

A Review on Regenerative Endodontics

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Abstract

Background and Aim: Regenerative endodontics with the goal of replacing the lost dental tissues is developing fast. The aim of this study was to review the recent progressions, known limitations and advantages of regenerative treatments compared to other possible treatment modalities.

Materials and Methods: We searched MedLine and PubMed systematically for articles published from May 2000 until May 2013 in English which were relevant to tissue engineering and regenerative procedures using the keywords “cell and tissue based therapy”, “regeneration”, “stem cell” and “tissue engineering”. More valid papers were chosen.

Results: Stem cells, growth factors and an appropriate scaffold are the three essential elements required for tissue regeneration. Revascularization is an ideal regenerative treatment, which uses a fibrin scaffold. This scaffold is made of peri-radicular stem cells, a clot and latent growth factors in dentinal walls. The clinical outcome of revascularization is complete root formation in a premature necrotic permanent tooth. Thus, revascularization is a realistic and worthwhile approach in patients with fair to good prognosis.

Conclusion: Regenerative treatments with the aim of producing a completely formed permanent tooth are going to revolutionize dental science. Regeneration of a fully formed tooth is much more complicated than treatment of an open apex tooth and this issue requires further studies.

Key Words: Cell and tissue-based therapy, Regeneration, Stem cell, Tissue engineering

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Introduction

Root canal treatment (RCT) aims to prevent or treat apical periodontitis. To achieve this goal, pulpal health must be preserved in case of inflammation. In case of pulp necrosis, healthy periapical tissue must be preserved [1]. At present, researchers are searching for methods to regenerate healthy dentin-pulp complex. Most currently available treatments replace the lost tissue with neutral materials such as root canal filling with gutta percha or dental implantation with a satisfactory rate of success even in presence of infected periapical tissue [2]. Regenerative dentistry aims to biologically replace teeth and their surrounding structures. Advances in regenerative dentistry highly depend on

biological treatments. Many of the regenerative dentistry ideas are secondary to the advancements in tissue engineering. Tissue engineering focuses on spatial orientation of stem cells in presence of growth factors, morphogens and scaffolds to form a tissue or an organ [3-5]. This study aimed to review regenerative dentistry with special emphasis on regenerative endodontics, its advances, applications and current limitations.

Definition of regenerative terms:

Controversy exists in definition of regenerative terms. Apexification is a method to create a calcified barrier in open apex teeth with necrotic pulp tissue [6]. This method is different from revascularization since apexification does not attempt to re-

generate the vital tissue in the root canal system (RCS). Apexification treatment forms an apical barrier in order to place the root canal filling material over it. The second term is apexogenesis, which is defined as a treatment process for vital pulp that results in continuation of the physiological development of the root and formation of apex. One main difference of apexogenesis with revascularization is that apexogenesis is indicated for teeth with adequate blood supply since it does not cause revascularization or angiogenesis in the RCS [7]. Another term used to describe root development is maturogenesis. It refers to the favorable outcome of revascularization; which is the continuation of the physiological processes of root development [8]. Revascularization can be defined as reinstating the blood supply of the tissue. Use of this term was recently discussed in the Journal of Endodontics and the authors stated that this term does not completely describe the favorable outcomes of endodontic regenerative processes because the favorable outcome of regeneration is the formation of pulp-dentin complex rather than reinstating the blood supply of the RCS alone. They suggested using "induced or guided tissue regeneration" (GTR) as a more accurate term for this process [9]. Others stated that GTR would be a more appropriate term considering the fact that we do not know what type of tissue would occupy the dental pulp space [10]. Due to the lack of histological findings in this regard, selection of a fixed terminology would be difficult [11].

