Regenerative Endodontic

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Phylosophy

Immature permanent teeth with necrotic pulp/apical periodontitis are traditionally treated with apexification procedure using calcium hydroxide to induce apical hard tissue barrier formation or apical MTA plug before root canal filling

The immature permanent teeth are exposed to prolonged calcium hydroxide dressing, which could increase the risk of root fracture

an apexification procedure has no potential to restore the vitality of damaged tissue in the canal space and promote root maturation

REGENERATIVE ENDODONTIC

History

Pada tahun 1960 , Dr Nygaard-Otsby : Concept of tissue regeneration inside the root canal treatment

In 2016, the American Association of Endodontic : standard protocol for Regenerative Procedure

In 2004 Dr Banchs and Trope : a modified clinical regenerative protocols (minimal instrumentation, copious irrigation and antiobiotic paste) ----induce bleeding---blood clot----PRP and PRF to induce tissue regeneration

What is regenerative endodontic

Revascularization/Regenerative Endodontic (AAE)/ Revitalization (ESE)

Regenerative endodontics was pioneered by the experimental studies of Nygaard-Ostby (1961)

Bi*ologically based procedures* designed to replace damaged tooth structures, including dentine and root structures, as well as cells of the pulp-dentine complex" for immature teeth with pulp necrosis (Murray et al., 2007, Kim et al, 2018)

***Applies the concept of the triad of tissue engineering, stem cells, biomimetic scaffold, and bioactive growth factors in the canal space to regenerate the pulp tissue damaged* by infection, trauma or developmental anomalies (Nakashima 2005)**

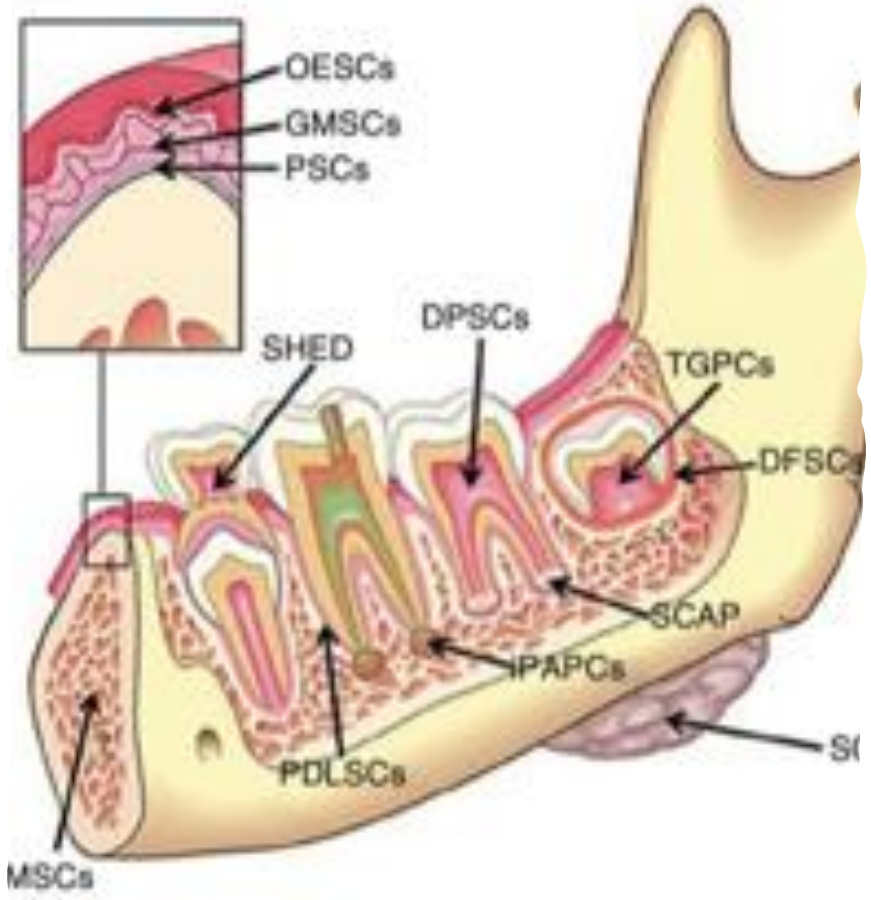
Purpose of Regenerative Endodontic

- regenerative endodontic therapy (RET) is **aimed to regenerate the pulp-dentine complex** damaged by infection, trauma or developmental anomaly of immature permanent teeth with necrotic pulp.
- To eliminate of symptom with evidence of bone healing (apexification)------Primary Goals
- To promote the continued formation of root (increased root wall and root length)-----Secondary Goals

