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Pulp capping materials and effect of biomaterials on angiogenesis

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Abstract: Regenerative endodontics has gained great interest recently in an attempt to fill the root canal space with living tissues instead of artificial materials. The most important goal is to provide a suitable environment for the regeneration of healthy tissue and restore the lost biological tissues. Based on the treatment site, this field is divided into two categories of dentin-pulp complex regeneration and dental pulp regeneration. The dentin-pulp complex regenerative procedures or vital pulp therapies include direct pulp capping, indirect pulp capping, and pulpotomy

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I.Vital pulp therapies

Direct pulp capping is defined as covering an exposed dental pulp with a protective agent and indirect pulp capping is referred to the application of a protective agent, on a thin layer of dentin over the nearly exposed dental pulp. The other treatment in vital pulp therapies is the pulpotomy which is the surgical removal of inflamed coronal part of the dental pulp in the exposed pulpal tissue to save the remaining healthy tissue. In dentin-pulp complex regeneration, clinicians attempt to provide an effective pulp capping with appropriate sealing ability, and maintain the vitality of irritated pulp tissues and promote the formation of a dentinal bridge and other tissues including neural cells.[1]

In these procedures, the progenitor dental pulp stem cells (DPSCs) are migrated, recruited, and differentiated into odontoblast-like cells, which have the ability to produce reparative dentin. The homeostasis of pulp should be restored by reestablishment of the vascular network, through up- or down regulation of pro- or anti-angiogenic growth factors, which guides the regenerative procedure toward survival or necrosis of pulp tissue. [1]

II.Non-vital pulp therapies

The second field of regenerative endodontics deals with regeneration of dental pulp tissue in necrotic teeth. The treatment involves complete removal of necrotic dental pulp tissue, which is referred to as pulpectomy. After canal preparation, the regenerative treatment, the revascularization process, begins with instrumentation of periapical tissue to cause bleeding into the canal space. The blood clot formed inside the canal provides a provisional matrix scaffold for the recruited stem cells from apical papilla. [1]

Beside the stem cells derived from apical papilla, other investigators have used tissue engineered DPSCs for transplantation into the empty canal. In this treatment protocol, the establishment of a functional vascular network in transplanted tissue is the challenging goal for a successful result. The formation of this vascular structure is possible through angiogenesis, which is defined as the formation of new blood vessels from pre-existing vasculature. [1]

Pulp tissue regeneration approach

A. Cell transplantation approach

For the cell transplantation method, the transplanted cells can be either collected from the host (autologous) and/or from other individuals (allogenic) or can be processed and grown in cultures to increase the numbers.

- In this method, exogenous stem cells are either directly injected to the needed anatomic site (cell injecting) of the host or applied by being loaded onto scaffolds: (cells seeded scaffolds) either incorporated with or without signaling molecules.

For cell injecting method, main problems at directly injection were the difficulty for adequate localization and the direct contact with the immune system which prevents efficacy of the therapy.

It has been reported that cells encapsulated into a delivery vehicle were able to proliferate and differentiate more precisely. Therefore, using a delivery vehicle to carry and deliver the material thought as a solution to increase the efficacy of the therapy. The cells seeded into scaffolds can allow superior control for stem cell delivery, saturation with time-release signaling molecules and control of stiffness, pore size and cell-substrate interaction. [32]

On the other hand, cell transplantation method can be complex according to the procedure patterns as tooth extraction, pulp extirpation, in vitro cell culture, selection of stem cell populations, storage and shipping. In addition, there is a risk for contamination, development of tumorigenesis during ex vivo cell manipulation, immunological rejection and the ability of injected cells to maintain their phenotype. [32]

B.Cell homing/Cell-free approach

Cell homing/Cell-free approach is developed to overcome the drawback of cell transplantation approach and it is considered as a better alternative method.

In cell homing method the main purpose is to induce regeneration by the chemotaxis, proliferation and differentiation of host endogenous stem cells to injured tissue via biological signaling molecules loaded onto scaffolds. Furthermore, signaling molecules used for this method should promote angiogenesis, migration of endogenous stem cells and mineralization. Cell types responding to the signaling molecules during cell homing are found as DPSCs, SCAP and BMMSCs. [32]

There are two ways to attract the host's stem cells:

1.To apply signaling molecules and scaffolds into the root canals. Therefore, platelet-rich plasma (PRP) application can be thought of as a cell-free approach which can be considered as a combination of signaling molecules. However, it has disadvantages like drawing blood from the patient and additional centrifuge and purification processes.