Development of regenerative processes:

Hermann [12] first suggested the application of calcium hydroxide (CH) for vital pulp therapy and Nygaard Ostby [13] described the revascularization technique to reinstate the dentin-pulp complex in permanent teeth with necrotic pulp. In the past decades, clinical application of dental regenerative treatments has greatly advanced. The currently used regenerative treatments include guided bone regeneration (GBR) [14], GTR [15], distraction osteogenesis [16], application of platelet rich plasma (PRP) to increase bone height [17], application of Emdogain to reconstruct periodontal tissue [18], application of recombinant human bone morphogenetic proteins (rh BMP) to increase alveolar ridge height [19] and use of fibroblast growth factor 2 (FGF2) for periodontal tissue re-

generation [20]. In early 21st century, success of these treatments was confirmed in dentistry and particularly in endodontics. For instance, using a scaffold and stem cells, pulp, dentin and enamel may be regenerated [21-23]. Tooth crown may be regenerated using the embryonic stem cells, derived from blastocyst-stage early mammalian embryos, and bone marrow stem cells [24]. When mixed with fetal epithelial cells, bone marrow stem cells can differentiate into ameloblasts [25]. Cells isolated from tooth bud can be mixed with agar and collagen to form crown, root and periodontal structures [26]. Stem cells isolated from the extracted third molars may be used for regeneration of root and periodontal ligaments [27]. Regenerative dentistry has emerged as a developing branch of dental science.

Whole tooth regeneration:

Nakahara [18] theoretically described three methods for regeneration of a whole tooth using stem cells:

- Culturing stem cells on an appropriate scaffold: This method may be controlled by adding specific growth factors or signaling molecules.

- Mimicking the natural development of teeth: In this method, an artificial tooth bud is implanted in an animal body. However, it must be noted that natural development of a human permanent tooth takes years. Regeneration of a whole tooth using patient's own cells is not clinically feasible.

- Regeneration of a functional dentin-pulp complex in permanent teeth in patient's mouth: This method preserves normal functions such as formation of reparative dentin and innervation system.

Dental pulp is a relatively small space compared to complex organs like the heart or liver. Also, it has a relatively simple cellular structure with a nucleolus of connective tissue surrounded by a layer of odontoblast cells [28]. Thus, from the perspective of tissue engineering, dental pulp is a relatively simple tissue to regenerate [21]. Thus, the third method described above is more feasible than the other two. Mesenchymal dental pulp stem cells are located in the perivascular area of the pulp adjacent to the odontoblastic layer and act as a cell reservoir for replacement of odontoblasts [29]. It is possible to regenerate loose connective tissue by local release of angiogenic growth factors like platelet-derived growth factor (PDGF) and vascular endo-

thelial growth factor (VEGF) that play a key role in coordination of angiogenesis and formation of connective tissue. This process is similar to what happens in the granulation phase of wound healing [30]. PRP increases PDGF and VEGF by approximately 3-6 times; it has also been reported to enhance wound healing. Thus, it can be used as both a growth factor and a suitable scaffold for pulp regeneration [31]. Studies have confirmed that application of human VEGF significantly decreases angiogenesis in human pulp implanted in immunocompromised mice [32].

General view:

Formation of crown, root and periodontium occurs via consecutive morphological stages (bud stage, cap stage and bell stage) regulated by mutual interactions between ectodermal stem cells and mesenchymal cells. Ectodermal stem cells differentiate into ameloblasts while ectomesenchymal stem cells differentiate into odontoblasts [28]. An important requirement of pulp tissue regeneration is to achieve stem cells with the ability to differentiate into odontoblasts [21, 29]. The three main elements for tissue regeneration include stem cells, growth factor and appropriate scaffold.

1. Stem cells:

Stem cells are relatively undifferentiated cells with the ability to proliferate and regenerate and can differentiate into different types of specific cells [5]. Differentiation potential is an important characteristic of stem cells and they are divided into 4 groups based on their differentiation potential: 1. Totipotent, 2. Pluripotent, 3. Multipotent and 4. Unipotent (from the highest to the lowest differentiation potential) [34, 35]. Studies mostly focus on totipotent stem cells that have the highest differentiation ability. However, due to ethical-legal considerations and difficult accessibility, postnatal stem cells (such as bone marrow cells) have greater clinical applications because they are more easily accessible.

