REGENERATIVE ENDODONTICS

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CONTENTS

Scaffold

Growth factors

Potential technologies for regenerative endodontics

- Root canal revascularization
- Postnatal stem cell therapy
- Pulp implantation
- Scaffold implantation
- Injectable scaffold delivery
- Three dimensional cell printing
- Gene therapy

Clinical outcomes

overview of clinical cases

Potential complications/undesired outcomes

Conclusion

INTRODUCTION

- The term 'revascularization' was first used by ***Iwaya et al. (2001***).

- Later, revitalization instead of revascularization was proposed as a more applicable term as the tissues regenerated in the canal space were not only blood vessels but also hard and soft tissues ***(Huang & Lin 2008)***.

- The term '**regenerative endodontics**' was adopted by the **American Association of Endodontists in 2007** (Murray *et al*. 2007), based on a tissue engineering concept.

- **Regenerative endodontic therapy** provides an alternative treatment approach that builds on the principles of regenerative medicine and tissue engineering.

REGENERATIVE ENDODONTICS

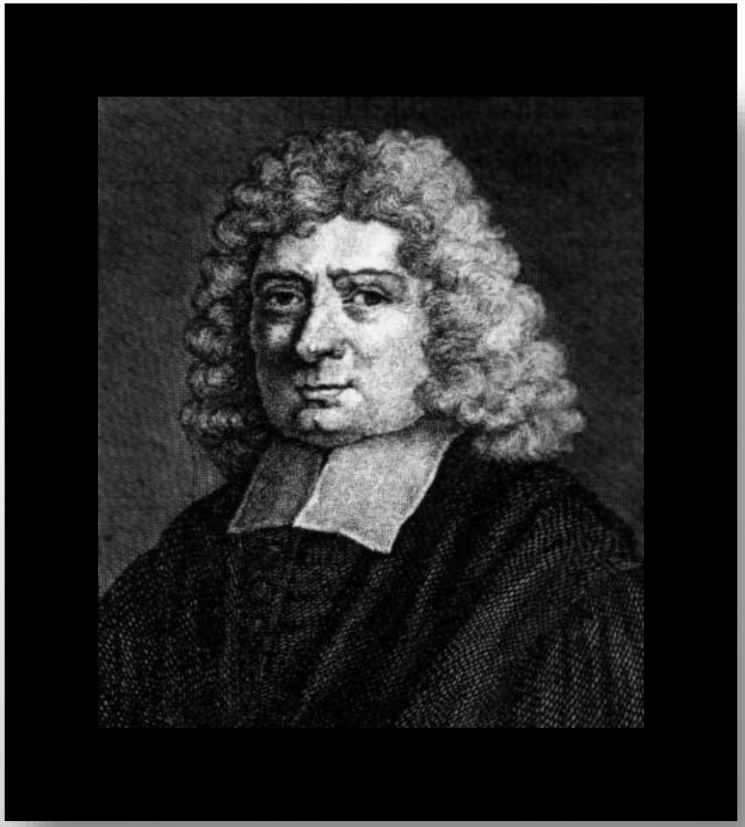
- ***REGENERATIVE ENDODONTIC PROCEDURE CAN BE DEFINED AS BIOLOGICALLY BASED PROCEDURE DESIGNED TO REPLACE DAMAGED STRUCTURES, INCLUDING DENTIN AND ROOT STRUCTURES AND CELLS OF PULP DENTIN COMPLEX.***

AAE

- Regenerative endodontics evolved out of early experiments on the role of the blood clot in endodontic therapy , coupled with an understanding that revascularization, or reestablishment of a vascular supply to existing pulp tissue, is essential for continuation of root development after traumatic injuries .

- Other contributing factors have been the expansion of stem cell research, in particular the discovery of mesenchymal stem cells with the potential to differentiate into odontogenic-like cell lines and the potential for therapeutic applications of tissue engineering .(AAE)

HISTORY



Regenerative dental procedures have a long history, originating around 1952, when Dr. B. W. Hermann reported on the application of $Ca(OH)_2$ in a case report of vital pulp amputation

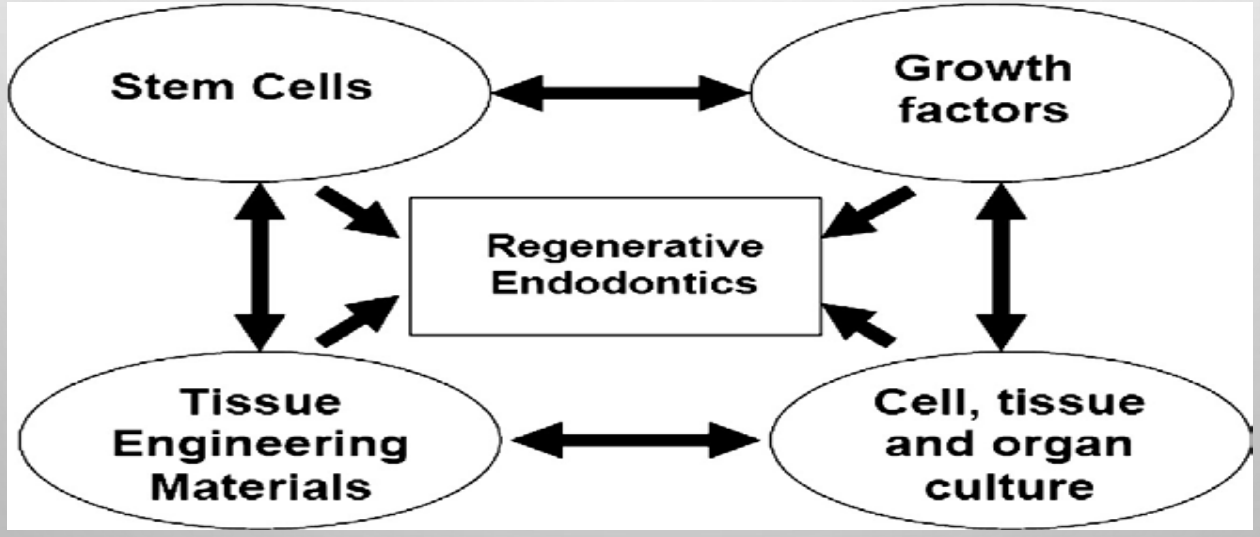
Professor Nygard Ostby evaluated a revascularisation method for re-establishing a pulp dentine complex in permanant teeth with pulp necrosis(1961).

1980 – Polymer chemist (Robert Langer) and an organ transplant surgeon (Joseph Vacanti) proposed that – it might be possible to regenerate a tissue or organ by seeding the cells that make this tissue into a biodegradable scaffold.

Tissue Engineering ????

- An interdisciplinary field that integrates the principles of biology and engineering to develop biological substitutes that replace or regenerate human cells, tissue or organs in order to restore or establish normal function (9).

- **stem cells, scaffolds** and **growth factors.**



Biological Basis for Regenerative Endodontic Therapy ??

- Historically, long-term calcium hydroxide treatment was used to induce apexification of the immature tooth with pulpal necrosis before placing an obturation material such as gutta-percha in the root canal system.

- While the success rate of calcium hydroxide apexification is reported to be as high as 95%, there are several associated problems.

Barrier formed - porous & not continuous

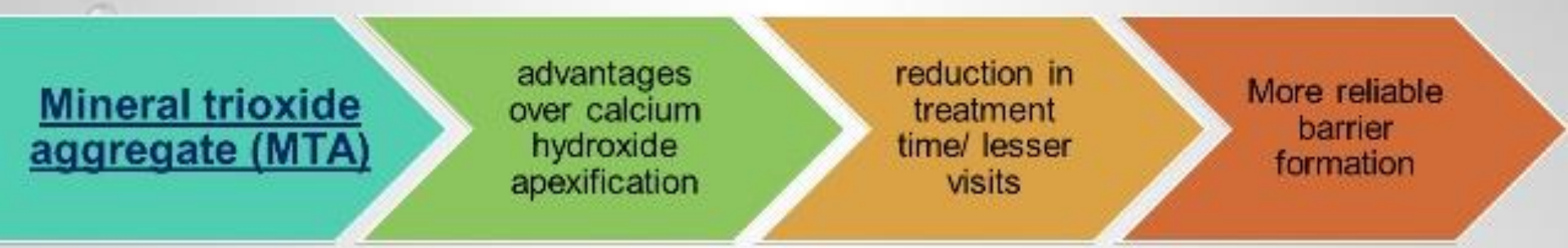
Strengthening or reinforcing of the thin fragile blunderbuss canals is not achieved

Calcium hydroxide induced apexification LIMITATIONS

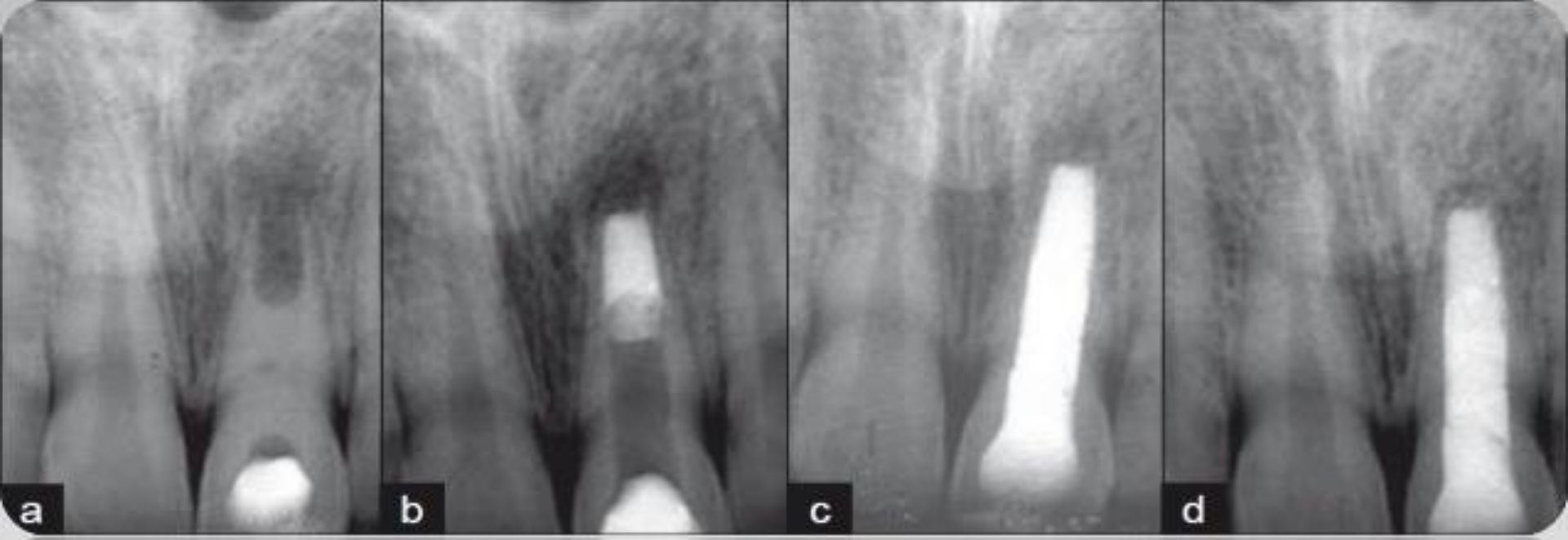
Long term therapy for barrier formation

3-24 months +multiple visits

Makes tooth brittle due to hygroscopic and proteolytic properties



- Studies on MTA apexification report that the success rate of the treatment is as high as 94%.
- Prospective clinical trials comparing MTA apexification to calcium hydroxide apexification report that the success rate of the former is comparable to or higher than that of the latter



However, neither of the apexification treatments fosters further root development and immature teeth remain vulnerable to cervical root fractures.

In contrast, regenerative endodontic therapy has the potential for increased root development, and thus, may confer a **better long-term prognosis**.

In addition, successful regeneration of the pulp-dentin complex would likely result in vital tissue capable of mounting an immune response and signaling tissue damage by sensory neurons.

Considerations for Clinical Regenerative Endodontic Procedures

Various regenerative endodontic treatment protocols have been associated with a successful clinical outcome and currently there is no single recommended protocol.

Common features of cases with successful clinical outcomes after REPs are:

- Young patient
- Necrotic pulp and immature apex
- Minimal or no instrumentation of the dentinal walls
- Placement of an intracanal medicament
- Creation of a blood clot or protein scaffold in canal
- Effective coronal seal

Based on Cvek's classification of root development (Cvek 1992)

- ✓ Immature permanent teeth with 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 apex) are suitable for RET (short root, thin canal walls and wide-open apex as apexification has no potential for root maturation (thickening of the canal walls and/or continued root development).

