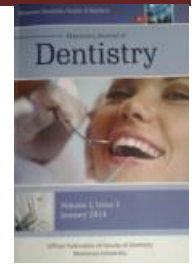# Tissue Engineering and Regenerative Endodontics past and Future

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Abstract:

The European Society of Endodontology and the American Endodontology Association have published the guidlines and regenerative endodontics clinical issues.Literatures are increasing in this interesting field. In regenerative endodontics, endogenous stem cells with inductive bleeding in periapicalregion and scaffoldsblood clot, platelet rich plasma/fibrin have been utilized. And this path has been demonstrated as a 'paradigm shift" and considered the first choice of treatment for pulp necrosis immature teeth. There are several steps to successfully originate regenerative endodontic treatment; firstly, clinical chief complain and symptoms resolution, secondly toothroot maturity and thirdly restore of neurogenesis.   The outcomes of these objectives are presented to be variable, and real pulp/dentine complex regeneration is a crucial goal that does not achieved yet.There is a previous concept regarding the Repair origin which considered to be primarily from osseous and periodontal tissues. The real regeneration of pulp is hoped to be an achievable goal with the concept of tissue engineering, embarking stem cells, scaffold and moleculessignaling together to achieve regenerative endodontics. This review outlines current and future implementation of regenerative endodontics. Proposed that regenerative endodontic has to be considered a first line of treatment for immature teeth and though teeth with immatureor mature root instead ofconventional endodontic treatment or MTA barrier procedures. For instance, there are still much unknown secrets about clinical and biological aspects until regenerative endodontics practicing dominantly among dentists.In this review we will describe how this compound and different signaling interactions that will impact cellular episodes post injury and provide new curative emulated that utilize the bioactive soluble molecules in dentin/pulp to optimize dentin-pulp complex regeneration.

Key words:
1. Regenerative endodontics.
2. Dental pulp stem cells.
3. Dentin.
4. EDTA.
5. Stem cells from exfoliated deciduous teeth.
6. Odontoblast differentiation.

Introduction

Dentistry is a pioneer in regenerative medicine for several years with using calcium hydroxide to promote repair of tissues following pulp exposured(1, 2) and with most newer agents such as mineral trioxide aggregate (MTA) (3) and tri-calcium silicate (Biodentine) (4).   Lack of clarification regardingmechanisms of action of these different agents have led in their applications being

near empirical, but they also played a major role in maintaining pulp vitality in diseased teeth[1].

Advances in biological and tissue engineering have been paired with pulp biology development over the past two decades,That supported our understandingmechanistic of dental tissue responses to

injury, healingwound and clinicaly intervention.   That will provide a platform for

emerging of new therapeutic strategies,   aiming to promote regeneration and repair of tissue and to preserve vitality of the tooth after pulpal disorders.Philosophies of regenerative endodontics 5 are comprehensive by provides a wi

de range of clinical approaches to translate their biological s creening   of pulp regeneration to improve   patient management approaches.

Accordingly,these strategies wide to iclude clinical protocol s, to harness the pulp's natural healing capacity by intervent ions aimed at revascularizing the root canal and optimizing action of canal irrigants for using the stem cells

in tissue engineering.Obviously some of strategies guarante e short   term translation, while other are long term therapeutic targets.   Nonetheless,   the over-riding emphasis in preservation

tooth vitalitywould be of interest to endodontic practice.

The article seeking to guideline some of the biological advance key underpin these new strategies in endodontic regeneration   therapy   and   to emphasize therapeutic translation opportunities.We   will describe how this compound and different signaling interactions that will impact cellular episodes post injury and provide new curative emulated that utilize the bioactive soluble molecules in dentin/pulp to optimize dentin-pulp complex regeneration.

Pulp and dentin biology and its implementation in Regenerativeendodontics

In sophisticated processes under strict temporospatial contr ol the embryonic development physiology of the dentin pul p complex is coordinationed(26). While, the post-natal regeneration of suchstructures provide a lower regulation degree. Particularly, regenerative environment of dentin-pulp complex is aninfluence of the bioactive molecules released with multi-cellular signals from dentin/pulp Besides theinteractions with inflammatory cascades(63). The limited temporospatialprocesswithactivated regulation of moleculessignaling, in comparing with embryonic tooth development, confirms pathologicalconsequenceshappening with repair/regeneration. This can be explained why true physiological regeneration of a pulp tissue is a challeng ing target. The release of bioactive molecules, soluble proteins and growth factors is aggravated by the disease dynamics mainly the enzymatic activity accompanied with the degradation progression of the disease (64, 65). Moreover, dental intervention bydentist like endodontic irrigantmaterials, disinfectants, and intracanal medicaments also can enhance cellular signaling interplay in dentin/pulp environments.