2.To induce transport of both signaling molecules and stem cells into the root canals. This method is consisting of the disinfection of the root canals without mechanical preparation, application of antibiotics and blood clotting which is a simple technique of revascularization.

Blood clot acts as a scaffold, bleeding of the apical area can directly transport the signaling molecules and stem cells or inflammation caused by this trauma can induce mi-

gration of the stem cells through the root canal. However, this method is used for dentin-pulp complex regeneration of immature permanent teeth in the clinic, the outcome of the treatment is inconsistent.[32]

In addition to regeneration of dental pulp tissue, apexogenesis and apexification are other endodontic procedures that are performed in immature permanent teeth. Apexogenesis is the procedure that enables the immature permanent teeth to continue root end development, while the apexification provides a calcified barrier at the end of immature root by biocompatible material next to periapical tissue. It has been reported that the revascularization process occurs through the angiogenesis events derived from the periapical tissues that grow into the engineered pulp tissue. Furthermore, the immature teeth with open apices are the best candidates for these regenerative procedures.[2]

III. Pro-angiogenic and Anti-angiogenic Factors

- Vascular endothelial growth factor(VEGF) :is one of the major proangiogenic growth factor secreted by many cells as dental pulp stem cells, macrophages and tumor cells.

  Actions

  1. Proliferation and migration of endothelial cells and maturation of sprouted capillary vessels

  2. Activation of intrinsic tyrosine kinases

  3. Differentiation of DPSCs into endothelial cells and odontoblasts. [3]

- FGF: Fibroblast growth factor is proangiogenic growth factor inducing angiogenesis by interacting with various endothelial cell surface receptors causing proliferation and migration of endothelial cells.[4]

- PDGF: platelet derived growth factor is proangiogenic factor induces angiogenesis by up-regulating VEGF production and modulating the proliferation and recruitment of perivascular cells. The hDPSCs express this growth factor as well. It was demonstrated that the dentin matrix contains a higher amount of PDGF than other growth factors [5]

- Angiopoietins:

  Angiopoietins have 2 major forms including Ang-1 and Ang-2, which interact with specific receptors on endothelial cells. Dental fibroblasts express Ang-2 along with other growth factors such as VEGF, FGF-2, PDGF. These factors play a critical role in initiation and stabilization of angiogenesis through competitive interactions with the receptor.[6]

  - Matrix metalloprotienase (MMPs):

    Are a group of important enzymes in angiogenesis. These biological molecules have a key role in degradation of the ECM of vessel walls, allowing the migration of endothelial cells. [6]

  - Bone morphogenetic proteins (BMPs):

    Bone morphogenetic proteins (BMPs) belongs to transforming growth factor-β family. BMPs play a critical role in embryonic and postnatal development, and also in maintaining homeostasis in different organs and tissues by regulating cell differentiation, proliferation, survival and motility. Also, BMPs have important role in angiogenesis either di-

rectly regulate the functions of vascular endothelial cells or indirectly via regulation of angiogenic factors, such as vascular endothelial growth factor (VEGF).[7]

- Transforming growth factor beta (TGF-β)

The TGF-β regulates various cell activities inside the cell, including the growth and division (proliferation) of cells, the maturation of cells to carry out specific functions (differentiation), cell movement (motility), and controlled cell death (apoptosis).[8]

- Biochemical Inhibitors:

It was reported that DPSCs can produce antiangiogenic factors including endostatin, thrombospondins (TSP) and angiostatin. It was found that the dentin matrix components in low concentrations have proangiogenic impact, whereas in high concentrations they have inhibitory effects on angiogenesis events of dental pulp. Hyperglycemia also has negative influence on immune system function and angiogenesis. There is now a large family of endogenous inhibitors of angiogenesis whose function in tooth development and regeneration needs evaluation. These include thrombospondin-1(TSP-1) and thrombospondin-2(TSP-2), whose expression plays a significant role in vascular homeostasis and avoid pathologic neovascularization. Another important physiologic antiangiogenic agent is platelet factor 4 is effective inhibitor of angiogenesis process and are useful agents for therapeutic purposes. [9]

Stimuli of angiogenesis:

❖ Mechanical Stimulation: Higher capillary shear stress increases the expression of VEGF and angiogenesis. An increase in the number of microvessels in pulp tissue was detected in orthodontically moved teeth that was due to the increased expression of the angiogenic growth factors. Thus, mechanical alteration in tooth has a significant impact on local angiogenesis and tissue regeneration. [3]