- ✓ Immature permanent teeth at stage 4 (nearly completed root formation with open apex) can be managed with either RET or an apical MTA plug and root canal filling because the canal walls have enough thickness and strength.

- ✓ Immature permanent teeth with a necrotic pulp requiring post for adequate coronal restoration are not suitable for RET and better treated with apical MTA plug and root canal filling.

OBJECTIVES

a)Regenerate pulp-like tissue, ideally, the pulp-dentin complex

b)Regenerate damaged coronal dentin, such as following a carious exposure

c)Regenerate resorbed root, cervical or apical dentin.

Regenerative Endodontics: A Review of Current Status and a Call for Action *Peter E. Murray, BSc(Hons), PhD,* Franklin*

SUBSEQUENT REGENERATIVE DENTAL PROCEDURES

- ✓ Guided tissue or bone regeneration (GTR, GBR) procedures
- ✓ Distraction osteogenesis
- ✓ Application of platelet rich plasma (PRP) for bone augmentation
- ✓ Emdogain for periodontal tissue regeneration
- ✓ Recombinant human bone morphogenic protein (rhBMP) for bone augmentation
- ✓ Preclinical trials on the use of fibroblast growth factor 2(FGF2) for periodontal tissue regeneration.

Tissue engineering triad

- Stem cells
- Scaffold
- Growth factors

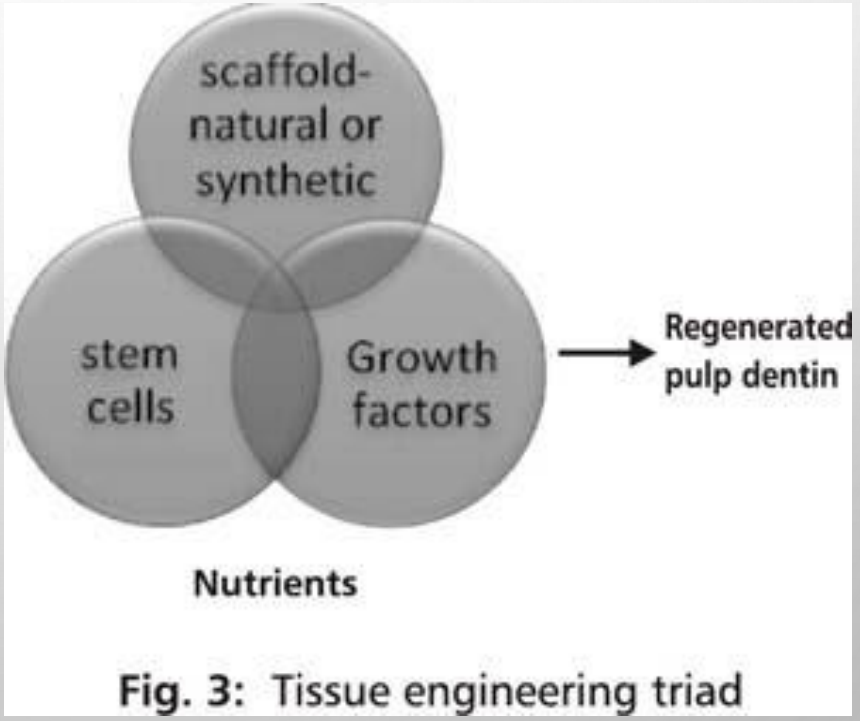
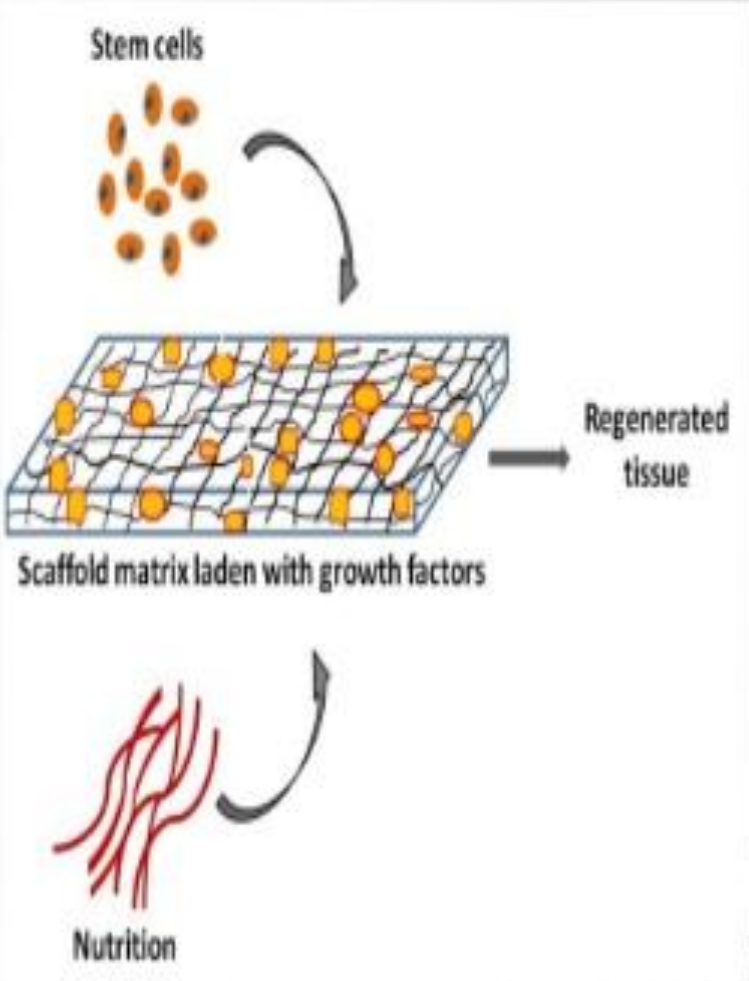


Fig. 3: Tissue engineering triad

Three major components of pulp regeneration

- A reliable **cell source** capable of differentiating into odontoblasts
- **Growth factors** that are capable of stimulating cellular proliferation and directing cellular differentiation.
- An appropriate **scaffold** to promote cell growth and differentiation



Stem Cells

- ✓ The most valuable cells for regenerative medicine are stem cells.
- ✓ All tissues originate from stem cells.
- ✓ ***A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues.***

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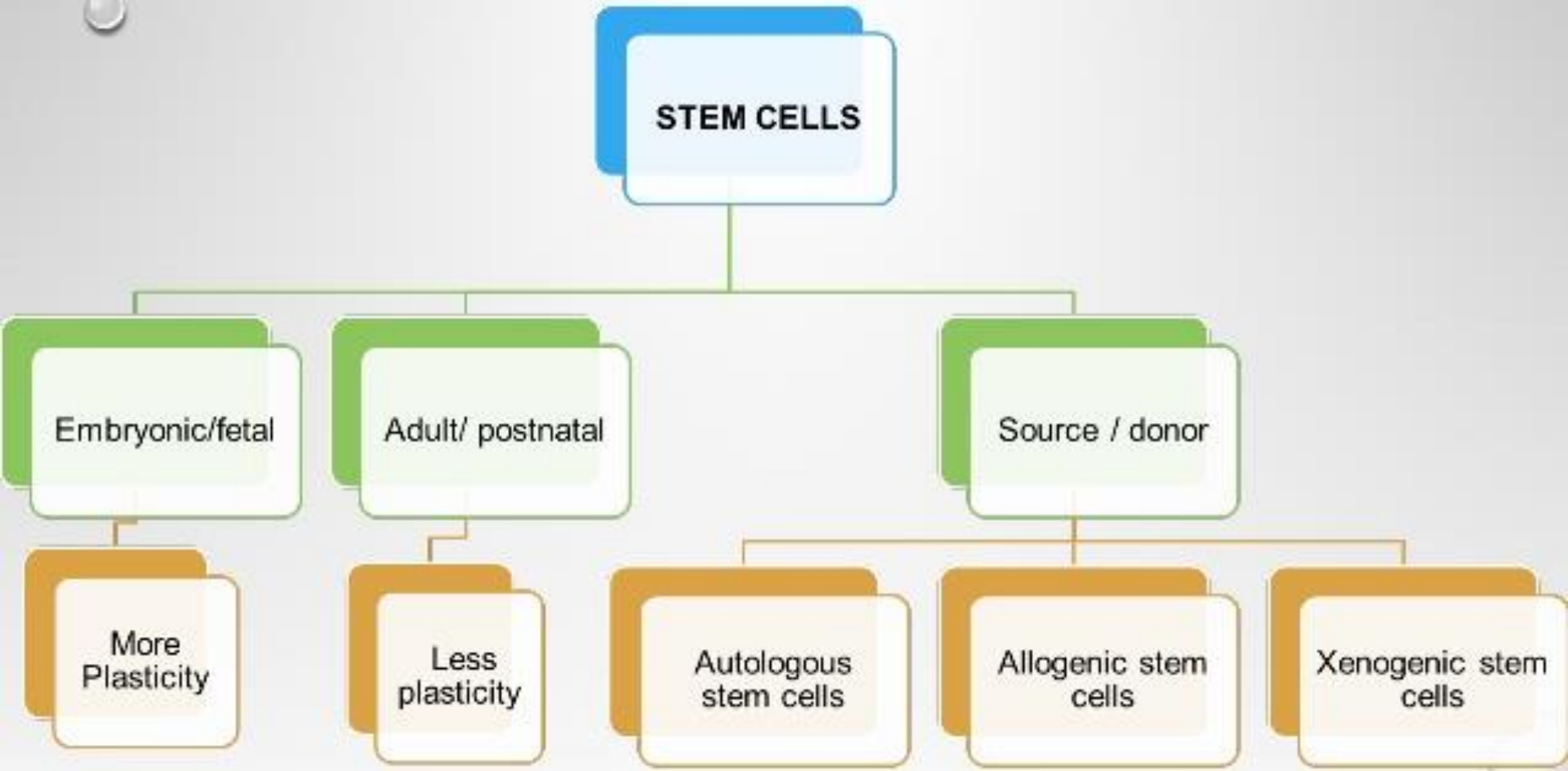
CLASSIFICATION OF STEM CELLS (GENERAL)



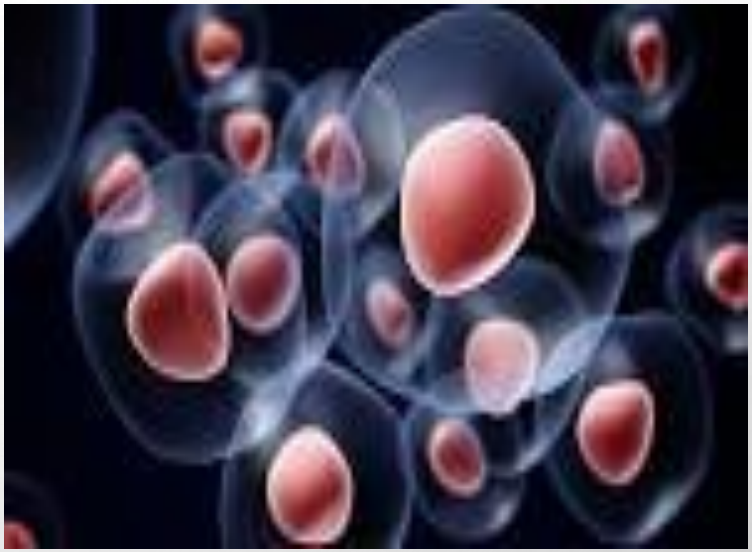
- ***The greater plasticity of the embryonic stem cells*** makes these cells more valuable among researchers for developing new therapies . However, the sourcing of embryonic stem cells is controversial and is surrounded by ***ethical and legal issues,*** which reduces the attractiveness of these cells for developing new therapies.

- The application of **postnatal stem cell therapy** was launched in **1968,** when the first allogenic bone marrow transplant was successfully used in the treatment of severe combined immunodeficiency.

- Postnatal stem cells have been sourced from *umbilical cord blood, umbilical cord, bone marrow, peripheral blood, body fat, and almost all body tissues* , including ***the pulp tissue of teeth***

Stem cells are often categorized by their sources

- Autologous stem cells
- Allogenic stem cells
- Xenogenic cells



AUTOLOGOUS STEM CELLS

- Bone marrow harvesting of a ***patient's own stem cells*** and their re-implantation back to the ***same patient*** represents one clinical application of autogenous postnatal stem cells.

- Stem cells could be taken from the bone marrow, peripheral blood, fat removed by liposuction, the periodontal ligament, oral mucosa or skin.

- An example of an autologous bank is one that store umbilical cord stem cells.

- Autologous stem cells have the fewest problems with immune rejection and pathogen transmission (45).