Stem Cell Behaviorand Pulp Cell Niche

MesenchymalStem Cells (MSCs)exist within pulp niches, thatprepared a micro-environment able to maintain the cells stemness and in undifferentiated state. Pulp nichesmaintenance is due to deep interactions between several factors dental stem cells, pulp's cells from different lineages, extracellular matrix, and soluble molecules involvingproteins and growth factors. That niches are traditionally offeringstability to dental pulp microenvironments, which exposed to distortionon different stages of disease and tissue repair. There are different makers that control the niches behavior within the pulp. To exemplify, Notch signaling nichesis expressed in dental pulp niche and correlated functionally with fate of stem cells in the pulp post dental injury(66). Within the pulp tissues, a stem cellperivascular niches have been recorded (67,68), thatillustratesthe transfer of vascular-based stem cell to pulp. Stem cell in blood vessels of apical papilla (69) indicates the recruitment of these subpopulation from apical papilla in the regenerativeendodontics (70, 71) immature (72) or mature permanent necrotic tooth(71).

Reactionary dentinogenesis:

A true evidence that prove the direct interaction between the niche origin of stem cells within the pulp and its role in maintaining balance of stem cells behavior in normal physiological condition and the reactive events in disease cascade is clearly seen in a trial that detect the Pulp cells growth rate on pulp extra cellular matrix and a mineralizing inductive conditions. On pulp mimic environment the growth of pulp cells decreased significantlymaintaining a stem cells like phenotype(73), whereas on the mineralizing condition the pulp matrix enhance the production of mineralization structure and the cells shows a differentiation potential. Collectively, pulp stem cells can maintain their stemness in an environment mimic its native niche. On the other hand the released bioactive molecules, growth factors can induce the differentiation capacity of stem cells within the pulp tissue as a reactive mechanism to the shift in niche state as in disease conditions (74). A directimplementation of endogenous reactive regenerative signals within the dentin-pulp structure is the reactionary dentinogensis, which is secreted as tertiary dentin matrix by resident odontoblast cells as result of a stimulus. This stimulus may be whether exogenous in nature or endogenous from dentin pulp matrix components during pathological affection. Recent articleconfirms that TGF-beta1 a growth factor detected in the dentine matrix of unexposed carious tooth of ferret using affinity chromatography can stimulate the reactionary dentin formation after stimulation of the surviving odontoblast. Moreover TGF-beta1 is previously correlated to the differentiation of stem cellsto odontoblast-like cells, in addition to control their secretory activity. Hypothetically we can say dental caries maysolubilize the underlying dentin matrix exposing growthfactors which in turn induce the modulation of odontoblast and resident stem cells to compartmentalization and secret dentin matrix during reactionary and reparative dentinogenesis.

Treatment options for immature or mature permanent necrotic tooth:

There are different approaches that can be considers a valuable treatment options for enhance endogenous regenerative endodontics, apexfication, apexogenesis and revascularization protocols are the traditional path for this process, whereas strategy of cell homing therapy by growth factors with scaffoldor Cell based therapy using stem cells with biological scaffold are too be the new era in Regenerative endodontic as a relevant clinical treatment.

Apexification refers to non-vital pulp therapy method of inducing a calcified barrier at the apex of incomplete root formation prior to traditional root canal treatment that allow root canal to be sealed.The apexification techniques, by using various formulations of calcium hydroxide to induce closure, are stressed. Most of the literature and authors clinical experience concluded that successful treatment of an immature pulpless tooth can result partly from the antibacterial and calcification-inducing action of calcium hydroxide.

Apexification can be achieved in two procedures: first, by calcium hydroxide dressing to stimulate apposition of biologic hard tissue barrier this known as a long-term procedure, or second which is more recent and short term procedure with creating an aggregation Mineral trioxide (MTA) artificial apical plugfollowed by obturating materialcompaction and finally, filling with coronal restoration.

Under these conditions, MTA has demonstrated strong seali ng effects[2, 3].

Unfortunately, these teeth tend to be at high risk for fracturi ng, even after treatment.[4, 5]

Apexification mostly performed in incisors that lost vitality with carious exposure, traumatic injury and anatomic variations tooth such a dens invaginatus with an immature root. Moreover, the immature tooth treated by apexification procedures assure healing of apical periodontitis, but does not achieve the target of continuing root development or restoring functional pulp tissue. [5, 6]The best aim for treatment of the necrotic pulp of immature permanent tooth would be to restore pulpal function with the continuing root growth and a curing of apical parodontitis.

