontoblastic differentiation by pre-programming in vivo. (81,82) In this other strategy, some products can help this differentiation.

5.2.2.1.2.2.2. Cell-homing strategy

Blood clot is a possibility of cell-homing scaffold. (18, 19, 21, 23, 25, 26, 29, 33) This is the consequence of the bleeding step; it can be considered as material of biological interest.

Growth factors or other molecules can also be used, with the capacity to cause migration or differentiation of stem cells. (76,83) EDTA is an example that we will see later in the protocols. But we can also mention the work of Suzuki T. et al. (2011) (collagen + FGF) et Zhang L-X et al. (2015) (SDF1). (84,85) Other studies show that the combination of these molecules can increase the effect. (73,86,87)

PRF/PRP or PPP are others products, which blood derivatives with the capacity to release tissue and endothelial growth factors (e.g. TGF-b) and inflammatory cytokines (e.g. interleukins 1, 6, 10) (88–90) acting as an "immunological nodule" according to Pantaleo

S et al. (2021) (65) In this sense, fibrin facilitates tissue regeneration. (20,25,31) More studies are needed, but of those we have, adding PRF would give better results than using a simple blood clot, or collagen, or hydroxyapatite. However, there is no significant difference between PRP and FRP with our studies, which needs to be confirmed by further studies.

5.2.2.1.2.3. Installation of a tight coronal seal (MTA, Biodentine ™, CSBC, CEBC).

The authors agree with the use of an intra-canal biomaterial : Biodentine™ (tricalcium silicate, zirconium oxide, calcium carbonate), MTA (calcium silicate and calcium hydroxide) or calcium sulphate-based cement. (54,55)

These products show a similarity in composition with the presence of calcium. Calcium allows, among other things, remineralization. For example, CEBC or CEMC appears to have the ability to induce and activate cell differentiation and pro-regenerative gene expression. CEMC used by Asgary S. et al. (2011) (22) allows stem cells to be attracted. (91) Biodentine™ and MTA also have this property. (92) The use of CSBC (Cavit G) has been used for this effect (19,21) but few studies have analysed the effect of sulphate cements in these conditions.

However, there are differences in the final properties. For example, Biodentine™ and MTA has shown positive effects in various RSs studies. (29,33,93,94) However, although MTA has interesting mechanical and biostatic properties, it may discolour the crown. (95) The literature shows that Biodentine™ has superior mechanical properties, and is more attractive in terms of time, handling, cost, and remineralization. (96) The biomineralization and calcium release capabilities of Biodentine™ are higher than those of MTA. Also, its push-out bond strength is greater. The cement's saleability is comparable to that of calcium-enriched mixture cement. (92) Biodentine™ seems to be a good compromise. With all this data we can observe some similarities in the protocols, which show a primary consensus, although more studies are needed; but given the weakness of comparative articles between RSs, it is impossible to conclude that one RS is better than another.

5.2.2.2. RSs : factors limiting the possibility of regenerative treatment

5.2.2.2.1. Internal factors

Among the limitations, the one that catches our attention is the age of the patient. The groups presented in our study show an age variation. Several studies show that RSs perform better in "young" subjects. (49,97) Mature teeth display a reduced potential for regeneration. (40) This may be due to capacity_cellular: in old population, the decrease in glial density of fibroblast (98), decreased autophagy and differentiation of SCs, and increased oxidative stress (99) and the lipofuscin in odontoblasts (pigment of degeneration). But also, this may be due to the diameter of the apex too : whose dimension is, as we have seen above, linked to the regenerative capacity.

Another internal factor is related to the regenerative capacity of the pulp itself : although some authors report outcomes with teeth regaining clinical, radiographic, and even sensitivity improvement, the question is whether is realistic or possible to have regeneration of a pulp-like tissue, or whether it will only be a partial recovery. (10) Nerve recovery, angiogenesis, cell differentiation and chemical signalling at the pulp level are among the regeneration processes to be elucidated in detail. This question leads to the confirmation that more studies, especially histological ones, are needed to definitively define the type of created tissue. (100)

5.2.2.2.2. External factors

At this level, these will be factors that are independent of the patient but of the clinical practitioner. The protocols use products and tools that each have a specific interest and effectiveness. (17–33) The research for interesting scaffolds, efficient and pro-regenerative drugs, and the improvement of variable analysis techniques and new diagnostic tools will allow us to go even further in this area. (11,40)

6. CONCLUSION

With all the limitations of this study, we respond to the hypotheses: 1. Regenerative strategies are a valid therapeutic alternative, under specific circumstances, for mature teeth. 2. It is possible to standardize protocols, and further studies are needed to consolidate procedures in this field.

This data allows clinicians to have a practical alternative to conventional treatment. This information allows scientists to do more research to overcome the limitations and move forward. The next step is to look for all these solutions and answer to these questions, for more understanding and predictability on the subject.

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