- Harvesting the patient's own cells makes them the least expensive to obtain and avoids legal and ethical concerns

- One potential disadvantage of harvesting cells from patients is that surgical operations might lead to postoperative sequelae, such as donor site infection

To accomplish endodontic regeneration, the most promising cells are autologous postnatal stem cells, because these appear to have the fewest disadvantages that would prevent them from being used clinically.

ALLOGENIC STEM CELLS

- Originate from a ***donor of the same species*** .
- Examples of donor allogenic cells include blood cells used for a blood transfusion , bone marrow cells used for a bone marrow transplant and donated egg cells used for in vitro transplantation
- The most serious disadvantages of using pre-existing cell lines from donors to treat patients are the risks of immune rejection and pathogen transmission

XENOGENIC CELLS

- Isolated from ***individuals of another species***.

Eg : Pig tooth pulp cells have been transplanted into mice, and these have formed tooth crown structures

- Many problems remains , such as the high potential for immune rejection and pathogen transmission from the donor animal to the human recipient.

Stem cell potency

TABLE 1. Types of stem cells

Stem cell type	Cell Plasticity	Source of stem cell
Totipotent	Each cell can develop into a new individual	Cells from early (1–3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5–14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and postnatal stem cells including dental pulp stem cells

Different populations of adult stem cells have been identified in tissue compartments in the oral region.

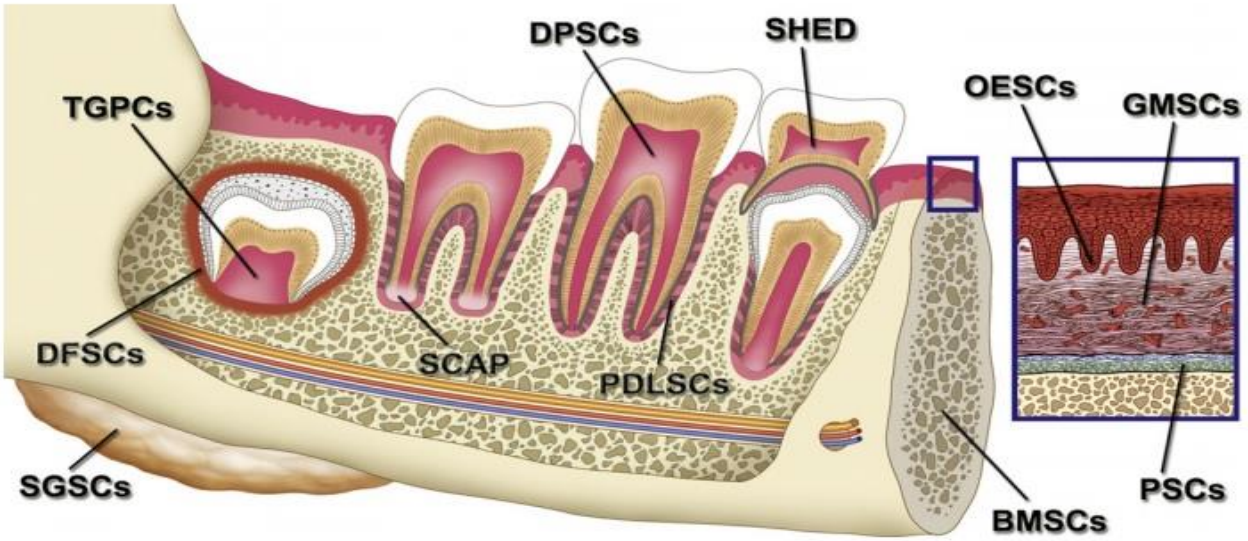


Fig. 2. Sources of adult stem cells in the oral and maxillofacial region. BMSCs: bone marrow-derived MSCs from orofacial bone (see Section 2.1.2); DPSCs: dent[illegible] pulp stem cells; SHED: stem cells from human exfoliated deciduous teeth; PDLSCs: periodontal ligament stem cells; DFSCs: dental follicle stem cells; TGPCs: too[illegible] germ progenitor cells; SCAP: stem cells from the apical papilla (see Section 2.1.3); OESCs: oral epithelial progenitor/stem cells; GMSCs: gingiva-derived MSCs (see Section 2.1.4), PSCs: periosteum-derived stem cells (see Section 2.1.5); SGSCs: salivary gland-derived stem cells (see Section 2.1.6).

Although stem cells have been identified in most oral tissues, the stem cells more likely to be involved in REPS are localized around the periapical region. These include SCAP, PDLSCs, BMSCs, and DPSCs (if vital pulp is still present apically).

SOURCES OF POST NATAL STEM CELLS

Potential Stem cell sources for development of pulp and dentin

- ***Dental pulp stem cells (DPSC):*** **derived from third molar.**
- ***Stem cells from human-exfoliated deciduous teeth (SHED):*** **stem cells are present within the pulp tissue of deciduous teeth.**
- ***Stem Cells from apical papilla (SCAP).***
- ***Bone marrow derived mesenchymal stem cells(BMMSC).***
- ***Dental follicle progenitor cells(DFPC)..***
- ***Periodontal ligament stem cells (PDLSC).***

- Two populations of stem cells involved in tooth formation are

✓**Epithelial stem cells**

✓**Mesenchymal stem cells**

- Mesenchymal stem cells located in a perivascular niche in the dental pulp, periodontal ligament, dental follicle and bone marrow may be potential sources for cell based therapies in regenerating the tooth.

BONE *MARROW STEM CELLS (BMSCS)*

- Both BMSCs and DPSCs can differentiate into osteoblasts, chondrocytes, adipocytes and odontoblasts exhibiting the ability to generate osteoid or odontoid structures, although BMSCs display a lower odontogenic competence than DPSCs.

- BMSCs have demonstrated good ability to form tooth supporting periodontal structures like cementum, periodontal ligament and alveolar bone suggesting their potential use for treating periodontal diseases.

- Because of their limited potential to generate odontoblasts, their use in pulp dentin regeneration may be limited and remains to be further explored

HUMAN DENTAL PULP STEM CELLS (HDPSCS)

- Gronthos *et al*, were the first to successfully isolate a population of cells from human dental pulp which exhibited high proliferative capacity, multilineage differentiation potential, ability to self renew and form dentin pulp complex

- Dental pulp stem cells have gene expression profiles and differentiation capacity similar to BMSCs and under appropriate conditions can differentiate into osteoblasts, odontoblasts,chondrocytes and adipocytes .

- They also have an ability to differentiate into neural like cells under neurogenic induction medium.

- Dental Pulp stem cells/ progenitor cells are found to reside in the central cell rich zone of the pulp particularly in the perivascular and perineurosheath regions.

STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH (SHED)

- Exfoliating deciduous teeth contain living pulp remnants and are good sources of cells which are highly proliferative, clonogenic and have multi differentiation potential.

- These cells have been termed as stem cells from human exfoliated deciduous teeth (SHED) and were isolated and characterized by Miura *et al*.

- SHED offers attractive advantages over other post natal stem cells, as they are derived from a source which is non invasive, readily accessible, naturally being disposed and with very limited ethical or legal concerns.

- SHED is turning into a favourite choice for commercial stem cell banks where autologous stem cell sources may be stored for future use.

- Compared to BMSCs and hDPSCs, SHED shows a higher proliferation rate and higher self renewal capabilities. They exhibit differentiation ability to convert into adipocytes, neural cells and odontoblasts but not into osteoblasts.

- They exhibit an osteoinductive potential in which the host cells are stimulated to differentiate into bone forming cells.

STEM CELLS FROM THE APICAL PAPILLA (SCAP)

- A newly discovered population of stem cells by Sonoyama *et al* who termed them as stem cells of apical papilla (SCAP).

- In immature teeth, when the roots are still developing, dental papilla assumes a position apical to the pulp tissue and the epithelial diaphragm.

- This apical papilla is loosely attached to the apex of the root from where it can be easily detached.

- Apical papilla is less cellular and vascular compared to dental pulp
- SCAP Compared to DPSCs shows a proliferation rate higher by 2-3 folds.
- SCAP exhibits osteogenic, dentinogenic, adipogenic and neurogenic differentiation capabilities when exposed to the respective stimuli.

PERIODONTAL LIGAMENT STEM CELLS (PDLSC)

- McCulloch reported the presence of progenitor/stem cells in the periodontal ligament of mice in 1985.

- The isolation and identification of multipotent MSCs in human periodontal ligaments were first reported in 2004

- Seo and colleagues demonstrated the presence of clonogenic stem cells in enzymatically digested PDL and further showed that human PDLSCs transplanted into immunodeficient rodents generated a cementum/PDL-like structure that contributed to periodontal tissue repair.

- Later work showed that PDLSCs differentiation was promoted by Hertwig's epithelial root sheath cells in vitro.
- PDLSCs have the capability to differentiate into cementoblast like cells, adipocytes, and fibroblasts that secrete collagen type I.
- As with BMMSCs, PDLSCs can undergo osteogenic, adipogenic, and chondrogenic differentiation.
- PDLSCs have also been shown to differentiate into neuronal precursors.

Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: A morphological report. Journal of Biomedical Materials Research Part A, 87A(4), 986–993. Trubiani, O., Orsini, G., Zini, N., Di Iorio, D., Piccirilli, M., Piattelli, A., & Caputi, S. (2008).

DENTAL FOLLICLE PROGENITOR CELLS(DFPC)

- The dental follicle is a loose vascular connective tissue that contains the developing tooth germ, and Dental Stem Cells .
- DFPCs were first isolated from the dental follicle of human third molars.
- Because DFPCs come from developing tissue, it is considered that they might exhibit a greater plasticity than other DSCs.
- Different cloned DFPC lines have demonstrated great heterogeneity.
- DFPCs shown to differentiate into cementoblast like and osteogenic like cells.

NEUROGENIC POTENTIAL OF DENTAL PULP STEM CELLS

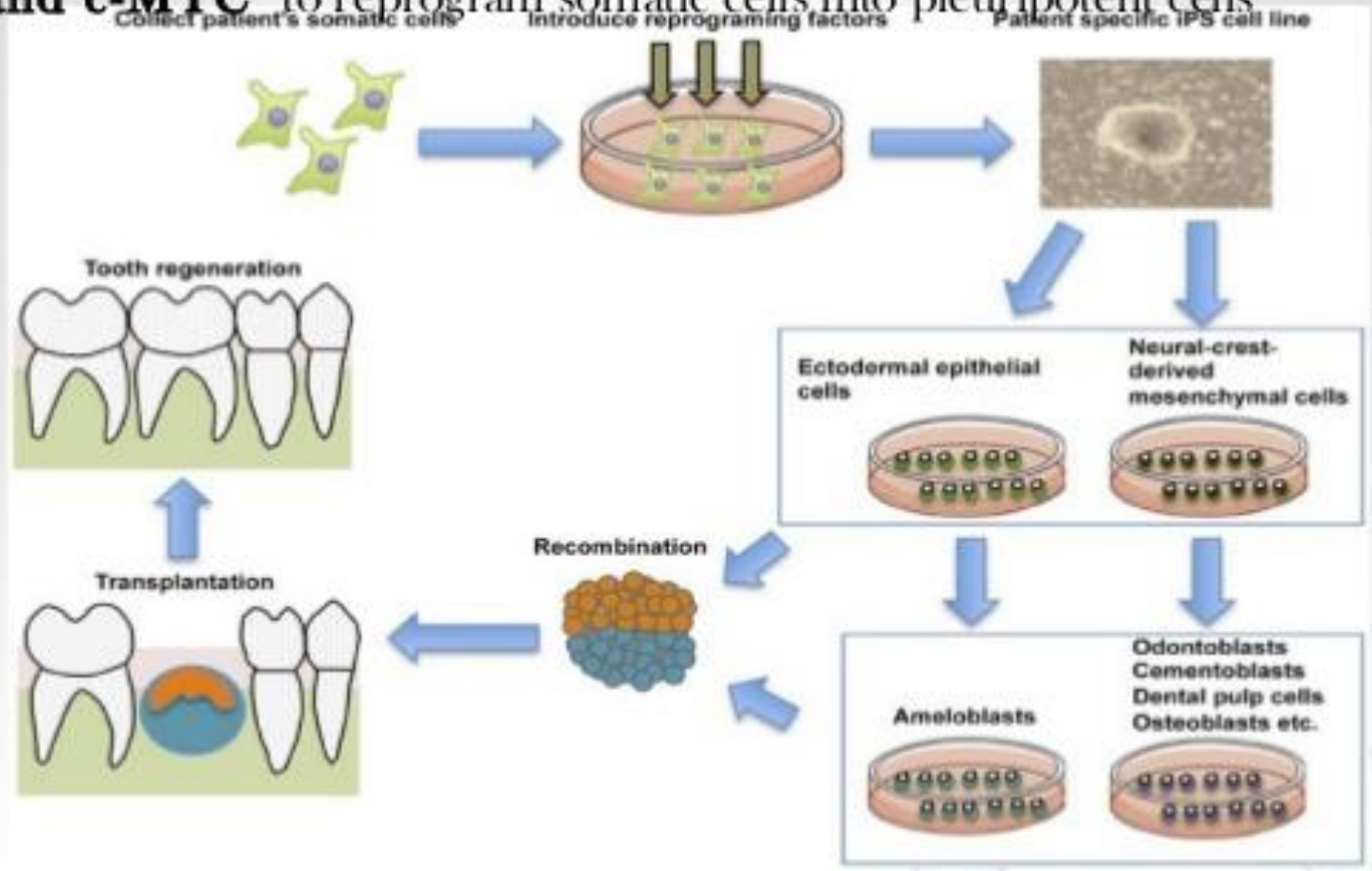
- The fact that dental pulp stem cells are derived from neural crest mesenchyme raises hope that they may be good sources of stem cells to treat neural tissue injuries or degenerative diseases.