Another approach is Apexogenesis, which is a procedure of encouragement physiological development to form root end in a vital pulp therapy to preserve the vital pulp and continue root formation with apical closure.Extract much of the coronal pulp to the orifice level , then putting calcium hydroxide paste on amputation site as a wound dressing[7-11].

A restorative base material is then imposed on the calcium hydroxide to be completely mounted.

To assure an optimum long term seal, a coronal restoration must be completed. The patient should re-evaluted for the first year after three months then every six months over the course of 2-

4 years to repote the root development is done without any root resorption,pulp necrosis or any peri-radicular pathosis.

The vital tissue responded by self limiting inflammatory reaction, then proliferation of cells and collagen formation. Mineralization of the newly formed collagen starts with dystrophic calcification, followed by tubular dentin formation [11]. Since it cannot determine the vitality of pulp or the quality of the remained pulp tissue,once root development become completed, the root canal therapy should performed[12, 13].

Recently, regenerative endodontic procedures have strong entered. It allows over apexification, root maturation to be continued by generating vital tissue.[16].

Revascularization as alternative treatment for immature permanent teeth was Endodontic regenerative treatment (Revascularisation) can cause dentine wall thickening and root development in immature permanent tooth.

In addition, stem cells are totipotent cells that can proliferate and generate cells that differentiate into specialized cells. In the near future, a complete vital tooth from a single stem cell will probably be produced. the two kinds of stem cells are embryonic and adult stem cells (pos-tnatal)[17]

For pulp revascularisation it is rather important to have mature stem cells. These cells can find in many areas of the dental element: in the pulp, in the apical papilla, and in the periodontal ligament[17, 18].

Furthermore, the pulp product of neural crest migration, is likely to be a very good candidate for the nerve regeneration [17]

Finding ways of saving the vitality of dental element stem cells as much as possible and of promoting their differentiation is essential.

These cells are capable of producing a highly vascular and conjunctivly rich living tissue.It can colonize the pulp space.

Such stem cells must eventually differentiate into newly formed odontoblasts which induce hard tissue apposition. This latter nature is still unknown[19]

Revascularization of pulp also enables the stimulation of apical and immature tooth root growth:

Revascularization technique would allow the growth of rootThus the remaining thin and fragile walls will be avoided thus reducing The risk of root fracture [20],as it may happened with apexification.The preservation of dental vitality provides better protection against future potential infections.

Revascularization can be used with necrotic immature permanent teeth. Even if the vitality of pulp has lost, residual pulp stem cells can besurvived.Apical stem cells of papillas can survive an apical lesion with plenty of blood[17, 20-22]Growth factors play crucial role in DPSC migration, proliferation and differentiation inregenerative endodontic[23, 24].

The dentin matrix contains several growing factors, including the transforming growth factor (TGF), an Insulin-like growth factor-1 (IGF-1), vascular-endothelial growth factor (VEGF) ,basic fibroblast growth factor (bFGF), epidermal growth factor and platelet derived growth factor (PDGF).[25].

Application of dental materials will release these bioactive molecules from the dentine matrix[26], acids[27] or chelating agents[28] with the dentine matrix demineralization.Such molecules can still contribute to cellular responses even when released at low concentrations.[28].

Regenerativeendodontic as a clinical treatment strategy
(A) Cell homing therapy growth factors with scaffold
(B) Cell based therapy (stem cells with scaffold)

Bone morphogenic proteins and transforming growth factors sub-family proteins has a crucial role to play in the invitro induction of dental derived stem cells into odontoblast with dentin slice scaffold signals. Calcium chloridepromotesDPSCs differentiation into odontoblast and increase the expression of Bone morphogenic protein 2 (BMP2) in the cells that modify the odontogenic markers dentin sialophosphoprotein (DSPP), while the Alkaline phosphatase activity significantly suppressed. This happened as a consequence of multiple molecular activities including increase BMP 2 activity, which in turn mediate Smad1/5 nad Erk 1/2 pathways with modification in converge at Runx2 gene inducing odontoblast differentiation [29]. Activated WNT/β-catenin signaling, which is mediated by p38 mitogen-activated protein kinase (MAPK) is also a proposed pathway enhanced by BMP2 to differentiated DPSCs into odontoblast cells[30].BMp2 also promotes the phosphorylation of Smad1/5/8 and activation of Smad6/7. In addition to induction of the DSP and DMP-1 proteins [31].Role of BMP4 has arole to play in the odontoblast differentiation as well it remarkably enhance the differentiation of DPSCs into odontoblasts-like cells. [32]