❖ Biochemical Stimulation: The biochemical stimulation of angiogenesis is due to the production of proangiogenic and antiangiogenic factors including growth factors such as bone morphogenetic proteins (BMPs). The DPSCs can secrete proangiogenic factors such as vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) [3]

IV. Pulp capping materials and the effect on Angiogenesis in Dentin-Pulp Complex Regeneration

- 1. Calcium hydroxide: The introduction of vital pulp therapies including direct or indirect pulp capping date back to 1939. An ideal pulp capping material should provide easy handling, antibacterial effect, good sealing ability, and induce dentinal bridge formation. Among several materials, calcium hydroxide was one of the most common material used in pulp capping. Due to its alkalinity, $Ca(OH)_2$ has antibacterial activity and stimulates dentin formation. Calcium hydroxide has been considered the "gold standard" for the capping of exposed pulp.[10]

- Composition:

✓ Calcium hydroxide cements are paste/paste systems. One paste contains calcium hydroxide and the other contains salicylate. Salicylate is a weak acid that is reacts with the calcium hydroxide by acid–base reaction which is responsible for setting, the reaction forms an amorphous calcium disalicylate, for example, Dycal [11]. A visible light cured calcium hydroxide was introduced to overcome the limitations of the chemical cure calcium hydroxide; that is, they set on command, improved strength and minimal solubility in water. A visible light-cured (VLC) calcium hydroxide liner consists of calcium hydroxide and barium sulfate dispersed in a urethane dimethacrylate resin containing initiators and accelerators activated by visible light VLC calcium

hydroxide liners are mainly indicated for indirect pulp capping and as a cavity liner under all types of restoratives, for example, Calcimol (Voco GmbH, Cuxhaven, Germany) and Lime-lite (Pulpdent Corporation, Watertown, MA, USA).[11]

- The effect of $Ca(OH)_2$ on dentin-pulp complex regeneration and angiogenesis:

✓ It was reported that $Ca(OH)_2$ increases the recruitment, migration, proliferation of DPSCs, and periodontal ligament stem cells (PDLSCs) through the expression of STRO-1 and CD146 markers. [12]

✓ It was also found that the regenerative effects of $Ca(OH)_2$ are due to calcium ion release and the high pH value. Calcium ions promote the migration of pulp progenitor cells, increase the synthesis of biomolecules such as fibronectin and bone morphogenic proteins (BMPs), and participate in mineralization. The alkaline pH can present antibacterial and anti-inflammatory effects, activate transforming growth factor β (TGF-β), increase the activity of alkaline phosphatase (ALP), and enhance the dissolution of dentine extracellular matrix (ECM).[1, 13]

✓ The pro-angiogenic effects of $Ca(OH)_2$ is mainly attributed to the release of growth factors preserved in the dentin matrix including TGF-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF). It was also found that chelating agents as (EDTA) can dissolve the dentin matrix and release pro- angiogenic factors.[13]

✓ Dentine matrix also contains vascular endothelial growth factor (VEGF), which is one of the most important factors in angiogenesis events. It has been suggested that $Ca(OH)_2$ can activate notch signaling pathway. In dental pulp tissue, notch signaling pathway is activated due to injury, and participates in angiogenesis through the proliferation and migration of endothelial cells, smooth muscle, and arterial-venous differentiation.

❖ During healing 3 zones are formed: -

Zone of obliteration: The pulp tissue immediately in contact with the calcium hydroxide is usually completely distorted because of the caustic effect of the drug. This zone consists of debris, dentinal fragments, hemorrhage, blood clot, and particles of calcium hydroxide.

Zone of coagulation necrosis: A weaker chemical effect reaches the subjacent, more apical tissues and results in a zone of coagulation necrosis and thrombosis, also called layer of 'firm necrosis'.

Zone of demarcation: A line of demarcation develops between the deepest level of the zone of coagulation necrosis and the subjacent vital pulp tissue. Exposed human dental pulp will heal with hard tissue bridging.

❖ Disadvantages:

• Highly soluble in oral fluids.

• Lack of adhesion.

• Presence of porosities known as "*tunnel defects*" in reparative dentin bridge. Therefore fail to provide barriers, which act as pathways for microleakage and bacterial reinfection.[14]

2. Bonding Agents:

• Dental adhesive systems were suggested for use as an indirect pulp capping material to overcome the disadvantages of calcium hydroxide as they produce superior adhesion.