Regenerative component

- Stem cells
- Growth Factors
- Morphogens
- Scaffold

Stem Cells



tooth germ progenitor cells (TGPCS);

dental follicle stem cells (DFSCS);

salivary gland stem cells (SGSCS);

stem cells of the apical papilla (SCAP);

dental pulp stem cells (DPSCS);

periapical progenitor cells

from human exfoliated deciduous teeth

periodontal ligament stem cells (PDLSCS),

bone marrow stem cells (BMSCS)

oral epithelial stem cells (OESCS);

derived mesenchymal stem cells

Stem cells

- **as a distinct subpopulation of undifferentiated cells with self-renewal and differentiation potential**
- **They can be classified as *pluripotent or multipotent cells***
- **Pluripotent stem cells have : the capacity of becoming specialized cells and belong to all three germ layers, Embryonic stem cells are the best example of pluripotent cells**
- **all adult mesenchymal stem cells are more restricted in their capacity to differentiate, only forming tissues of**

Tissue Compartments in oral region

- **stem cells of the apical papilla (SCAP),**
- **inflammatory periapical progenitor cells (iPAPCs),**
- **dental follicle stem cells (DFSCs),**
- **dental pulp stem cells (DPSCs),**
- **tooth germ progenitor cells (TGPCs),**
- **salivary gland stem cells (SGSCs),**
- **stem cells from human exfoliated deciduous teeth (SHED),**
- **oral epithelial stem cells (OESCs),**

Regenerative Endodontics are localized around the periapical region

- stem cells of the apical papilla (SCAP),
- inflammatory periapical progenitor cells (iPAPCs),
- dental pulp stem cells (DPSCs),
- periodontal ligament stem cells (PDLSCs),
- bone marrow stem cells (BMSCs),

Regenerative Endodontics are localized around the periapical region. These include SCAP, PDLSCs, BMSCs, iPAPCs, and DPSCs (if vital pulp is still present apically)

Growth Factors/Morphogens

- **Dentine is composed of collagen fibers (90%, collagen type I) and noncollagenous matrix molecules (proteoglycans, phosphoproteins, and phospholipids)**
- **The collagen fibers act as *a grid or matrix*, and this structure behaves as a scaffold upon which mineralization can occur**
- ***Dentine phosphoprotein* (DPP) and *dentine sialoprotein* (DSP) are the most abundant dentine-specific proteins among the noncollagenous proteins of organic matrix.**
- **Dentine is today considered a reservoir of growth factors and cytokines. These growth factors/ cytokines are secreted by the odontoblasts during primary dentinogenesis, becoming sequestered and "fossilized" into the dentine after biomineralization**

Morphogens

- **It is also important to clearly keep in mind that a second level of regulation exists during dental development**
- **Msx1 is expressed in polarized preodontoblasts**
- **Msx2 is present in mature odontoblasts**
- **Several growth factors have also been evaluated for their ability to trigger the differentiation of selected mesenchymal stem cell populations into odontoblastlike cells**
- **several case studies have reported that patients taking**

Morphogens + growth factors

- Differentiation of Selected mesenchymal stem cell
- Example : Patient with long comsumption of corticosteroid (pulp space decreased, increase thickness 5 fold times) , Medical drug : Statins and Dexamethason with Vit D
- If growth factors stay alone ----maximal differentiation
- If combines need evalution with clinical trial

Scaffold

- **Tissues are organized as three-dimensional sructures, and appropriate scaffolding is necessary to provide a spatially correct position of cell location and regulate differentiation, proliferation, or metabolism while promoting nutrient and gaseous exchanges**
- **The most important component tissue engineering**
- **Scaffolds can be classified as either natural or synthetic**
- **Natural scaffolds include collagen, glycosaminoglycans, hyaluronic acid (HA), demineralized or native dentin matrix, and fibrin Synthetic scaffolds include poly-L-lactic acid**

Properties of Scaffold

- Easy delivery
- Adequate mechanical properties
- Controllable biodegradation
- Incorporation of growth factors

Regenerative Procedures

- Bleeding
- Blood clot
- Scaffold

Scaffolds

Platelet Rich Plasma

- Growth factors rich
- Degradasi overtime
- Three dimensional fibrin matrikx

Platelet Rich Fibrin

- Three dimensional architecture conductive with stem cells proliferation and differentiation
- Contain bioactive moleculs