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On seeding Stem cells from Human Exfoliated Deciduous Teeth (SHED) on human dentin slice/ scaffold in-vitro it induced the expressionof odontoblastic differentiation markers (DSPP, DMP-1, MEPE) also when these cells transplanted subcutaneouslyinimmune-deficient Nude mice as a vasculature bed host. On the contrary, SHED cultured in Dentin slice/scaffolds free of proteins, or scaffolds without a dentin slice, it does not shows these markers. Moreover SHED express the various BMP receptors as BMPR-IA, BMPR-IB, and BMPR-II which confined with the endogenous activity of this proteins that secreted in-vitro after dentin slice conditioning by EDTA 17% of even adding this soluble protein exogenously in the culture media to induce odontogenic differentiation. Obviously, the knockout of BMP-2 signaling decline the expression of markers of odontoblastic differentiation by SHED cultured in dentin slice/ scaffolds. To sum up, BMP-2 derived from dentin conditioned by different chelating agents -derived BMP-2 is essential to induce the differentiation of SHED into odontoblasts like cells.[33]

The bone morphogenetic proteins (BMPs) have a paramount importance inphysiological tooth development. A recent scientific article investigate this role theyprepared deficient mice model in both *BMP2* and BMP*4*. The first and second molars in the *Bmp2* and *Bmp4* double knockout mice displayed reduction in dentinwith enlarged pulp chambers compared to negative non-knocked controls. Knockout mouse dentin matrix in first molars show significant decreases in dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), and bone sialoprotein (BSP). Knockout mouse odontoblasts primary cellshave a sharp increase in type I collagen mRNA production, thatindicatethe direct correlation between the loss of BMP signaling and the rate of collagen gene expression in these cells. [34]

Osteogenic/odontogenic Inductive media that mainly consists of Dexamethasone, β-glycerophosphate, ascorbic acid and $1\alpha25(OH)_2$vitamin D3 have a great role in the signals regarding the differentiation of native mesenchymal dpsc into secretory odontoblast like cells. Dexamethasone(Dex), which is a synthetic glucocorticoid induce osteogenic differentiation in vitrosignificantly and inhibit cell proliferation. furthermore, Dex powerfully stimulate alkaline phosphatase (ALP) activity and interplay in the primary signals regarding the expression of the transcript encoding the specific odontoblastic marker, dentin sialophosphoprotein.[35], ascorbic acid 2 phosphate, also promote collagen and extracellular matrix formation invitro during culture with dental stem cells[36] in addition to, β-glycerophosphate that was shown to induce the production and mineralization nodules in mesenchymal stem cells' cultures [37],

Theimpact of vitamin D3 metabolism as $1\alpha,25(OH)_2D3$ metabolite, on differentiation of DPSCs to odontoblast is obviously remarkable. Treatment of DPSCs with $1\alpha,25(OH)2D3$ at a concentration of 0.01μM or o.1μM significantly up regulateddentin sialophosphoprotein (DSPP) and dentin matrix protein1 (DMP1)markers,in addition to the odontogenesis-related genes. Also, $1\alpha25(OH)2D3$enhanced the alkaline phosphatase (ALP)

activity that directly correlated to mineralizing nodules formation. In addition, $1\alpha,25(OH)2D3$ induced activation of extracellular signal-regulated kinases (ERKs),and this pathway confirmed to be important in the odontogenic differentiationprocess aswhen ERK is knocked out up regulation of DSPP and DMP1 is ameliorated and the mineralization enhanced by $1\alpha,25(OH)2D3$ is vanished . These results demonstrated that $1\alpha,25(OH)2D3$ promoted odontoblastic differentiation of HDPCs via modulating ERK activation.It promoted the odontoblast-like cell properties, including odontoblast-related and may be useful for inducing odontogenesis and facilitating regeneration of dentin.[38]Another factors besides using appropriategrowth factorsand soluble proteins is to bio mimic the microstructure and micro geometry ofdentine structure as in the dentin-pulp niches and this depicts the importance to choose the suitable scaffold with suitable microstructure and micro geometryto induce the odontoblast differentiation. These study findings demonstrated thatthe development of a odontogenic phenotype cellresemble native odontoblasts can be only done on surfaces having accessible dentin tubules.[39]