- DPSCS, SHED, SCAP and DFSCs possess differentiation properties of MSCs and NSCs.

- These stem cells have expressed a variety of neural cell markers like nestin, tubullin, GAD (glutamic acid decarboxylase), Neu N (neuronal nuclei), GFAP (glial fibrillary acidic protein), NFM (neurofilament M) .

INDUCED PLEURIPOTENT CELLS

This technique was developed using a quartet of **trascription factors- OCT3,SOX2, KLF4 and c-MYC** to reprogram somatic cells into pleuripotent cells



STEM CELL ISOLATION

- *SIZE-SIEVED ISOLATION*

Enzymatic digestion of whole dental pulp tissue in solution of 3% collagenase type I for 1 h at 37°C is done. Through process of filtering and seeding, cells with diameter between 3 and 20 μm are obtained for further culture and amplification. Based on this approach, small-sized cell populations containing a high percent of stem cells can be isolated.

STEM CELL COLONY CULTIVATION

Enzymatic digestion of the dental pulp tissue is done to prepare single cell suspension cells of which are used for colony formation containing 50 or more cells that is further amplified for experiments.

MAGNETIC ACTIVATED CELL SORTING (MACS)

Is an immune-magnetic method used for separation of stem cell populations based on their surface antigens (CD271, STRO-1, CD34, CD45, and c-Kit). MACS is technically simple, inexpensive and capable of handling large numbers of cells but the degree of stem cell purity is low.

FLUORESCENCE ACTIVATED CELL SORTING (FACS)

Is convenient and efficient method that can effectively isolate stem cells from cell suspension based on cell size and fluorescence. Demerits of this technique are a requirement of expensive equipment, highly-skilled personnel, decreased viability of FACS-sorted cells and this method is not appropriate for processing bulk quantities of cells.

STEM CELL IDENTIFICATION

(a)Staining the cells with specific antibody markers and using a flow cytometer in a process called fluorescent antibody cell sorting (FACS);

(b) immunomagnetic bead selection;

(c) immunohistochemical staining; and

(d) physiological and histological criteria, including phenotype (appearance), chemotaxis, proliferation, differentiation, and mineralizing activity.

(e)FACS together with the protein marker CD34 is widely used to separate human stem cells expressing CD34 from peripheral blood, umbilical cord blood, and cell cultures.

STORAGE OF STEM CELLS

CRYOPRESERVATION :

- It is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures.
- Cells harvested near end of log phase growth (approximately. 80–90% confluent) are best for cryopreservation.
- Liquid nitrogen vapour is used to preserve cells at a temperature of -196^{0}c .
- In a vial 1.5 ml of freezing medium is optimum for 1–2 × 10^{6} cells.

MAGNETIC FREEZING

- This technology is referred to as cells alive system (CAS)

- It works on principle of applying a weak magnetic field to water or cell tissue which will lower the freezing point of that body by up to 6–7°C.

- Using CAS, Hiroshima University (first proposed this technology) claims that it can increase the cell survival rate in teeth to 83%.

- CAS system is a lot cheaper than cryogenics and more reliable

(DENTAL STEM CELL AND DENTAL TISSUE REGENERATION)

Table 1 Characteristics of different types of dental stem cells

Types	Tissue sources	Markers	Differentiation potency
DPSC	Adult human dental pulp	STRO-1, CD146	Odontoblast-like cells, osteoblasts, adipocytes, neural cells
SHED	Pulp of exfoliated deciduous teeth	STRO-1, CD146/MUC18, CD90, CD29, CD44, CD166, CD105, CD13	Odontoblasts, osteoblasts, adipocytes, neural cells
PDLSC	Periodontal ligament	STRO-1, CD146, CD73, CD90, CD105	Osteoblast-like cells, adipocytes, collagen-forming cells
SCAP	Apical papilla	STRO-1, CD146, CD24	Odontoblasts
DFC	Dental follicle	STRO-1, CD105, CD90, nestin, notch-1	Periodontal ligament cells, osteoblasts, cementoblasts

MORPHOGENS

- Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation.
- Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific .
- GROWTH FACTORS → CONTROL STEM CELL ACTIVITY SUCH AS

stimulating stem cells to synthesize and secrete mineralized matrix.

by increasing the rate of proliferation

inducing differentiation of the cells into another tissue type

- Platelet derived growth factors
- Bone morphogenetic proteins (BMPs)
- Vascular endothelial growth factor
- Fibroblast growth factors
- Transforming growth factor b
- Insulin like growth factor

Colony stimulating growth factors

Epidermal growth factor

Insulin like growth factor I or II

Interleukins IL– 1 to 13

Nerve growth factor

Stromal cell derived growth factor

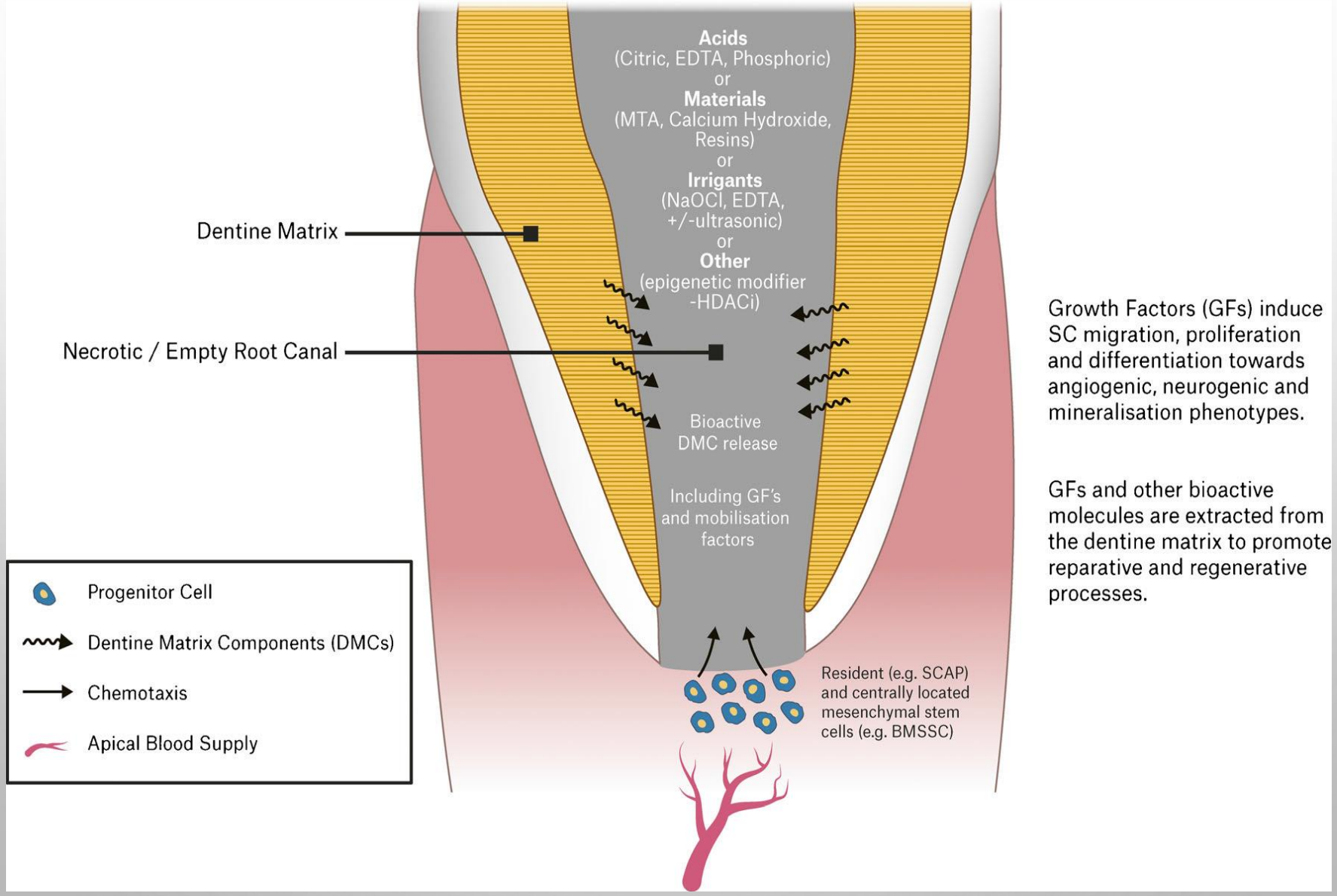
REPAIR & REGENERATION	PDGF, TGF, BMP, VEGF.FGF, IGF
ANGIOGENESIS	FGF2, PDGF, VEGF
NEURONAL GROWTH	NGF
DIFFERENTIATION	TGF2, PDGF, FGF2, BMP 2,4,7,11,IGF, NGF
PROLIFERATION	FGF2, SD-1, TGFß1, VEGF, PDGF
CHEMOTAXIS	SDF-1, TGFß1, PDGF, FGF2

Abbreviation	Factor	Primary Source	Activity	Usefulness
BMP	Bone morphogenetic proteins	Bone matrix	BMP induces differentiation of osteoblasts and mineralization of bone	BMP is used to make stem cells synthesize and secrete mineral matrix
CSF	Colony stimulating factor	A wide range of cells	CSFs are cytokines that stimulate the proliferation of specific pluripotent bone stem cells	CSF can be used to increase stem cell numbers
EGF	Epidermal growth factor	Submaxillary glands	EGF promotes proliferation of mesenchymal, glial and epithelial cells	EGF can be used to increase stem cell number
FGF	Fibroblast growth factor	A wide range of cells	FGF promotes proliferation of many cells	FGF can be used to increase stem cell numbers

IGF	Insulin-like growth factor-I or II	I - liver II– variety of cells	IGF promotes proliferation of many cell types	IGF can be used to increase stem cell numbers
IL	Interleukins IL-1 to IL-13	Leukocytes	IL are cytokines which stimulate the humoral and cellular immune responses	Promotes inflammatory cell activity
PDGF	Platelet-derived growth factor	Platelets, endothelial cells, placenta	PDGF promotes proliferation of connective tissue, glial and smooth muscle cells	PDGF can be used to increase stem cell numbers
TGF- alpha	Transforming growth factor-alpha	Macrophages, brain cells, and keratinocytes	TGF-□ may be important for normal wound healing	Induces epithelial and tissue structure development

TGF-β	Transforming growth factor-beta	Dentin matrix, activated TH1cells (T-helper) and natural killer (NK) cells	TGF-β is anti-inflammatory, promotes wound healing,inhibits macrophage and lymphocyte proliferation	TGF-1 is present in dentin matrix and has been used to promote mineralization of pulp tissue
NGF	Nerve growth factor	A protein secreted by a neuron's target tissue	NGF is critical for the survival And maintenance of sympathetic and sensory neurons.	Promotes neuron outgrowth and neural cell survival

DENTIN MATRIX AS AN ENDOGENOUS SOURCE OF GROWTH FACTORS



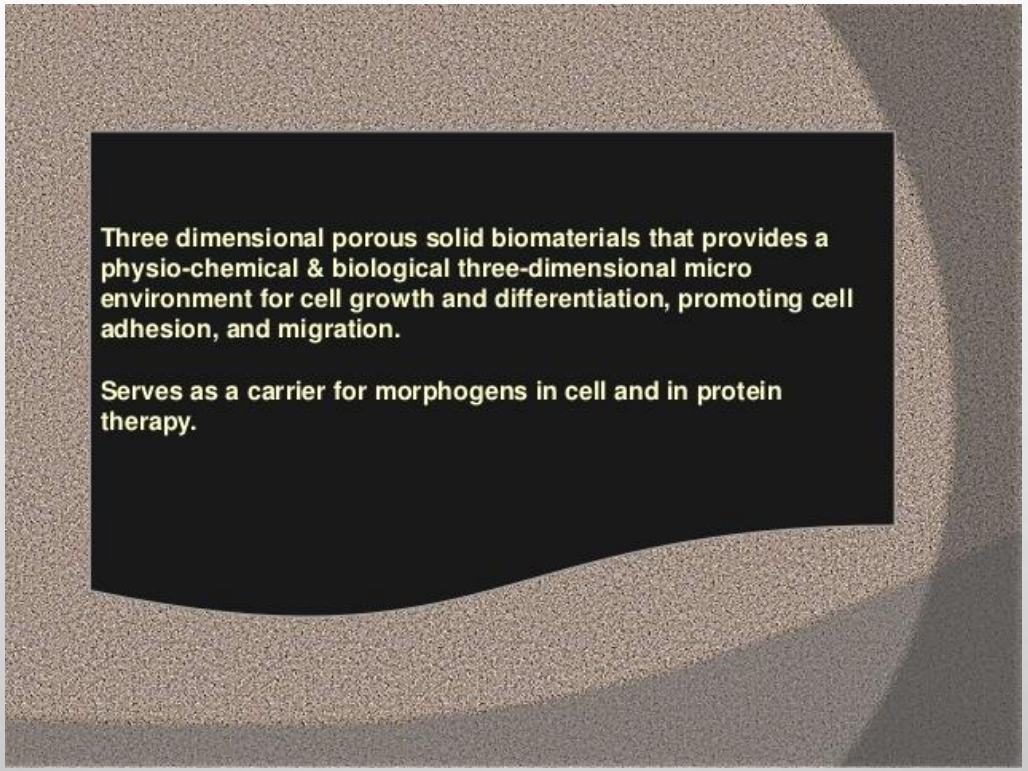
Growth Factors (GFs) induce SC migration, proliferation and differentiation towards angiogenic, neurogenic and mineralisation phenotypes.