• However, they have poor outcome due to its cytotoxic effect and absence of calcific bridge formation.[15]

- Effect on angiogenesis:

- By comparing the effect of dentine adhesive resin and $Ca(OH)_2$ on the pulp cells, it was found that the adhesive resin causes more inflammation of pulp tissue due to its missing antibacterial efficacy and foreign body reactions.

- Decrease the release of FGF and has no effect on PDGF therefore it cannot induce the formation of an acceptable tertiary dentine bridge. [16]

3.Laser:

- Pulp capping therapy using lasers results in good prognosis for the tooth however, the sealing of exposed pulp with one of the dental materials after laser treatment is still required.

- For Example: CO_2, Diode and Nd: YAG lasers.

Mechanism of action:

- Sterile field is provided by the bactericidal effect of the laser. Area of coagulation is created by a superficial necrosis, with an underneath area of reversible damage, this stimulates odontoblasts to produce reparative dentin.[17]

Effect of laser on angiogenesis:

It was reported that low-level laser therapy caused higher proliferation of DPSC and higher gene expression of VEGF with the lower energy densities (0.7, 1.5, and 3 J/cm^2) but not with higher ones (9 J/cm^2), so it could be concluded that low-level laser therapy could be a useful tool to promote angiogenesis and dentinogenesis of the dentin-pulp complex when parameters are optimized.[18]

4.Calcium silicate based materials:

4.a. MTA:

- Composition:

The MTA is composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate and bismuth oxide.

❖ Two types of MTA were available: White and Gray MTA.

The grey version is due to the addition of iron. Tooth discolouration has been reported with the use of grey MTA. Therefore, the use of white MTA has generally been recommended. [14]

- Mechanism of action:

The primary reaction product of MTA with water is calcium hydroxide. Therefore, the formation of calcium hydroxide explains a similar action with the calcium hydroxide paste.

The second reaction is depending on MTA is calcium silicate-based materials. Calcium silicate-based materials are bioactive materials capable of forming apatite by using calcium silicates or calcium aluminates. these formed deposits can stimulate dentin bridge formation and remineralization by promoting the release of cytokines and interleukins from white blood cells.[14]

The differences between the MTA and Ca (OH) 2:

MTA has a higher sealing ability, lower solubility, higher-strength and stability, MTA can set in a moist environment, prevent bacterial infiltration, produce thicker dentin bridge formation with a lesser inflammatory response, less hyperemia, and less necrosis of pulp tissue compared to calcium hydroxide. Therefore, many clinical reports demonstrated that the success rate of direct pulp capping was higher with MTA than with calcium hydroxide. [14]

Disadvantages of MTA:

It has shown high solubility, demonstrating a 24% loss after 78 days of storage in water, the presence of iron in the grey MTA formulation may darken the tooth, prolonged setting time of approximately 2 hours and 45 minutes, the handling is difficult and high cost.

Effect on angiogenesis:

By comparing $Ca(OH)_2$ and MTA, it was found that MTA had positive effects on angiogenesis and differentiation of dental pulp cells when it was placed in direct contact with dental pulp. It was also reported that MTA, as a direct pulp capping agent, can induce the expression of VEGF, osteocalcin and dentin sialoprotein. The in vitro culture of hDPSCs with MTA facilitated their differentiation, and also increased the expression of angiogenic factors as angiopoietin-1 (Ang-1).[19]

4.b. Biodentine:

Biodentine is a new class of dentin material which has been recently introduced as a pulp capping material. It was developed as a silicate-based restorative material from the addition of calcium chloride to MTA. It is represented in a capsule form.

•Composition:

The powder: Mainly contains tricalcium and dicalcium silicate ($3CaO\ SiO_2$ and $2CaO\ SiO_2$), the principal component of Portland cement, calcium carbonate ($CaCO_3$). In addition, zirconium dioxide (ZrO_2) serves as a contrast medium.

The liquid: Consists of calcium chloride ($CaCl_2.H2O$), which is used as a setting accelerator and water-reducing agent in aqueous solution with an admixture of polycarboxylate (a super plasticizing agent). [13]

Effect on angiogenesis:

It was reported that the induction effect of Biodentine cement on differentiation of DPSCs is through the mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase II (CaMKII) pathways. The angiogenic effect of this cement was evaluated and it was suggested that Biodentine can induce early miner-

alization in dental pulp due to an increase in release of TGF-β1, a pro-angiogenic factor produced by pulp cells.[13]

4.c. TheraCal

Theracal is a Light-curable resin-modified calcium silicate-based materials. It is used as a pulp capping agent and as a protective liner for use with restorative materials, cement, or other base materials. This is material has been classified as a 4th generation calcium silicate material.