Many stem cells are capable of differentiating into cells that express phenotypic markers of neural cells, muscle cells, adipocytes and odontoblasts [36]. In addition to dental pulp mesenchymal stem cells in the perivascular area, researchers have been able to differentiate three different types of postnatal mesenchymal stem cells to pre-odontoblast cells.

A. stem cells from the apical papilla (SCAP)

B. Dental follicle progenitor cells (DFPC)

C. Bone marrow derived mesenchymal stem cells [37]

Many of these studies have been conducted on patients younger than 25 years. However, it must be noted that stem cells have been isolated from the dental pulp of patients as old as 41 years. No previous study has evaluated formation of pre-odontoblast cells in patients older than 50 years and there is a gap of information in this respect [29].

Regeneration ability is one characteristic of stem cells. This characteristic may be assessed by measuring population doubling (PD) time [38]. PD time of apical papilla cells has reported to be over 80 [39]; which is a very high rate among the isolated postnatal stem cells [38].

2. Growth factors:

Several growth factors have been evaluated for their ability to induce and mediate the differentiation of mesenchymal stem cells to pre-odontoblast cells. Significant reduction in the size of pulp chamber radiographically and five times increase in predentin thickness have been reported in patients receiving long-term corticosteroids.

It appears that use of corticosteroids is related to increased activity of human odontoblasts [40]. Studies have confirmed that application of dexamethasone significantly increases the differentiation of human dental pulp stem cells to pre-odontoblast cells [41]. This has particularly been observed in simultaneous application of dexamethasone and vitamin D3 [42]. As soon as the combination of growth factors changes, differentiation of pulp cells changes completely. Cell populations can express odontoblastic, chondrocytic or adipocytic markers when exposed to different combinations of growth factors. These findings emphasize the importance of growth factors in guided differentiation of these cells [43].

Studies on the effect of growth factors alone or in combination with different medications on differentiation of pre-odontoblasts show that a growth factor alone cannot cause the highest cell differentiation possible and a combination of growth factors is required in clinical assessments. Also, it has been stated that dexamethasone, insulin and statins can also induce differentiation of cells to odontob-

lasts [44]. This suggests that patients using statins may show narrowing of pulp chamber similar to those taking corticosteroids [40, 45, 46]. Human demineralized bone has long been used for tissue healing after surgical procedures. Human demineralized bone provides ideal growth factors as well as a scaffold for differentiation and function of osteoblasts [47]. Recent evidence confirms that human demineralized dentin induces pre-odontoblast cell differentiation [48]. Human dentin contains several non-collagen proteins like transforming growth factor beta 1 (TGFB1) [49]. One method of extracting TGFB1 from human dentin is by using EDTA. The amount of TGFB1 extracted by using EDTA is much more than that released by treatment of dentin with CH, sodium hypochlorite, citric acid or mineral trioxide aggregate (MTA) [50].

3.Scaffold:

The third important element in tissue engineering is presence of a physical scaffold. Tissues are organized in the form of three-dimensional (3D) structures. Presence of a suitable scaffold is necessary to:

- Enable spatial arrangement of cells
- Regulate the differentiation, proliferation and metabolism of cells [51]

Scaffolds are divided into natural and synthetic types. Natural scaffolds include collagen, glycosaminoglycan, demineralized dentin and fibrin [52]. Synthetic scaffolds include polylactic acid, polyglycolic acid, polylactic co-glycolic acid, poly-epsilon-caprolactone, hydroxy apatite, tricalcium phosphate, bio-ceramics, titanium, alginate or derivatives of polyethylene glycol [53].

A suitable scaffold must selectively attach to the cells and aggregate them. Also, it must contain growth factors and disintegrate within a specific time period. Thus, a scaffold is much more complex than a simple network for cell arrangement [5]. Clinically, it appears that PRP has many of these characteristics. PRP is autologous and its preparation is relatively easy. It is rich in growth factors, disintegrates over time and creates a 3D fibrin matrix [54]. Combination of scaffolds with special growth factors is important for optimal regeneration of pre-odontoblast cells. This is an important field of research for development of regenerative

endodontics as a clinically predictable process [55].