Different chelating agents and irrigants protocols for regeneration

Irrigators play an important role in primary disinfection in r egenerative endodontic procedure.They should have the hig hest bactericidal and bacteriostatic impact and have limited cytotoxicity in stem cells and fibroblasts to encourage survi val

and proliferative capacity.Compared to conventional irrigati on use in endodontic therapy, this is a very different argume nt where the chemical effect on host stem cells is not consid ered.therefore all aspects of procedure including irrigator must be evaluated. Chlorohexidine (CHX) in nonsurgical endodontic procedur es is an effective irrigation against persistent microorganis mszzAlthough CHX is a potencial bactericidal and bacterio static agent, CHX is known to remain cytotoxic precipitate following irrigation with NaOCl.[53, 52]

In August 2011, Trevino E.G. et al. concluded that SCAPs did not produce viable cells byusing of irrigation protocols contained 2% Chlorohexidine [40]sodium hypochlorite use as irrigator reference in endodontic. It has a solvent effect on necrotic tissue and has a widely pr oven antiseptic effect [41]

Sodium hypochlorite (NaOCl) was used in regenerative end odontic procedure as a main root canal irrigation solution fo r the disinfection of immature teeth[42]It is a very effective bactericidal agent however it has cytotoxic and negative effect on stem cell survival, attachment, and differentiation[40, 43-46] but can be prevented by a low concentration(1.5% or less) followed by 17% EDTA irrigation [47]

EDTA chelators are weak acids reacting with the minerals of dentinal wall. EDTA-chelating provide better wettability and a removal of the smear layer[48, 49] This increased cell attachment can be due to increased fibro nectin adhesion, which is a key adhesive protein and is pref

erably adsorbed on hydrophilic surroundings[50].using of EDTA in regenerative endodontic procedures as a final irrigant can promote survival of stem cell [40] and allow Intimate adhesion of stem cells on the dentin [28] by release bioactive growth factors that are in the dentin matrix [51, 52]the remnants of EDTA solution may prevent cellular proliferation and shows higher cytotoxic effects [53] Atalla and Calvert [54], Koulaouzidou [55] and Sousa smg et al observed a similar cytotoxic aggressiveness of the EDTA solution. Moreover, Silveira et al. [56], also EDTA solutions showed aggressive on tissues, with evidence of intense exudation and hemorrhagic areas . This dictate the research about a biocompatible material that perform the same action of EDTA but has little harmful effect on stem cells.

Bioactive properties of dentin structure:

This diverse molecules category includes growth factors, Chemokines, Cytokines, extracellular matrix molecules as well as bioactive peptides, representing its difficulty of signaling cellular event the could

be positioned.The corresponding discussion on the bioactive molecules and their capacity role in a regeneration of dentine /pulp should concentrate rather than just simple catalog the present molécules on the signaling of bioactive events associated with regeneration.While extensive studies

have examined biological activities of individual molecule on dentin/pulp regeneration, the micro-environement at tissue-

injury sites represents a local dissolution of multitude of bioactive molecules,

therefore it is important to note that, if present individually or summation and indeed synergistic action of these molecules may differ considerably (22).

Although the dental pulp also contains a diverse number of bioactive molecules, their long-

term bioavailability can be constrainted with faster pulp extracellular matrix turnover and the fact that in pulpal necrosis cases, this sources may not be available. Dentine can therefore provided as a source of growth factors with other bioactive molecules and has

essential role in repairing and regeneration

The nature of binding between bioactivemolecules and dentin structure: ref 62

Dentine inertness represents immobilization and sequestration or fossilization of the bioactive molecules in the matrix .
In health,these molecules are primarily stay in ' fossilized 'state. But if injury and disease occur, will release bioactive molecules during matrix dissolution. This maybe oversimplification as the immobilization/association mechanisms of the different bioactive molecules of the dentin matrix varies.

In certain cases, these molecules are fairly unspecific, perhaps ionic binding, with the dentinal mineral phase. However, the bonding may be more specific

in nature for other molecules (e.g., the specific interaction of TGF-β1, although no other isoforms, with decorin/biglycan in dentin)(62). Thus, with a variety of conditions, different bioactive molecules from dentin can be dissolved, enabling modulating release of these molecules.

Our understanding of various tissue pools and relative solubility of different molecules in dentin/pulp are still very limited. Therefore, a deep understanding may provide powerful means to enhance release these molecules potentiating natural repair processes.