Hydrogels

- Three dimensional hydrophilics polymers
- The Form is gel/gelation
- Properties of chemical : change PH and Osmolarity
- Properties of physical : temperatures changes
- Highly tunable
- Biocompatible
- The most interest in RET because can be easily injected into narrow root canal
- Example : Puramatrix

Case Report 1

- **A necrotic immature mandibular second premolar with Materials Science, Tohoku University Graduateperiapical involvement in a 13-year-old patient was treated. InsteadSchool of Dentistry, Sendai,3Department ofof the standard root canal treatment protocol and apexification,Operative Dentistry and Endodontics, Iwateantimicrobial agents were used in the canal, after which the canalMedical University, Morioka, Japanwas left empty. Radiographic examination showed the start of api-cal closure 5 months after the completion of the**

Histopathologic

Inflamed Pulp

- In a study of pulp and apical tissue response to deep caries in immature permanent teeth, apical papilla showed extremely r**educed cellularity or lack of cells,** and **Hertwig’s epithelial root**

Necrosis pulp

- the necrotic pulp tissue was c**olonized by bacteria**l biofilm and the apical papilla could not be discerned, and **HERS was absent**

Cvek's classification of root development

Cvek 1992

of root development" (for immature teeth, necrotic pulp)

- at the stage 1 (less than 1/2 of root formation with open apex),
- stage 2 (1/2 root formation with open apex) and
- stage 3 (2/3 of root development with open

suitable for RET

f the short root,

Fig. 1. Examples of teeth with respect to the stage of root development. A, B, C, D and E, stages 1, 2, 3, 4 and 5, respectively; 1–4 = *immature teeth, 5 = mature teeth.*

Are all immature permanent teeth with necrotic pulps indicative for RET?

- **Stage 1, 2 and 3** are suitable for Regenerative Endodontic Treatment (short root, thin canal walls and wide open apex
- Stage 4 (nearly completed root formation with open apex) can managed with either **RET or an apical MTA plug** and root canal filling
- Immature permanent teeth with necrotic pulp requiring post adequate coronal restoration **are not suitable for RET**

Size of apical diameter

- if apical foramen of the tooth was smaller than 1 mm, revascularization was unpredictable
- it was shown that the apical foramen smaller than 1 mm did not prevent revascularization and ingrowth of vital tissue into the pulp cavity
- In a clinical study, it was demonstrated that regenerative procedures were successful with apical diameter as small as 0.5 mm
- it was also found that regenerative endodontic procedures were suitable for the patients ranging from age 9 to 18 years (Estefan et al. 2016).

Irrigants

- The use of sodium hypochlorite before EDTA conditioning reduced transforming growth factor (TGF)-b1 release significantly (Galler et al. 2015). This effect is probably due to damage to the proteins including dentine growth factor
- Sodium Hypochlorite is the most commonly used antiseptic irrigating solution in root canal therapy
- Antimicrobial activity of sodium hypochlorite **less effective in immature teeth** when compared to mature teeth
- The AAE clinical considerations for r**egenerative procedure** recommends the uses **1.5% NaOCl then 17% EDTA**

Calcium hydroxide

- Calcium hydroxide is recommended as intracanal medication in RET because of its good antimicrobial property
- Calcium hydroxide has a high pH 12.5–12.8, which is not a favourable environment for most bacteria to survive
- calcium hydroxide can hydrolyse the lipid moiety of gram-negative bacterial lipopolysaccharide (LPS), thus resulting in the release of free hydroxy fatty acids and degradation of LPS
- Calcium hydroxide in RET was tested against survival of stem cells from apical papilla in vitro rather than killing of the intracanal bacteria in vivo

Triple Antibiotic Paste (AAE,2016)

- Infected Necrotic pulp
- Eliminate bacteria
- The use of antibiotics agent to sterilize
- When the antimicrobial agents are combined such as double or triple---- Unknown
- It assumed that antimicrobial combination would prevent **polymicrobial infection and have synergistics effects**
- **Increased the risk of adverse effects, antagonism and bacterial resistance, systemic allergic reaction**
- **The use of antibiotics, its effect on the survival of stem cells**
- **It recommended to use triple antiobiotic paste at a concentration no greater than 1 mg/ml (to avoid damage of stem cells)**