Even after selection of a suitable cell source, growth factors and proper scaffold, the complex must be somehow transferred to the RCS. Thus, a proper delivery system is also required.

All cells in the body must be within 0.1 mm distance from the blood vessels for adequate supply of oxygen and nutrients [56]. If cells are injected in the entire length of the root canal system, most of them will be destroyed due to tissue hypoxia. An alternative technique is to inject a mixture of cells-scaffold-growth factors into the apical one millimeter of the root and then fill the rest of the RCS with a combination of scaffold and growth factors [55]. Since dental pulp is composed of a core of connective tissue surrounded by a layer of odontoblasts, transfer of cells and growth factors inside a scaffold may play a special role in promotion of dentin formation without root canal calcification.

Further studies are required in order to properly answer this question. After providing the necessary conditions for differentiation of stem cells to odontoblasts, the main issue would be the identification of the differentiated cells. Identification of cells differentiated into odontoblasts is not easy since they very much look like osteoblasts (due to producing mineralized nodules and expression of several proteins such as dentin sialoprotein).

However, dentin sialoprotein surface area in odontoblasts is approximately 400 times that of osteoblasts [57]. Assessing only one or two characteristics of a cell cannot definitely identify it or determine whether the resultant cell is an actual odontoblast or not [36]. Phenotype of the cell must be taken into account as well. However, among odontoblasts, phenotype of cells located in the apical (squamous) is different from that of coronal (long columnar) cells [33]. Molecular studies have identified many genes specifically expressed by odontoblasts and this knowledge is expected to help future studies provide the necessary conditions for differentiation of mesenchymal cells to actual odontoblasts [36]. Accurate identification of cells depends on both cell morphology and expression of different genes. In order to be highly accurate, cells producing mineralized nodules and express dentin sialoprotein are considered to be pre-odontoblasts [58].

Differentiation of human dental pulp cells to pre-odontoblasts is confirmed via the followings:

-Deposition of mineral matrix confirmed by Von Kossa staining

-Immunostaining of human dental pulp cells [59]

Differentiation of dental pulp cells to adipocytes is specified by the following:

-Accumulation of neutral lipid vacuoles stained by oil red O [60]

Differentiation of dental pulp cells to cartilaginous tissue is specified by the followings:

-Secretion of cartilage-specific proteoglycans stained with blue alkaline

-Immunostaining for type II collagen [61]

A summary of regenerative endodontic research projects:

Many studies have used cell culture techniques for detection of key growth factors regulating the differentiation of pre-odontoblasts [62-66]. However, further studies on animal models are required to assess pulp tissue regeneration. In a previous study, human root apex filled with a combination of human stem cells, growth factors and scaffold was implanted in the body of immunocompromised mice and it was demonstrated that the formed pre-odontoblast cells had human origin [67]. The key difference of revascularization and tissue engineering (that focuses on the transfer of cells-growth factors and scaffold) is in that revascularization focuses on reinstating blood supply to the empty space inside the root canal system.

Almost 50 years ago, Nygaard Ostby [68] assessed the ability to regenerate pulp tissue inside the RCS. His technique was based on the role of blood clot in wound healing. He evaluated nine patients aged 21-42 years with 17 teeth. After access cavity preparation and extraction of the pulp tissue, a file was passed through the apex to cause bleeding and the root canals were filled with Kloroperka paste and gutta percha. Necrotic pulp teeth were treated similar to the vital pulp teeth with an exception that after canal irrigation, sulfathiazole and 4% formaldehyde were used as intracanal medications in between treatment sessions. The teeth were then extracted and histologically analyzed, revealing intracanal calcification. One month after treatment, periodontal tissue was healed. Ten months after treatment, periapical bone had been formed. Blood clot in the RCS was gradually replaced with the granulation tissue and then with

fibrous connective tissue. No newly formed dentin was observed.

Although the newly formed tissue was evident on some of the dentin walls, it appeared to be cementum rather than newly formed dentin. Recent histological studies confirm the deposition of cementum [38, 67, 69, 70]. The ingrowth of this newly formed tissue was limited to the apical three millimeters of the RCS.