GFs and other bioactive molecules are extracted from the dentine matrix to promote reparative and regenerative processes.

- Irrigation, as part of chemical disinfection, has attracted particular attention as it has the potential to release a range of DMCs including GFs beneficial to cell migration, proliferation, and differentiation .Irrigating with 17% EDTA can release TGF-β family members from the extracellular matrix of dentin .

- Sodium hypochlorite, however, may have a deleterious effect on SCAP cell survival and differentiation ability, leading to suggestions that the final rinse in cell homing procedures should be with a 17% EDTA solution

SCAFFOLDS


Three dimensional porous solid biomaterials that provides a physio-chemical & biological three-dimensional micro environment for cell growth and differentiation, promoting cell adhesion, and migration.
Serves as a carrier for morphogens in cell and in protein therapy.

Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds.

Scaffolds usually serve at least one of the following purposes:	allow cell attachment and migration
	deliver and retain cells and biochemical factors
	Enable diffusion of vital cell nutrients and expressed products
	Provides structural support and shape to construct

IDEAL REQUIREMENTS OF A SCAFFOLD

- A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout whole structure of both cells and nutrients

- Should allow effective transport of nutrients, oxygen, and waste

- Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal

- The rate at which degradation occurs has to coincide with the rate of tissue formation
- Should be biocompatible

- Should have adequate physical and mechanical strength.

Based on degradability of matrices[11]	Based on form[12]	Based on presence or absence of cells[13]
Biodegradable scaffolds Permanent or biostable scaffolds	Solid blocks Sheets Porous sponges Hydrogels (injectable scaffolds)	Cell free scaffolds Scaffolds seeded with stem cells

Based on origin[1]	
Biological or natural scaffolds	**Artificial or Synthetic scaffolds**
Platelet rich plasma[14] Platelet rich fibrin[15] Collagen Chitosan Glycosaminoglycans/hyaluronic acid Demineralized or native dentin matrix[1] Blood clot[16] Silk[17]	Polymers[1] PLA PGA PLGA PCL Bioceramics[1] Calcium/ phosphate materials Bioactive glasses Glass ceramics

PLA: Polylactic acid; PGA: Polyglycolic acid; PLGA: Polylactic-coglycolic acid; PCL: Polyepsiloncaprolactone

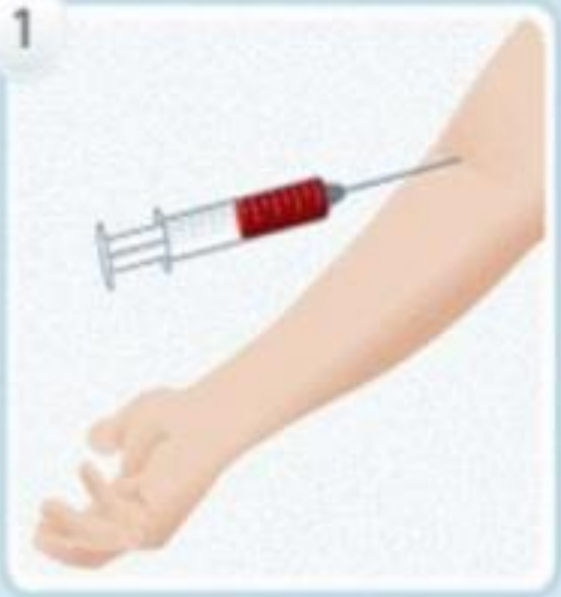
PLATELET RICH PLASMA

- Autologous first generation platelet concentrate with a rich sourcs of growth factors.
- Introduced in 1997 by Whitman
- Potential substitute scaffold
- Easy to prepare, rich in growth factors, 3D fibrin matrix that helps to entrap growth factors.

- Platelet concentration – Exceeds 1 million/mL – 5 times more than that of normal platelet
- It is a concentrated suspension of different growth factors like PDGF, TGF-b, IGF, VEGF, epidermal growth factor, epithelial cell factor
- Released via degranulation of alpha granules & stimulate bone & soft tissue healing
- Disadvantage – Drawing blood in young patients, need special equipment & reagents to prepare, increased cost of treatment.

PROCESS OF PRP THERAPY

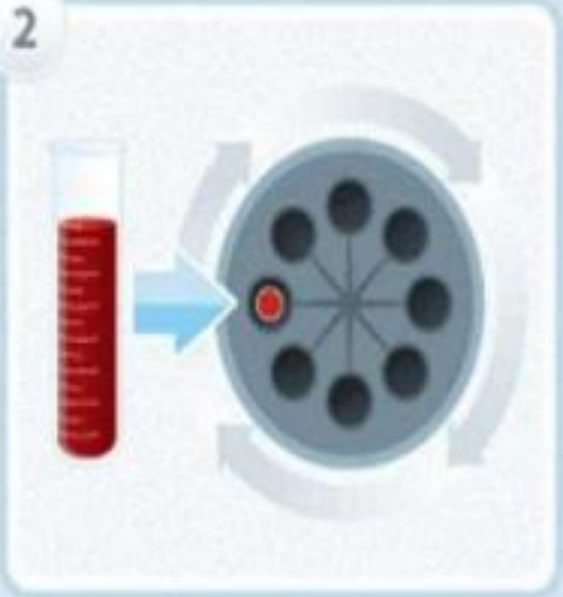
1



Collect blood

30-60ml of blood is drawn from the patient's arm.

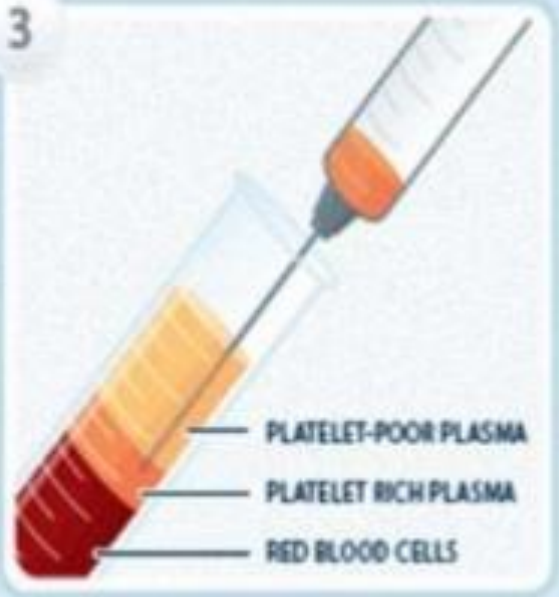
2



Separate the platelets

The blood is then placed in a centrifuge. The centrifuge spins and separates the platelets from the rest of the blood components.

3



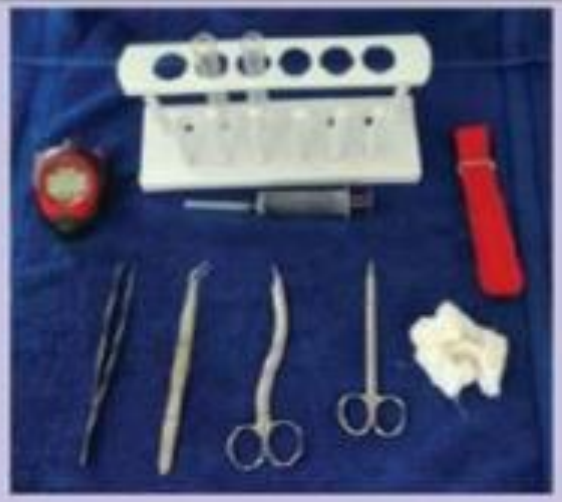
Extract platelet-rich plasma

Extract 3-6ml of platelet-rich plasma.

PLATELET RICH FIBRIN

- Second generation platelet concentrate
- Developed by Choukroun et al., in 2001
- Resorbable fibrin matrix enriched with platelets and leukocytes.
- Rich source of growth factors – VEGF, IGF – Slowly released

- Blood is drawn & collected into test tubes without an anticoagulant centrifuged instantaneously.
- Tabletop centrifuge – 10 min @ 3000 rpm for 12min @ 2700 rpm.
- Resultant product –
  - Acellular PPP @ peak level
  - PRF clot @ intermediate level
  - Red fraction of RBC'S @ base level



Armamentarium

Collection of Blood

Centrifuge Machine

Layers Of Centrifuged Blood

PRF Clot

PRF Membrane

BLOOD CLOT

- The utilization of a blood clot to regenerate dental pulp tissues was first practiced by Ostby and resulted in a growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals.
- The blood clot consists of fibrin matrix that traps cells necessary for tissue regeneration.
- It also provides a suitable pathway for cells from the periapical area including macrophages and fibroblasts to migrate into the root canal and enhance the new tissue growth.
- The rich content of growth factors allows the blood clot to play an important role in cell differentiation and thus, promotion of tissue regeneration.
- The limitation of the blood clot as a scaffold for pulp regeneration comes from the fact that the composition of a clot is variable. The concentrations of cells trapped in a clot might differ leading to unpredictable outcomes.

BLOOD CLOT/PRP/PRF

Blood clot	PRP	PRF
Lesser cytokines compared to PRP and PRF	Lesser cytokines than PRF	Maximum concentration of the cytokines
Rate of clot formation corresponds to inherent body clotting time	Addition of thrombin for conversion of fibrinogen to fibrin in PRP leads to drastic activation and rapid polymerization leading to dense network of monofibers poor in cytokine concentration	Slow physiological polymerization allows the formation of flexible three-dimensional fibrin network that supports cytokine enmeshment and cellular migration
Slower healing compared to PRP and PRF	Slower healing compared to PRP. Limited bone and dentine regeneration	Faster and stronger healing kinetics than PRP
Not rich in growth factors	Maximum release of morphogens occurs before the actual cell ingrowth, fewer signaling molecules are left for osteoblasts and odontoblasts from the surrounding tissues	Releases its growth factors steadily with the peak level reaching at 14 days corresponding to the growth pattern of periapical tissues
	Inhibits differentiation of BMSC	Shows proliferation and differentiation of BMSCs
	Fibrin matrix susceptible to washout in surgical field	Stronger stable fibrin matrix

PRP: Platelet-rich plasma; PRF: Platelet-rich fibrin; BMSCs: Bone mesenchymal stem cells

COLLAGEN

As a scaffold, collagen allows for easy placement of cells and growth factors and allows for replacement with natural tissues after undergoing degradation.

Advantages

- biocompatible, biodegradable,good tensile strength, simulates natural ECM of dentin,high alkaline phosphatase activity, allows soft tissue and hard tissue formation, forms a trap for osteoinductive factors.

- Collagen may also be processed into a variety of formats, including porous sponges, gels, and sheets, and can be cross linked with chemicals to make it stronger or to alter its degradation rate.

Disadvantages

- mechanically weak and undergoes rapid degradation
- undergoes contraction (shrinkage)

CHITOSAN

- Produced commercially by deacetylation of chitin,which is a structural element in exoskeleton of crutaceans.(crabs and shrimps)

- Formation of pores in scaffolds: influencing mechanical and biological properties

- Advantages:
  - nontoxic,easily absorbable.antibacterial,increased alkaline phosphatase activity,fibroblast and odontoblastic proliferation,
  - It is a porous scaffold that can be molded into any shape and its hydrophilic property enhances cell attachment and proliferation.