Triple antibiotic paste

Ciprofloxaxin

Metronidazole

Clindamycin Hydrochloride

RET

Root canal disinfection and Irrigants

- Preservation of stem cells is important in RET
- The presence of prior infection could negatively affect the process of pulp tissue regeneration by damaging tissue forming cells as well as stem cells in the periapical tissues
- intra-radicular infection should be controlled for possibly pulp tissue regeneration to occur in RET (Fouad 2017).
- Sodium hypochlorite is the most commonly used antiseptic irrigating solution in root canal therapy
- It has been shown that sodium hypochlorite was very effective against biofilm formed by 5 different root canal bacterial isolates
- 5.25% sodium hypochlorite was able to eliminate single-species biofilm in 30 s

Regenerative vs Apexification Procedure

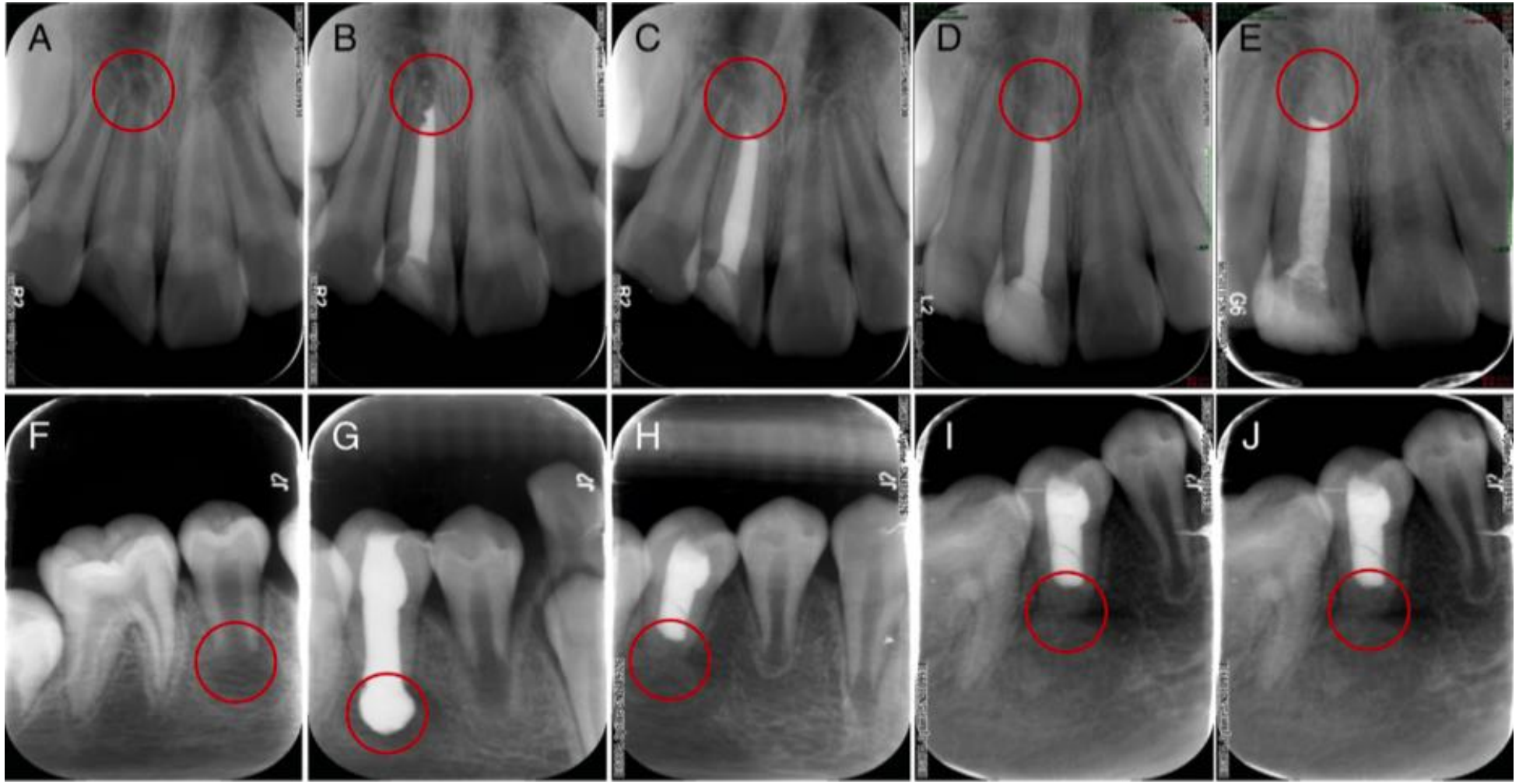
Regenerative

- Anestesi, Isolation, Access opening, Irrigation (NaOCl, Saline and EDTA)
- The canal dried with paper point, 0,1 mg triple antibiotics delivered into root canal, temporary filling
- After 3 weeks, If no symptom, all the medication removed and iriigation
- Bleeding was induced by overinstrumentating with a #25 file
- Allowed reach 3-4 mm below the CEJ to form a blood clot
- An Asorbable colagen barier was place of the top blood clot dan white MTA

Apexification

- Anestesi, Isolation, Access opening, Irrigation (NaOCl, Saline and EDTA)
- The canal dried with paper point, calcium hydroxide paste was placed into the canal, temporary filling
- One week later, the tooth was reaccessed after isolastion with rubber dam
- Calcium hydroxide paste was removed using EDTA 17%
- The root canal was dried with paper point
- Calcium hydroxide paste was injected into the canal before the tooth was sealed with glass ionomer cement.

Cases of Apexification



Case of RET

Research

TABLE 3. Clinical Symptoms and Root Canal Morphology at the 12-month Follow-up

Treatment	Asymptomatic, *n* (%)	Resolution of radiolucency, *n* (%)	Increase of root length, *n* (%)	Increase of root thickness, *n* (%)	Apex closure, *n* (%)	Survival rate, *n* (%)
RET (*n* = 69)	69 (100)	69 (100)	56 (81.16)	57 (82.60)	46 (65.21)	69 (100)
Apexification (*n* = 34)	34 (100)	34 (100)	9 (26.47)	0 (0)	28 (82.35)	34 (100)

Type of change of the root morphology

- Type I : Continued Root Formation and Decreased Apical Foramen Size
- Type II : Increased Root Length and no change apical foramen size