Bioactive component released from dentin by chelating materials:

Bioactive molecules contained within dentin can also be involved in the signaling of dental tissue repair events such as transforming the growth factor beta1 (TGF-b1).The authors evaluated the amount of TGF-b1 released from dentin slices *in vitro* after treatment with 10% ethylenediaminetetraacetic acid (EDTA)[57].

With the growth factors present in dentine extracellular matrix, transforming growth factor β1 (TGF-β1), bone morphogenetic proteins (BMPs), and vascular endothelial growth factor (VEGF) are reported to play an essential roles in wound healing and repair. TGF-β1, BMP2, and VEGF were detected in the conditioned media of dental pulp stem cells over a 4d culture period. DME stimulation has led to upregulation in output of the growth factors, in particular with EDTA and citric DMEs, which may be efficient in differentiation induction via downstream signal transduction[23].

To provide a strong differentiation the preservation of the biological activity of these molecules in dentin by mineral before their release enables a continuing supply of these molecules, thus avoiding the short half-life and the non-human origin of exogenous molecules.

These bioactive molecules ready released by the various tissue preparation agents,

materials widely used in endodontics and medicaments indicates the opportunities for translational regenerative strategies exploiting these molecules with little change to current clinical practice.The identification of several

bioactive molecules sequestered inside the dentin and dental pulp, which include growth factors, cytokines, chemical and matrix molecules, enhance an opportunity to present key signaling molecules that promote repair and regeneration after injury.

Biological events associated with endodontics regeneration:

(a) Recruitment of Pulp Stem/Progenitor Cell

Recruiting stem cells/progenitor throughout perivascular or other niches ara an important step in regeneration and tissuerepairing guidance after disorders. Chemotactic molecules, including dentines /pulp matrix derived molecules , can be released on tissue injury area that is of relevancy for recruitment (Fig. 2).Perivascular stem cell niches are valuable recruiting opportunities following carious injury, as more pulp vasculature proximally to odontoblast layer (75, 76). It has been reported that dentin as well as pulp matrices are containing molecules and chemotactic properties. (53, 65, 77). While growth factors with established cell homing characteristics could be attributed to some of these activities (53), different molecules are probably involving. For instance, the additional activation and generation of C5a was identified

as that of the molecules related to lipopolysaccharide induced pulp stem cell recruitment(77).Matrix-inhabited chemotactic

molecules give desirable potential mediators for stem - progenitor cell employment since releasing them when dissolution of carious tissue (18, 79) with using of irrigant as EDTA (24, 80, 81)in endodontics procedure would probably result in chemotactic gradient providing indications for the spatial application of stem cells on dentinal wall in regenerative endodontic therapy.

(b) Dentinogenic Cell Differentiation

There are a number of similarities between terminal differentiations of primary odontoblasts during tooth development and differentiation of odontoblast like cellthroughout reparative dentinogenesis (25). However, the distinction of new generations of odontoblast-like cells must be accepted as being a pathological rather than a physiological event and there areinsufficient in tightly controlled temporo-

spatial of odontoblast differentiation observed during teeth development. Consequently, the phenotypes odontoblast like cells and matrices secreted can be considerably heterogeneous. This can be noticed as differences in the natural tubular dentin at the morphological level. (85). In addition, the mineral composition variations of the variousmesenchymal stem cell populations like the DPSCs and SCAP seem to occur , which also indicate heterogeneity depending on cell types involving in differentiation stage.

There are lack of encovering about "true" odontoblast like cell from other types of the mineralized cells like osteoblast that recognized by molecular markers. Nevertheless, it's necessary to note that odontoblast like cells are not single, well defined phenotype and these are importance consequences for a robust identifier. While 1^{ry} odontoblasts exhibit a profile for molecular markers, such as the nestin , dentin sialophosphoprotein, dentin matrix protein-1 (DMP-1) and matrix extracellular phosphoglycoprotein , these profiles cannot be specific for odontoblasts and are not generally a robust understands of defining odontoblast phenotype in fairly simple molecular characterization . Other identification propertieslike cell and matrix morphology, in particular the regularity of the tubular structure, will improve confident, inspite of it is not necessarily easy to apply those additional criteria. For example, the odontoblast's morphology diferent through it's secretory life cycle (87, 88).Also the density dentine tubular varies at different points in thickness due to odontoblast crowding while dentinogenesis is proceeding in a pulpal direction. Nevertheless, reliable characterisation strategies for the phenotype of odontoblast-like cells and experimental functional deletion approaches are essential for the resolution of which dentin matrix preparation components are critical for signaling of odontoblast like cell differentiation and how physiologically primary odontoblast is resembling the cells that result.