- Disadvantages:
  - low strength , difficult to accurately control the size of the hydrogel pores, chemical modifications of chitosan structure could induce toxicity.

GLYCOSOAMINOGLYCANS

- Hyaluronic acid (HA) is one of the glycosaminoglycans in ECM
- Important roles in maintaining morphologic organization by preserving extracellular spaces
- Excellent potential for tissue engineering.

Advantages

- It helps in differentiation of dental mesenchymal cells to odontoblasts, contributes to formation of dentin matrix and dental pulp
- biocompatible,biodegradable,bioactive,nonimmunogenic,and nonthrombogenic,
- plays a beneficial role in wound healing,
- can be used as an injectable scaffold and also as HA sponge

Disadvantages

- HA is highly water soluble, it degrades rapidly by enzymes such as hyaluronidase, especially when not in the form of hydrogel and lacks mechanical integrity in an aqueous environment.

- However, these drawbacks can be overcome by cross linking and modification of HA.

Silk

Silk-based biomaterial scaffolds have been extensively used for both soft and hard tissue engineering.

Advantages

- Biocompatibility, ability to support the attachment, proliferation, and differentiation of many different cell types.

- Silk fibroin (SF) is an enzymatically degradable material, which can be processed into water insoluble implants, injectable hydro gels, and porous sponges

- The ability of SF to support vascularisation with good anticoagulant activity and platelet response is encouraging for tissue engineering research and clinical therapy in dentistry.

- It has good mechanical strength, elasticity, biodegradability, morphologic flexibility, oxygen and water permeability, and a slow degradation rate that enables gradual replacement of fibroin with newly formed tissue

<u>Disadvantages</u>

Hard tissue formation consists of osteodentin. Complete degradation of silk scaffold occurs after 2 years.

ARTIFICIAL OR SYNTHETIC SCAFFOLDS

Polymers

A number of synthetic polymers such as polylactic acid (PLA), poly-l-lactic acid (PLLA), polyglycolic acid (PGA), PLGA, and polyepsilon caprolactone (PCL) have been used as scaffolds for pulp regeneration.

Advantages

- Nontoxic, biodegradable, and allow precise manipulation of the physicochemical properties such as mechanical stiffness, degradation rate, porosity, and microstructure.

- Synthetic polymers are generally degraded by simple hydrolysis, when natural polymers are mainly degraded enzymatically.

- PLLA is a very strong polymer and has found many applications where structural strength is important. Experiments were carried out by Sakai *et al*. and Cordeiro *et al*. showing PLLA scaffolds promoted dental pulp cell differentiation into endothelial cells and odontoblasts.

- PGA has been used as an artificial scaffold for cell transplantation, and degrades as the cells excrete ECM.

- PLA is an aliphatic polyester, more hydrophobic than PGA.

- PLGA WAS USED AS A SCAFFOLD TO DEMONSTRATE THAT DENTIN-LIKE TISSUE FORMED AND PULP-LIKE TISSUE COULD BE REGENERATED AFTER 3–4 MONTHS

- PCL IS A SLOWLY DEGRADING POLYMER THAT HAVE BEEN USED TOWARD TISSUE ENGINEERING EFFORTS IN BONE, EITHER ALONE OR COMBINED WITH HYDROXYAPATITE.

- <u>DISADVANTAGES:</u>
  - SYNTHETIC POLYMERS CAN CAUSE A CHRONIC OR ACUTE INFLAMMATORY HOST RESPONSE, AND LOCALIZED PH DECREASE DUE TO RELATIVE ACIDITY OF HYDROLYTICALLY DEGRADED BY PRODUCTS.

BIOCERAMICS

- Calcium phosphate ceramics such as hydroxyapatite (HA), betatricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP) : biocompatible and bioactive crystallized Materials; widely used as bone graft materials;great ability to form a strong direct bond with the host bone.

- Cells cultured on the porous form of ceramics could attach, proliferate, and expressed dentin sialophosphoprotein, which is a dentin marker.

- HA [(Ca10 (PO4)6(OH)2] :effective scaffold for regeneration of dentin and dentin-pulp complex.

- HA is a non-biodegradable ceramic while β-TCP [β-TCP $Ca_3(PO_4)_2$] is considered a biodegradable. However, the mechanical properties of TCP are inferior to those of HA.

- BCP has been developed from HA and TCP to display the advantages of the both ceramics. BCP was widely investigated as a possible scaffold for pulp and dentin tissue regeneration.

- When pulp-derived cells were mixed with HA or HA/TCP and transplanted subcutaneously in mice, bone and dentin-like mineralized tissues were generated.

BIOACTIVE GLASS (BG)

- BG is a group of synthesized surface reactive biomaterials that have an amorphous structure and high mechanical strength.

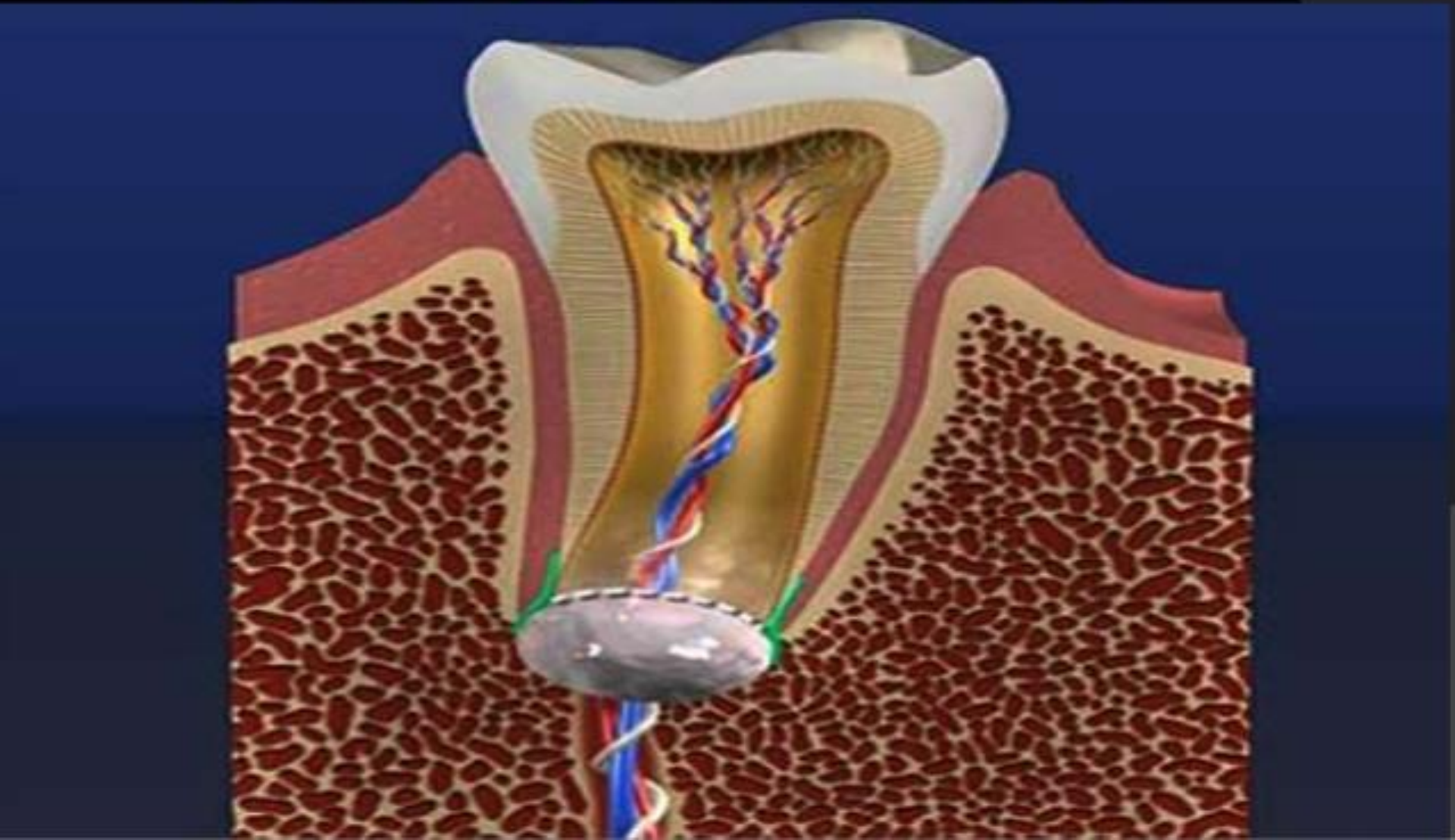
- Silicate BG (known by its commercial name:Bioglass) has been traditionally used in BG researches.

- For tissue engineering purposes, new BGs based on borate and borosilicate compositions have been suggested,the biocompatibility and controllable degradation rate of these new glass scaffolds have been reported.

- When degrades,BG will be converted into an HA-like substance that is able to bond to soft and hard tissues. The degradation also releases ions that contribute in osteogenesis and angiogenesis.

POTENTIAL TECHNOLOGIES FOR REGENERATIVE ENDODONTICS

ROOT CANAL REVASCULARIZATION



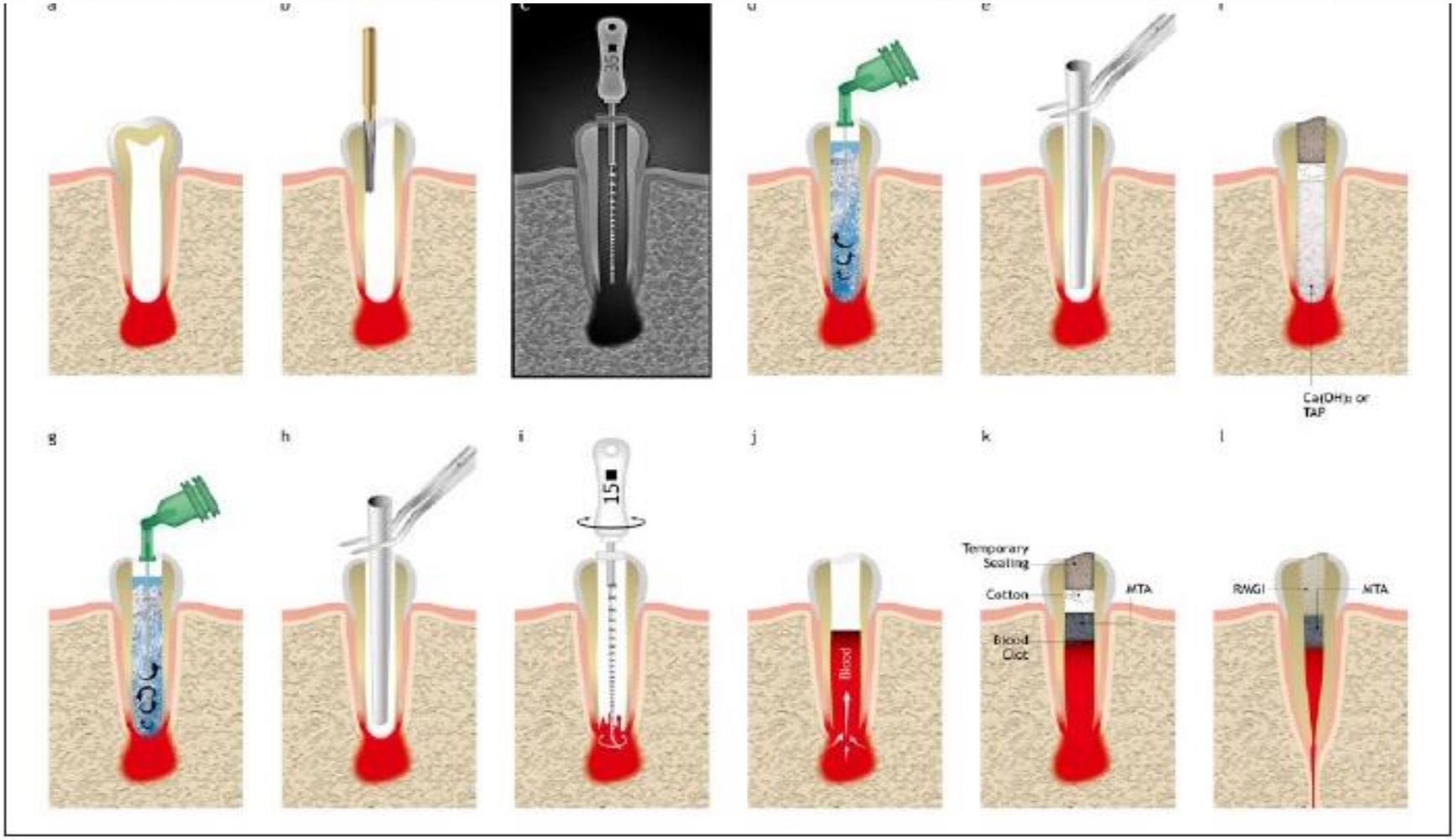


Figure 1. Schematic illustration of revascularization procedure. Revascularization is considered for immature teeth with open apices, pulp necrosis, and apical periodontitis (a). After accessing the opening (b), gentle irrigation limited to coronal part of the chamber is performed. A radiograph with K-file insertion (c) provides the approximate tooth length, which helps to determine a working length. Low concentration of NaOCl (1.5 or less than 3%, 20 mL/canal, 5 min) is used for disinfection (d), following which saline or 17% EDTA is used. After copious irrigation and canal drying with paper point (e), intracanal medicaments, such as $Ca(OH)_2$ or TAP were placed, and covered with temporary filling material (f). After confirming the absence of any signs of infection, the final step is initiated. Final irrigation is performed with sterile saline and 17% EDTA (g). After the canal has dried (h), a pre-curved K-file is introduced 2 mm past the apical foramen and rotated to induce bleeding (i). Blood fills the canal from the bottom and the blood clot can be identified after 15 min (j). After the blood clot is confirmed, capping materials such as MTA are placed over the blood clot (k). Regeneration of pulp-dentin leads to root development with thickening, lengthening, and apical closure, as well as maintenance of tooth vitality (l).

- Revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation have been documented .

- An important aspect of these cases is the use of intracanal irrigants (NaOCl and chlorhexdine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for several weeks.

- The selection of various irrigants and medicaments is worthy of additional research, because these materials may confer several important effects for regeneration in addition to their antimicrobial properties.

- For example, tetracycline enhances the growth of host cells on dentin, not by an antimicrobial action, but via exposure of embedded collagen fibers or growth factors

- The revascularization method assumes that the root canal space has been disinfected and that the formation of a blood clot yields a matrix (e.g., fibrin) that traps cells capable of initiating new tissue formation.

Advantages

- Approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology.

- The regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct.

Disadvantages

- Potential risk of necrosis if tissue become reinfected.

There are three issues relating to the current clinical protocol with regard to unfavorable outcome and those reasons are as follows:

(1) insufficient bleeding
(2) incomplete disinfection
(3) ectopic tissue formation instead of pulp-dentin regeneration

- Although case reports are largely from teeth with incomplete apical closures, it has been noted that reimplantation of avulsed teeth with an apical opening of approximately 1.1 mm demonstrate a greater likelihood of revascularization .

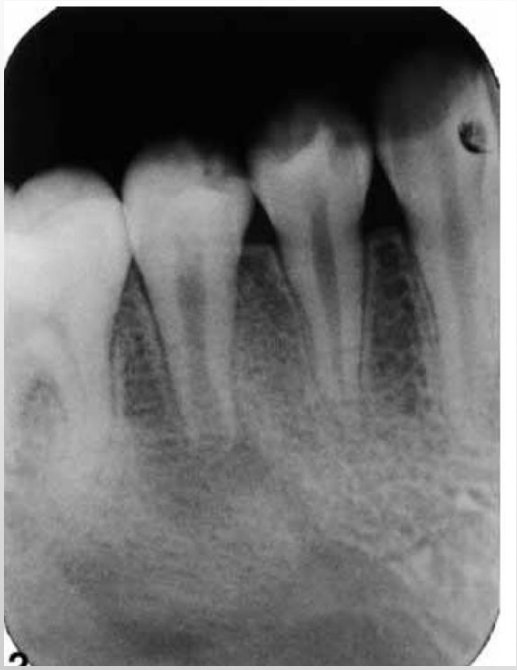
- This finding suggests that revascularization of necrotic pulps with fully formed (closed) apices might require instrumentation of the tooth apex to approximately 1 to 2mm in apical diameter to allow systemic bleeding into root canal systems.

Kling M, Cvek M, Mejare I. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endod Dent Traumatol 1986;2:83–9.

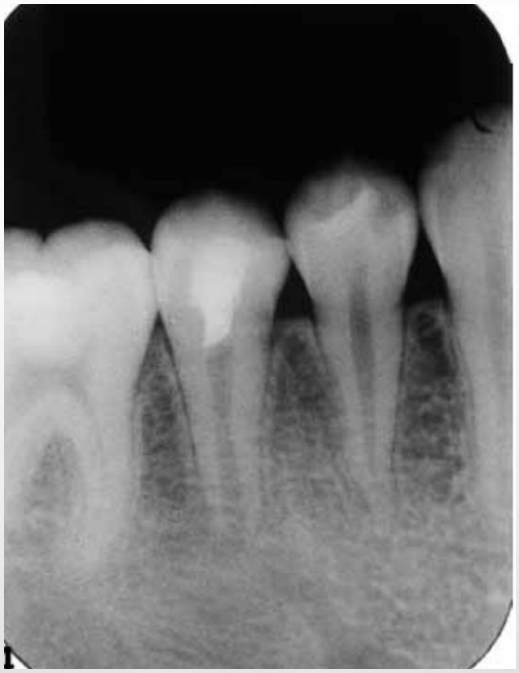
REVSCULARISATION STUDY POTOCOLS

In 2001, Iwaya and colleagues showed the revascularization potential of an immature permanent tooth.

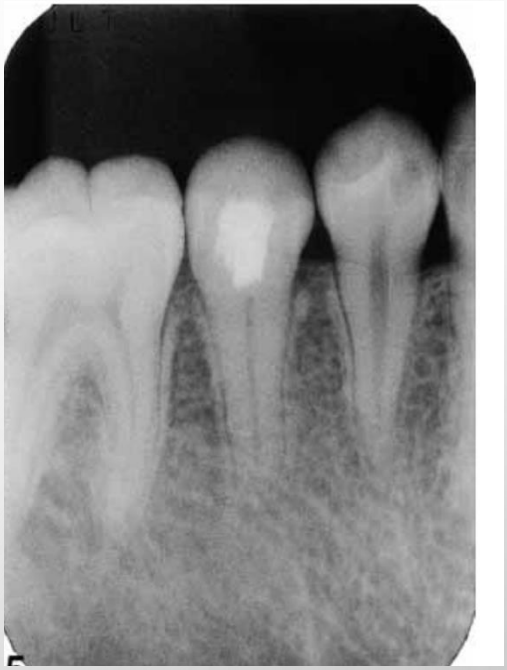
A 13-year-old girl presented with a diagnosis of necrosis and chronic apical abscess on tooth 45. The tooth was accessed and allowed to drain. The patient returned and the sinus tract had healed. The coronal portion of the root was irrigated with 5% NaOCl and 3% hydrogen peroxide over a 4-week period. No instrumentation was performed. Metronidazole and ciprofloxacin were placed in canals for disinfection and the tooth was temporized. At the final visit, calcium hydroxide was placed and the access restored with glass ionomer and composite. Root development continued and the tooth was fully formed at 30 months.



Initial presentation



5 month recall



30 month recall

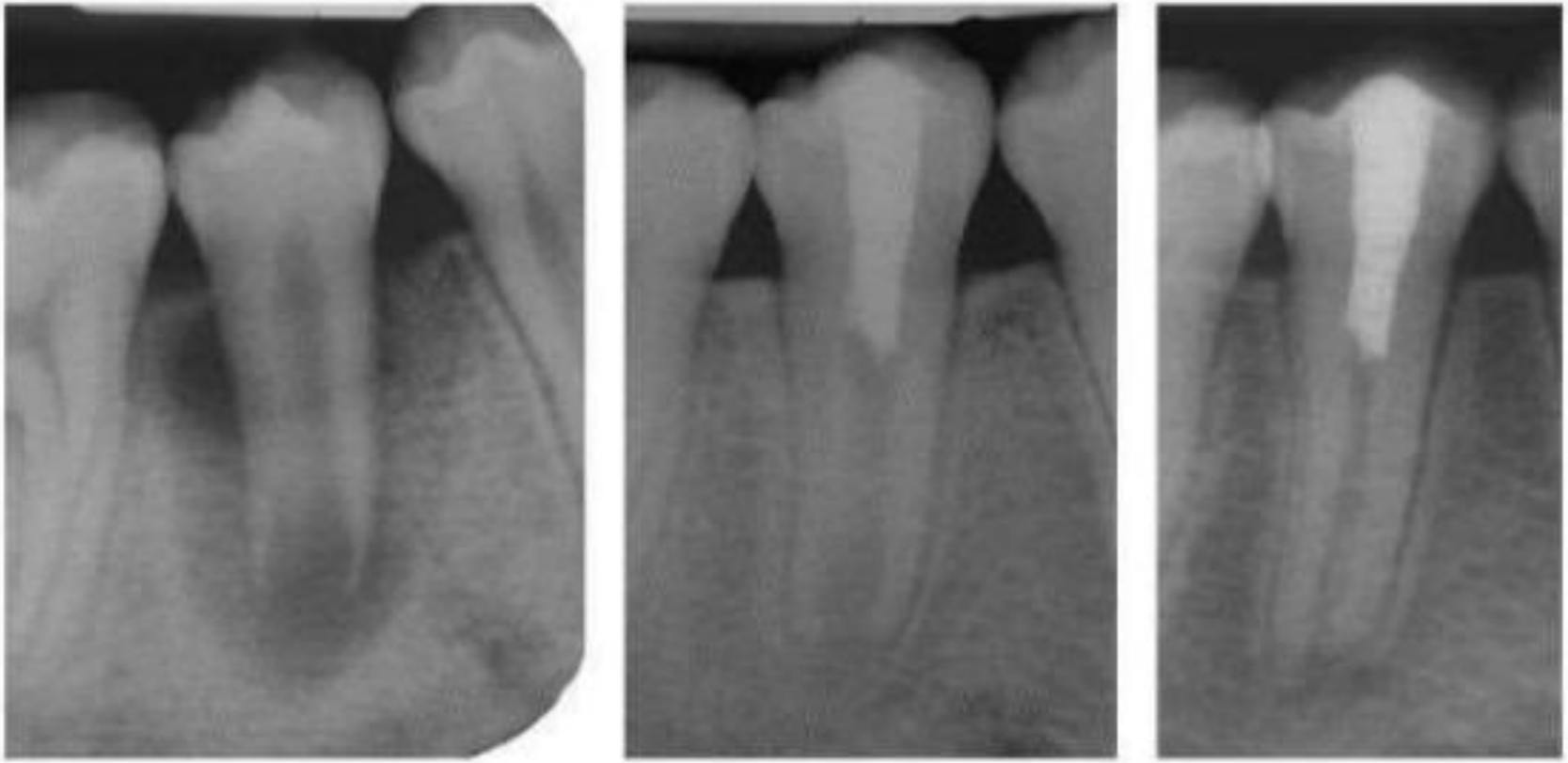


Fig. 8. Immature tooth with a necrotic infected canal with apical periodontitis. The canal is disinfected with copious irrigation with sodium hypochlorite and tri-antibiotic paste. After 4 weeks the antibiotic is removed, and a blood clot created in the canal space. The access is filled with an MTA base, and bonded resin above it. At 7 months the patient is asymptomatic, and the apex shows healing the apical periodontitis and some closure of the apex. At 24 months apical healing is obvious, and root wall thickening and root lengthening have occurred, indicating that the root canal has been revascularized with vital tissue. (*Adapted from* Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod 2004;30:196; with permission.)

FOLLOW-UP

- The follow-up of clinical cases of revascularization is mandatory to verify clinical success.

- A period of approximately 6 months is required, after the treatment, to evaluate success and to identify treatment progress.

- *Chueh et al.* showed that complete root formation of necrotic, immature teeth associated with periapical lesion was achieved only after a follow-up period of between 10 and 13 months.

- According to Chen et al.57 immature teeth diagnosed with pulp necrosis and apical periodontitis may present four types of revascularization outcome:

- ✓ **Type I - increased dentin wall width and root-end development;**
- ✓ **Type II - insignificant continued root development associated with apical closure;**
- ✓ **Type III - root-end development without apical closure;**
- ✓ **Type IV - calcification (obliteration) of root canal;**
- ✓ **Type V - mineralized tissue barrier between MTA cervical plug and radicular apex.**

Pulp revascularization: an alternative treatment to the apexification of immature teeth
December 2014
RGO - Revista Gaúcha de Odontologia 62(4):401-410

CURRENT AAE PROTOCOL CONSIDERATIONS

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

Informed consent:
Two (or more) appointments
Use of antimicrobial(s)

Possible adverse effects: staining of crown/root, lack of response to treatment, pain/infection

Alternatives: MTA apexification, no treatment, extraction (when deemed non salvageable)

Permission to enter information into AAE database (optional)

FIRST APPOINTMENT:

- Local anesthesia, rubber dam isolation, access .