- Composition:

The material is single paste light cured calcium silicate based cement and composed of Portland cement (30-50%), resin (10-30%), and barium sulfate (1-10%). TheraCal is opaque and "whitish" in colour, so it should be kept in thin layers so as not to show through composite materials that are very translucent affecting final restoration shade.[20]

- Mechanism of action:

TheraCal may act as a scaffold for reparative dentine formation. Dentinal fluids are absorbed within it, resulting in the release of calcium and hydroxyl ions and the tooth responds to form apatite deposits. Moreover, the formed apatite deposits have a natural sealing ability that plays a crucial role in pulpal protection by the formation of an interfacial layer rich in minerals thus promoting remineralization of dentin bridge.

- Compared to conventional MTA materials, the resin-modified light-curable cement is superior as it polymerizes immediately after light-curing, prevention of materials washing out and superior physical properties.

- Disadvantages: TheraCal displayed lower calcium ion release and the presences of unpolymerized resin can exert a toxic effect on the pulp. [20]

- Effect on angiogenesis:

TheraCal showed toxic effects after 24, 48, and 72 hr. Moreover, TheraCal decreased secretion of TNF-α and IL-8 in hDPSCs.[21]

4.d. Endosequence Root Repair Material:

It is a recent nano-bioceramic material in both low and high viscosity form recommended for pulp capping, perforation repair, apical surgery, and apical plug and can be able to penetrate dentinal tubules and set using their moisture.

- Composition:

Calcium silicates, Monobasic calcium phosphate, Zirconium oxide, Tantalum oxide, fillers, thickening agents. No mixing is required due to its supply as a paste in a preloaded syringe or a moldable putty form.[22]

- Advantages:

Excellent sealing ability, highly biocompatible, dentin bridge formation, hydrophilic, improved handling characteristics over MTA and antibacterial effect.

Effect on angiogenesis:

ERRM promoted the vascularization, migration and differentiation of DPSCs through the release of VEGF and BMP growth factors from dentin matrix. [22]

5. Enamel matrix derivative (EMD)

EMD (Emdogain) is an extract derived from porcine foetal tooth material.

Composition:It is mainly consisting of amelogenins, a class of protein known to induce proliferation of PDL cells, acellular cementum and alveolar bone during tooth development.[23]

- Mechanism of action:

Enamel matrix proteins have been reported to increase the levels of mineralization markers (including bone sialoprotein and osteopontin) in odontoblasts. Based on this concept, the enamel matrix derivative (EMD) was introduced. The regenerative process of EMD consists of differentiation of odontoblasts with consequent dentine formation and pulpal wound healing without affecting the vitality of the remaining pulp in a manner similar to normal dentinogenesis. [23]

- Disadvantage:

It has poor sealing qualities and no effective hard tissue barrier formation.

Effect on angiogenesis:

Moreover, EMD has also been reported to contain growth factors such as transforming growth factor-beta 1 and small amelogenin peptides that are actively involved in cell signalling to stimulate matrix formation and mineralization. These growth factors are recognized as mediators in processes such as tissue homeostasis, inflammation, healing and neogenesis. Enamel matrix derivative (EMD) can also be used as a pulp capping material. It was found that EMD was more capable of inducing the differentiation and proliferation of human tooth germ stem cells (hTGSCs) compared with calcium calcium hydroxide and mineral trioxide aggregate (MTA). EMD can exhibit angiogenic effects by chemotactic effect on endothelial cells and stimulation of periodontal cells to produce VEGF. [1] [23]

6. CEM cement:

Calcium-enriched mixture (CEM) cement is one of the pulp capping agents composed of calcium oxide (CaO), sulfur trioxide (SO3), phosphorous pentoxide (P2O5), and silicon dioxide (SiO2), which has osteogenic, cementogenic and dentinogenic functions.

Effect on angiogenesis:The ability of CEM and MTA as capping agents was compared. It was concluded that CEM could increase the expression of FGF and bone morphogenic protein 2 (BMP-2) when compared to MTA.[24]

7.Natural pulp capping materials

a.Propolis

Propolis is collected from trees and shrubs by honeybees. The main chemical classes present in propolis are flavonoids, phenolics and other various aromatic compounds. Propolis has potent antimicrobial and anti-inflammatory properties. Propolis has shown to inhibit the synthesis of prostaglandins and supports the immune system by promoting

phagocytic activities, stimulating cellular immunity and augmenting healing effects. Moreover, it contains some elements as zinc and iron that are important for the collagen synthesis.