(c) Dentinogenic Cell Secretion and Its Control

Entirely focused on cell differentiation whileregeneration of dentin/pulp, but it is necessary to upregulation and control of consequence secretionsofcell differentiation. Dentine secretion's management takes place physiologicallywith down-regulation following completion of primary dentinogenesis then again upregulation take place during tertiary dentinogenic episode. Failure to control the odontoblast'ssecretions, pulp canal obliteration can ensue, with prominant consequences for teeth vitality and endodontics therapy (96). TGF-ß superfamily members able to upregulate odontoblasts matrix secretionswith physiological conditions (20) and repair/ regeneration model invitro (93). The purpose of signaling cell seems to activate of P38 mitogen-activated protein kinase (MAPK) pathway by phosphorylation (22). Identification of the moleculares switch for the secretory activities of odontoblasts offers exciting opportunities for therapeutic modulating these activities by combining local targeted with stimulating of growth factors or pharmacological control, with resultin more regulated mineral deposition rate.

(d) Neurogenesis and angiogenesis

Neurogenic incidents are more confined after injury and regenerative endodontic treatment. Thedifference of neuropeptides and neurotrophic molecules that explaned by odontoblasts and surrounding fibroblasts may have potential in area of tissue repair/regeneration by recruiting of nearby free terminal nerve (106, 111–114). In addition to the remaining pulpal cells, recruited MSCs like stem cells of apical papilla (SCAP) showed that axonal sprouting and targeting was mediated by a brain-derived growth factor mechanism (57). Interesting study showed a subpopulation of Dental Pulp Stem Cells with enhanced expression of brain derived neurotrophic factor have transplanted into root canals in a dog model for regenerative endodontics, resulting in formation of an innervated pulp like tissues. effecient innervation is the aim of endodontic regeneration, because neuron mediates protective nociception and modulate procedure of homeostasis as inflammatory and odontoblastic responses.
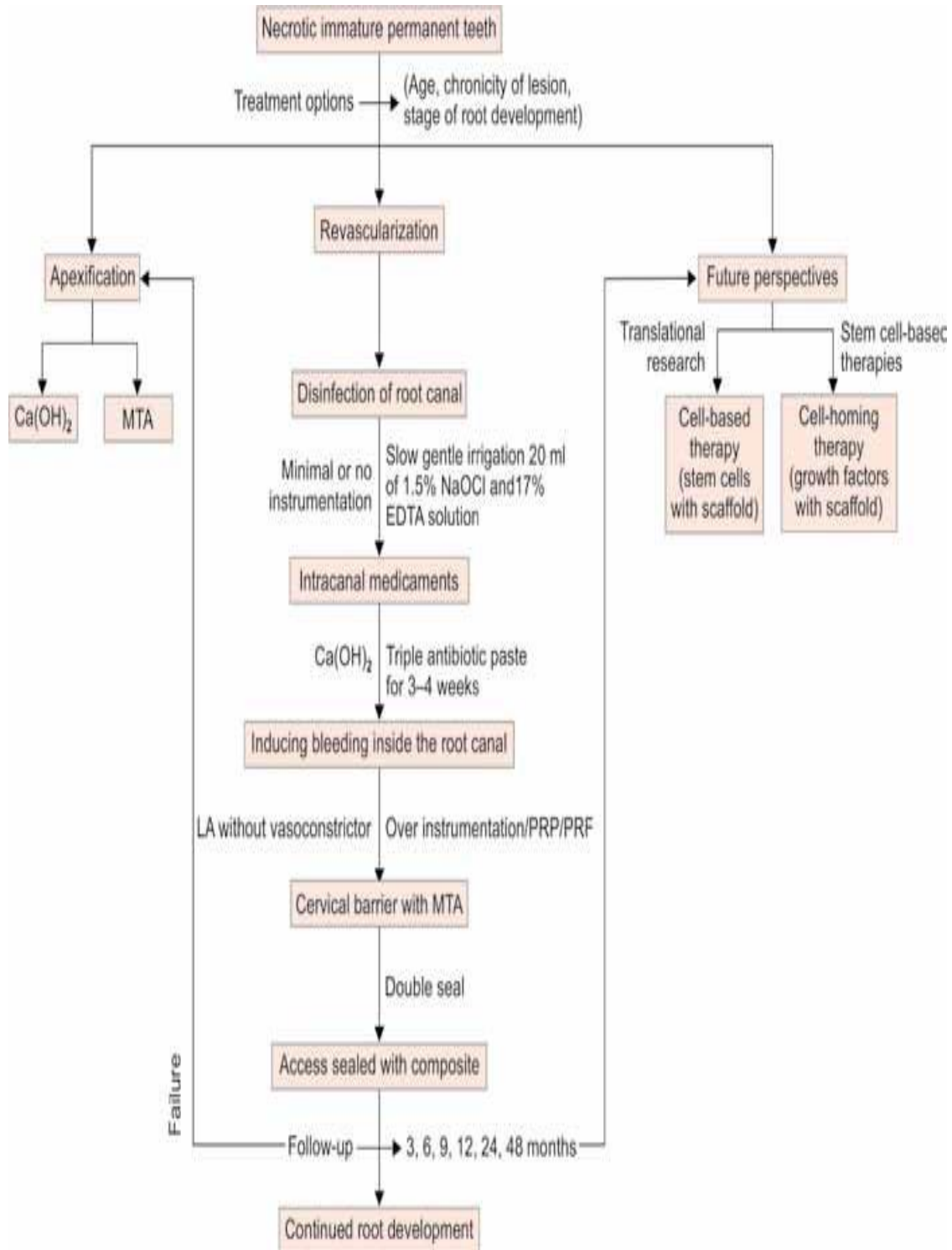
Figure 1:treatment option scheme for immature or mature permenant necrotic tooth