Necessary considerations in revascularization treatment:

A few points must be taken into account for revascularization treatment. Case selection is the first step. This treatment is suitable for a permanent tooth with undeveloped root that negatively responds to pulp tests [71]. The final goal of this treatment is pulp tissue regeneration in a permanent, closed apex tooth. However, it must be noted that the current revascularization protocols are not well developed for such complex (closed apex) cases. Another point is that the patient must sign a written informed consent form. This form must include adequate information about the number of treatment sessions (minimum of two sessions), risk of possible side effects and complications (such as risk of tooth crown discoloration by minocycline) [72], the possibility of no response to treatment, and alternative treatment options [73]. Clinical discoloration of tooth crown above the gingival margin may occur secondary to the application of minocycline to pulp chamber. This problem may be minimized and limited to areas below the cemento-enamel junction by using a drug delivery system [74]. In case of occurrence of crown discoloration, it may be improved or completely resolved by walking bleaching with sodium perborate [75]. An alternative treatment for such cases is apexification with mineral trioxide aggregate (MTA) [76]. In the first session, clinical data must be collected, pulp and periapical diagnosis must be confirmed and risks and benefits of this treatment along with other available treatment options must be thoroughly explained to the patients and/or their parents.

Clinical stages of revascularization treatment:

After obtaining a written informed consent, the tooth is anesthetized, isolated and access cavity is prepared. Minimum canal preparation must be done. But, a small size file must be necessarily used for RCS scouting and determination of working length. RCS is gradually rinsed with 20 mL of

5% sodium hypochlorite followed by 20 mL of 2%-12% CHX. Since canal disinfection is mainly based on chemical irrigation, the needle must be placed in the apical one-third and irrigation must be carried out using a needle with a closed-end tip and a side vent at a low speed to prevent passage of materials from the open apex. Next, the RCS is dried with sterile paper points and antimicrobial agents are transferred to the RCS.

Researchers use several medications for disinfection of the RCS including the triple antibiotic paste (TAP) (1:1:1 ciprofloxacin, metronidazole and minocycline), CH alone or in combination with antibiotics and formocresol [77, 78]. TAP is very efficient against dental pathogens and its efficacy has been confirmed by several studies. However, it is not FDA approved and can cause crown discoloration due to the presence of minocycline in its composition. Calcium hydroxide is an easily accessible alternative. However, it may be cytotoxic for stem cells. However, the TAP has been less commonly used for dental purposes in comparison with CH and formocresol and further investigations are warranted in this regard. Hoshino et al. [77] confirmed the efficacy of a combination of antibiotics (especially the combination of ciprofloxacin, metronidazole and minocycline) for elimination of bacteria from the infected dentin walls. The TAP eliminates the bacteria from the RCS of necrotic teeth with immature apex and provides a suitable environment for growth of blood vessels and stem cells in the RCS. The efficacy of this paste has been confirmed in a study on dogs and a 10,000 times reduction in viable bacterial count was reported as the result of its application [79]. In general, evidence suggests the use of TAP or CH.

Aside from the type of intracanal medicament, its place of application is also important. If the application of CH is limited only to the coronal half of the root canal, the amount of increase in the root canal wall thickness will be 55%. If the application of CH is limited to the apical half of the RCS, increase in wall thickness will be 3% only. This is probably due to the fact that the remaining CH exerts a cytotoxic effect on stem cells. Studies have shown that a minimum of 12-18 month period is required in order to be able to judge about the radiographic evidence of root development [80]. After the placement of the disinfectant, the cavity is

sealed by a sterile sponge and a temporary restorative material like Cavit. The patient is scheduled for a follow up visit at 3-4 weeks. In the second visit, the patient is examined for any sign of acute infection (swelling, pain, sinus tract, etc. that probably existed in the first session). The antimicrobial treatment is repeated if the problem still exists. In most cases, acute signs and symptoms disappear by the second visit. Since the bleeding required for revascularization is induced at the second session, the tooth should not be anaesthetized with an anesthetic agent containing vasodepressors; 3% mepivacaine may be used, which enhances the potential of bleeding in the RCS.