- Composition: Propolis is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other various substances, including organic debris.

- Effect on angiogenesis:In previous studies, showing the ability of Propolis to stimulate the production of transforming growth factor (TGF) Beta 1, which is important for the differentiation of odontoblasts. It also induces the synthesis of collagen by dental pulp cells. [25]

b.Eggshell powder

Eggshell has attracted research interest recently due to its bioactive effect in different biomedical applications. It is thought to have anabolic effects on human bone without side effect. This may be attributed to its unique chemical composition.

- Composition:

Contains 94 wt.% $CaCo_3$,1 wt.% $MgCo_3$, 1 wt.% calcium phosphate ($Ca_3(Po_4)_2$) and trace amounts of strontium (Sr) and fluoride (F).

- Mechanism of action:

It was reported that the natural biological origin of the eggshell makes it similar in its crystalline structure to the custom-made hydroxyapatite and human bone-like crystals. It has been reported that a significantly thicker dentin bridge formation with less pulp inflammation and fibrosis within the micro-sized eggshell powder when compared to calcium hydroxide paste. However, the material still needs further investigation to reveal it is a mode of action of the pulp tissue. [26]

c. Grapessed extract:

Grapeseed extract has been used in dentistry in multiple applications due to its anti-inflammatory, antibacterial, antioxidant and anticancer effect. Grape seed oil contains a large amount of phenolic compounds, including flavonoids, carotenoids. It has anti-angiogenic effect especially VEGF.[27]

V. The effects of canal irrigating solutions and disinfecting agents on angiogenesis:

V.1.NaOCl

The first step in dental pulp regeneration is the complete disinfection of root canal system. At first, root canal space should be irrigated with sodium hypochloride (NaOCl) which has proteolytic and antimicrobial activities which dissolves the organic debris and eliminate microorganisms inside the dental canal. It was suggested that NaOCl has toxic effects on on human bone marrow mesenchymal stem cells (MSCs).[28]

It was also reported that high concentrations of NaOCl can drastically effect the survival and differentiation of stem cells of the apical papilla (SCAPs) and significantly reduce the expression of dentin sialophosphorprotein (DSPP). It has been suggested that using lower concentrations like 1.5% and using 17% EDTA after NaOCl irrigation reduced the NaOCl negative effects, and increased the survival rate of SCAPs and expression of DSPP. [28]

V.2.Chlorhexidine gluconate

The other disinfecting endodontic solution used in regenerative procedures is 2% chlorhexidine gluconate (CHX). The effect of CHX on DPSCs was measured and no viable cell was detected in samples irrigated with 2% CHX. It was reported that 2% CHX, as well as 6% NaOCl, showed cytotoxic effects on DPSCs due to negative influence on their attachment to root canal wall surface.[29]

V.3.EDTA

EDTA is an endodontic irrigating solution with chelating activity, which is suggested to be added to canal irrigation protocol as a final rinse for smear layer removal or in combination with NaOCl and CHX solutions. Although, the addition of EDTA to other rinsing solutions can increase the viability of DPSCs, and positively affect stem cell's attachment to root canal wall, authors showed that the time of irrigation with EDTA should be 1 min, while after 3 min the microhardness of dentin can significantly reduce.

It was also noted that EDTA can induce DPSCs cell attachment and odontoblastic/osteoblastic differentiation. Also, it was indicated that EDTA can stimulate the release of pro-angiogenic growth factors in dentin matrix including TGF-β, VEGF, FGF-2, PDGF, and BMP-2.[1]

V.4.Triple antibiotic paste (TAP)

TAP is a disinfecting regimen containing three antibiotic pastes including: ciprofloxacin, metronidazole, and minocycline used for complete elimination of microorganisms inside necrotic root canal in regenerative procedures. It was indicated that the scaffolds containing 5%wt ciprofloxacin were safe for hDPSCs, and only 25%wt ciprofloxacin had cytotoxic effects on pulp stem cells. Also, it was suggested that ciprofloxacin could decrease the expression of inflammatory cytokine IL-6, and increase the expression of the IL-8.

It was indicated that metronidazole plus clindamycin had anti-agiogenic activity and could strongly interact with pro-angiogenic factors like FGF-2. It was demonstrated that minocycline has anti-angiogenic activity due to suppression of vascular endothelial growth factor (VEGF) expression. It has showed that minocycline can inhibit hypoxia-induced neovasculogenesis. [30, 31]

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