Table 1: Growth factors, soluble proteins and morphogens exist in dentin to play an important roles in repairing and regeneration

Important growth factors within dentin matrix	Regenerative roles
TGF-β1 (23, 24)	Involved in primary odontoblastic differentiation (25, 26) and promotes tertiary dentinogenesis (20)
TGF-β2 (23)	It's expression is upregulated on differentiation of DPSCs into a mineralizing phenotype (27)
TGF-β3	Promotes odontoblastic differentiation (28, 29)
BMP-2 (30)	Promotes odontoblastic differentiation in both *in-vitro* and *in-vivo* models (31) and the inductions of DSPP and increases of alkaline phosphatase activity (32)
BMP-4 (30)	Improves odontoblastic differentiation (33)
BMP-7 (34)	Promotes mineralizing phenotype in DPSCs (35, 36)
Insulin growth factor-1 (37, 38)	Promotes proliferation and differentiation of DPSC and SCAP into mineralize phenotype (39, 40)
Hepatocyte growth factor (41)	Promotes migration, proliferation, and survival of MSCs (42)
VEGF (24, 43)	Potent angiogenic factor (44, 45, 46) has been shown to enhance blood vessel formation on tooth slices implanted subcutaneously in SCID mice (47)
Adrenomedullin (48, 49)	Enhances odontoblastic differentiation by activation of p38 (22)
FGF-2 (24, 43)	Promotes stem cell homing (chemotaxis), stemness, and angiogenesis (44)
Platelet derived growth factor (23)	Promotes angiogenesis (50), chemotaxis of MSCs (51), modulates process of odontoblastic differentiation (52), which acts in synergy with other growth factors (53)
Epidermal growth factor (43)	Enhances neurogenic differentiation of DPSCs (54) and SCAP (55)
Placenta growth factor (43)	Enhances angiogenesis (44) and osteogenic differentiation of MSCs (56)
Brian derived neurotrophic factor (38)	Encourages neuronal development and axonal targeting(57)
Glial cell line derived neurotrophic factor (38)	Promotes regeneration of nerve *in-vivo* (58) andsurvival/proliferation of pulp cells (59). Expression improved throughout odontogenic differentiation(60).
Growth/differentiation factor 15 (38)	Promotes axonic regeneration and post-injury activity and plays a significant role in neurons.(61)

Table 1: Growth factors, soluble proteins and morphogens exist in dentin to play an important roles in repairing and regeneration

Conclusion:
Regenerative endodontics introduced as a new era in biological and dentaltherapy. At this moment, this Regenerative medicine based treatment is being recognized as the first line of treatment for non-mature teeth with pulp necrosis depend on the successes of documentarypublished casesrecordedin the literature. Our understanding of the different clinical protocols has modified toeliminate pulp infection, also to allow stem cell potentiality to be promoted in the canal and with the aid of variousreleased growth factors that fossilized in the dentine walls. Whereas repair rather than true regeneration is accomplished with current protocols, further research is needed in the area of stem cell-based pulp tissue engineering to allow true regeneration and improved treatment outcomes.

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List of figures and table:
Figure-1:treatment option scheme for immature or mature permanent necrotic tooth.
Table-1: Growth factors, soluble proteins and morphogens exist in dentin to play an important roles in repairing and regeneration.

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