- Copious, gentle irrigation with 20 mL NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (eg, needle with closed end and side vents, or EndoVac).

- To minimize potential precipitate in the canal, use sterile water or saline between NaOCl; lower concentrations of NaOCl are advised, to minimize cytotoxicity to stem cells in the apical tissues.

- Dry the canals

- Place antibiotic paste or calcium hydroxide.

- If the triple antibiotic paste is used:
  - (consider sealing pulp chamber with a dentin bonding agent to minimize risk of staining),
  - mix 1:1:1 ciprofloxacin/metronidazole/minocycline (or, if esthetics are crucial, then consider a 1:1 mixture of ciprofloxacin/metronidazole).

- Deliver into canal system via lentulo spiral, MAP system, or Centrix syringe

- If triple antibiotic paste is used, ensure that it remains below the CEJ (to minimize crown staining)Seal with 3 to 4 mm of Cavit, followed by immediate restorative material, glass ionomer cement, or another temporary material

- Dismiss patient for 3 to 4 weeks

SECOND APPOINTMENT

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

A REVIEW OF REGENERATIVE ENDODONTICS: CURRENT PROTOCOLS AND FUTURE DIRECTIONS Louis M. LIN 1 , Bill KAHLER J Istanbul Univ Fac Dent 2017;5

POSTNATAL STEM CELL THERAPY

- The simplest method to administer cells of appropriate regenerative potential is to inject postnatal stem cells into disinfected root canal systems.

- Postnatal stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone. A major research obstacle is identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp (e.g., fibroblasts, endothelial cells, odontoblasts).

- One possible approach would be to use dental pulp stem cells derived from autologous (patient's own) cells that have been taken from a buccal mucosal biopsy, or umbilical cord stem cells that have been cryogenically stored after birth; an allogenic purified pulp stem cell line that is disease- and pathogen-free; or xenogneic (animal) pulp stem cells that have been grown in the laboratory.

ADVANTAGES

- Quick
- Easy delivery
- Least painful
- Cells are easy to harvest

DISADVANTAGES

- Cells may have low survival rates
- The cells might migrate to different locations within the body possibly leading to aberrant patterns of mineralization.

PULP IMPLANTATION

- In pulp implantation, replacement pulp tissue is transplanted into cleaned and shaped root canal systems.

- The source of pulp tissue may be a purified pulp stem cell line that is disease or pathogen-free, or is created from cells taken from a biopsy, that has been grown in the laboratory.

- To take two-dimensional cell cultures and make them three-dimensional, the pulp cells can be grown on biodegradable membrane filters.

- Many filters will be required to be rolled together to form a three-dimensional pulp tissue, which can be implanted into disinfected root canal systems

The cultured pulp tissue is grown in sheets in vitro on biodegradable polymer nanofibers or on sheets of extracellular matrix proteins such as collagen I or fibronectin .

ADVANTAGES :

- Sheets of cells are easy to grow
- More stable than a injection of dissociated cells

DISADVANTAGES :

- The sheets are very thin and fragile

- Specialized procedures may *be* required to ensure that the cells properly adhere to root canal walls.

- Sheets of cells lack vascularity, so only the apical portion of the canal systems would receive these cellular constructs,with coronal canal systems filled with scaffolds capable of supporting cellular proliferation.

SCAFFOLD IMPLANTATION

- To create a more practical endodontic tissue engineering therapy pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization.

- This can be accomplished using a porous polymer scaffold seeded with pulp stem cells.

- A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development.

ADVANTAGES

- Structure support cell organization
- Some materials may promote vascularisation

DISADVANTAGES

- Low cell survival after implantation
- Must be engineered to fit root canal properly

INJECTABLE SCAFFOLD DELIVERY

- Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support.

- In root canal systems a tissue engineered pulp is not required to provide structural support of the tooth.

- This will allow tissue engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel.

- Hydrogels are injectable scaffolds that can be delivered by syringe.

- Hydrogels have the potential to be non-invasive and easy to deliver into root canal systems.

- The hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure

ADVANTAGES:

- Easy delivery
- May promote regeneration by providing substitute for extra cellular matrix.

DISADVANTAGES:

- Limited control over tissue formation
- Low cell survival

THREE DIMENSIONAL CELL PRINTING

- The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique.

- An ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue.

- The three-dimensional cell printing technique can be used to precisely position cells, and this method has the potential to create tissue construct that mimic the natural tooth pulp tissue structure.

- The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cell.

- The disadvantage of using the three-dimensional cell printing technique is that careful orientation of the pulp tissue construct according to its apical and coronal asymmetry would be required during placement into cleaned and shaped root canal systems.

GENE THERAPY

- One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization. However, a literature search indicates there has been little or no research in this field.

- Because of the apparent high risk of health hazards, the development of a gene therapy to accomplish endodontic treatment seems very unlikely in the near future.

DELIVERY OF REGENERATIVE ENDODONTIC PROCEDURES

- The method of delivery must also be efficient, cost-effective, and free of health hazards or side-effects to patients. A promising cellular source for regenerative endodontic procedures is autogenous stem cells from oral mucosa.

- The oral mucosa cells may be maintained using in vitro cell culture with antibiotics to remove infection . The cells may then be seeded in the apical 1 to 3 mm of a tissue engineering scaffold with the remaining coronal 15 mm containing an acellular scaffold that supports cell growth and vascularization.

- This tissue construct may involve an injectable slurry of [hydrogel+cells+X (growth factors, etc)] or [hydrogel + X (growth factors, etc.)], then this two layer method would be fairly easy to accomplish.

- Moreover, by seeding cells only in the apical region, there is reduced demand for large numbers of cells derived from the host. Instead, most of the cellular proliferation would occur naturally in the patient. This would reduce the need to grow large quantities of cells in the laboratory

HISTOLOGY FROM ANIMAL STUDIES

❖ The reported outcomes from case reports/series showing continued root development after revitalization/regeneration procedures, although encouraging, is not sufficient to demonstrate regeneration of pulp tissue.

❖ In a recent review by Andreasen and Bakland, based on an analysis of more than 1200 traumatized teeth and 370 auto transplanted premolars, 4 types of healing outcomes following regeneration procedures have been discussed:

(1) Revascularization of the pulp with accelerated dentin formation leading to pulp canal obliteration (PCO);

(2) Ingrowth of cementum and PDL;

(3) Ingrowth of cementum, PDL, and bone; and

(4) Ingrowth of bone and bone marrow.

Results from animal studies may provide a glimpse into possible outcomes in human teeth following revitalization procedure

- ***Huang and colleagues*** subcutaneously implanted 6- to 7-mm long human tooth fragments containing dental stem cells seeded onto a poly-D,L,-lactide and glycolide (PLG) scaffold into immunodeficient mice.

- ***Three to 4 months after*** the transplantation, the tooth fragments were harvested. The histology revealed the formation of well-vascularized soft tissue in the root canal space, and a continuous layer of dentin like tissue lined with odontoblast-like cells.

POTENTIAL COMPLICATIONS/UNDESIRED OUTCOMES

Although revitalization procedures are designed to heal bone and promote root development, potential complications and undesired outcomes must be addressed with patients and guardians before treatment.

- Potential tooth discoloration
- Adverse reaction to intracanal antibiotics
- Treatment failure.

TOOTH DISCOLORATION

- The likely cause for bluish-gray discoloration reported in some cases is minocylcline from medicaments placed in the canal space; the removal of which eliminated tooth discoloration.

- Another approach to prevent discoloration is to place a Root Canal Projector (CJM Engineering Inc, Santa Barbara, CA, USA) into the access, seal the coronal dentin with composite, and then remove the Projector and place the triple antibiotic paste in the canal.

- Discoloration can also be avoided by using Ca(OH)2 paste.
- Discoloration does occur after the placement of a minocycline-containing paste in the canal, sodium perborate bleach can be used in the tooth internally

- Another potential source of discoloration resulting from revitalization procedures is the placement of mineral trioxide aggregate (MTA).

- To avoid potential discoloration from MTA, the MTA could be placed apical to the aesthetic zone, but this placement would necessarily decrease the potential for revitalization in the coronal portion of the canal, thus eliminating the potential for increased root wall thickness in the cervical area.

- Alternatively, a different pulp space barrier, such as glass ionomer, could be used.

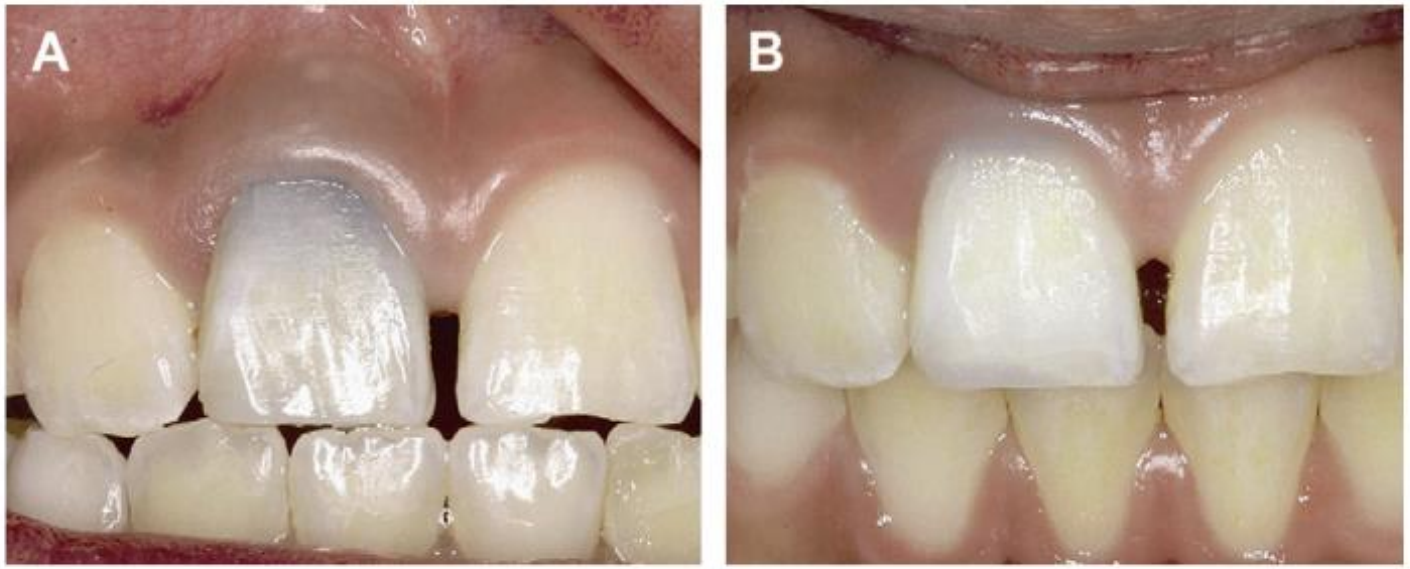


Fig. 5. Bluish discoloration caused by minocycline in triple antibiotic paste (*A*), and same tooth following internal bleaching with sodium perborate (*B*). (*From* Kim JH, Kim Y, Shin SJ et al. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod 2010;36:1086–91; with permission.)

ALLERGIC REACTION

- A potentially more serious consequence of using antibiotics in the canal space is an Allergic reactions have been reported after topical use of the antibiotics in the triple antibiotic paste.

- Nonetheless, patients and guardians should be informed of the possibility of a reaction and asked about related allergies

TREATMENT FAILURE

- Another potential outcome of the revitalization procedure is the failure of the apical bone to heal and/or tissue to grow into the canal space, or apical bone to heal.
- This may be evidenced radiographically by a persistent or enlarging apical radiolucency, and/or the lack or root development
- Additionally, a sign of treatment failure would be radiographic evidence of root resorption.
- Persistent pain, swelling, or sinus tract would also indicate failure of the treatment.
- If any of these signs or symptoms should occur, alternative treatments should be considered, including apexification, nonsurgical root canal treatment, or extraction

CONCLUSION

Several developmental issues have been described to accomplish endodontic regeneration.Each one of the regenerative techniques has advantages and disadvantages, and some of the techniques are hypothetical, or at an early stage of development.

The available case reports of pulp revascularization were generally reported on young patients (with high stem cell populations) and teeth with open apices.However, for regenerative endodontic procedures to be widely available and predictable, endodontists will have to depend on tissue engineering therapies regenerate pulp dentin tissue.

The future development of regenerative endodontic procedures will require a comprehensive research programs and their application to our patients.The unleashed potential of regenerative endodontics may benefit millions of patients each year.

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