- Type III : No Increased Root Length but a decrease in the size of the apical foramen
- Type IV : No significant root length increase nor decrease in the size of the apical foramen

Change of the root morphology

- Type I : Continued Root Formation and Decreased Apical Foramen Size
- Type II : Increased Root Length and no change apical foramen size
- Type III : No Increased Root Length but a decrease in the size of the apical foramen
- Type IV : No significant root

❖ Type I was regarded as the ideal outcome

❖ Type IV was defined as failure

❖ Type I, II, III were recognized was as a successful (there was no recurrence of apical periodontitis)

❖ Type I (dens evaginatus)

Research

TABLE 6. Classification of Root Morphology at the 12-month Follow-up

Types	Root length	Apical foramen size
I	Increased	Decreased
II	Increased	Unchanged
III	Unchanged	Decreased
IV	Unchanged	Unchanged

Research

- **Lin et al, Journal of Endodontic, 2017**

Clinical Research

TABLE 7. Outcomes of Root Morphology in the Regenerative Endodontic Treatment (RET) and Apexification Groups

	RET		Apexification	
Types	Dens evaginatus (n = 48)	Trauma (n = 21)	Dens evaginatus (n = 21)	Trauma (n = 13)
I	44 (91.6%)	7 (33.3%)	7 (33.3)	2 (15.4)
II	3 (6.3%)	2 (9.5%)	0	0
III	0	6 (28.6%)	14 (66.7)	10 (76.9)
IV	1 (2.1%)	6 (28.6%)	0	1 (7.7)
Success rate (%)	97.9*	71.4	100	92.3
Overall success rate (%)	89.8		97	

Clinical Outcome (AAE)

1

Primary Goal (essential) : The elimination of symptoms and the evidence of bony healing

2

Secondary Goal (desirable) : Increased Root Wall Thickness and/or Increased Root Length

3

Tertiary goal : positive response to vitality testing

Histological Outcome

- It was found that fibrous connective tissue and bone grew into the canal space
- Regenerate new pulp tissue
- The stem cells from the apical papilla might migrate intothe desinfected root canal space and differntiate into odontoblast to produce dentine in RET

Conclusion

RET is based on the concept of tissue engineering technology to regenerate the dentin-pulp complex in the canal space of immature permanent teeth damaged by caries, trauma and developmental malformation

RET has the potential to encourage continued root formation of immature teeth with necrotic pulp/apical periodontitis

The tissue formed in the canal space after RET i**s not pulp like tissue but periodontal like tissue**

References

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