Following isolation and re-preparation of a coronal access cavity, the tooth is gradually rinsed with 20 mL of 5% sodium hypochlorite. At the same time, the RCS must be agitated with a hand file to remove the antimicrobial agent. After drying the RCS with sterile paper points, a file is passed via the apex for a few millimeters to cause bleeding by up to 3 mm apical to the CEJ. A small piece of collagen plug may be placed in the RCS to act as a resorbable matrix and limit the application of MTA. Three millimeters of the MTA is placed and sealed with the restoration.

In most cases, a blood clot forms inside the RCS. The blood clot is actually a protein scaffold that enables 3D ingrowth of tissue [68]. Almost all available reports mention thickening of the root canal walls followed by closure of apex [81]. It must be noted that radiographic thickening of dentinal walls does not necessarily indicate dentin formation because histological assessments have not been done in clinical cases [82]. Based on histological results, increased thickness of root canal walls is due to the ingrowth of cementum, bone or a dentin-like substance [11, 83]. As mentioned earlier, a 12-18 month period is minimally required for clinical examination and radiographic assessment of root development.

Clinical assessment of treatment results:

RCT aims to preserve and restore the health of the root surrounding tissues [1]. The goals of revascularization treatment are beyond those of RCT. It also aims to reinstate the vital and functional tissue that is capable of completing root development in immature permanent teeth [84]. The success of revascularization treatment is based on the pres-

ence of radiographic and clinical evidence of the health of periradicular tissues and presence of a vital pulp in the RCS. Radiographic evidence of the pulp tissue function (or dental pulp-like tissue) includes continuation of root development in both length and thickness of the walls. Other criteria include presence of vital tissue in the root canal space, ensuring adequate blood supply by Laser Doppler Flowmetry, pulp tests like heat and cold tests and electric pulp testing and absence of clinical signs and symptoms [4]. Published articles on revascularization treatment have only mentioned increased dentinal wall thickness to be limited to the middle and apical one-thirds of the root. There is no evidence on the increased root thickness at the cervical third (which is susceptible to fracture) [80]. Future clinical studies must focus on the extension of revascularization to the cervical area, strengthening this area and decreasing the risk of root fracture.

After reviewing the revascularization methods, it seems necessary to discuss the benefits and limitations of it compared to those of other techniques for immature teeth with necrotic pulp. RCT of immature teeth is problematic. Root apices in these teeth are not well developed and cleaning and shaping of the apical third is difficult. Also, the thin and fragile dentinal walls are susceptible to fracture during preparation or obturation. Moreover, open apex increases the risk of leaking of the root canal filling material into the periapical tissues. An immature tooth with an open apex is conventionally treated with apexification requiring long term treatment with CH to induce the formation of an apical calcified barrier [85]. Long-term use of CH is among the drawbacks of conventional apexification and weakens the root structure. The main cause of tooth loss following apexification is root fracture [86-88]. Introduction of one-stage apexification by creating an artificial barrier with MTA decreases the number of treatment sessions as well as the duration of treatment. But, one stage apexification does not cause further root development [6,89-91]. One primary advantage of revascularization is further increase in root length and thickness of dentinal walls [84]. To date, no clinical trial has been published regarding revascularization and case reports do not provide definite evidence to support a specific treatment. However, in

comparison with preclinical studies, case reports have the advantage of being performed on actual patients. Thus, case reports carry a higher level of reliability [92]. Almost all reported cases are patients 8-18 years of age with teeth with immature apices. Age may be an important factor and some studies have discussed that younger patients have higher healing potential [93]. The regenerative power of stem cells is also higher in younger patients [94]. Moreover, a large-diameter immature apex enhances the growth of granulation tissue in the RCS and can provide a rich source of mesenchymal stem cells of the apical papilla. However, stronger evidence is required to cast a judgment on the more efficient treatment.

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