Regenerative endodontics: A state of the art

Rashmi Bansal, Rajesh Bansal

ABSTRACT

Scientific advances in the creation of restorative biomaterials, in vitro cell culture technology, tissue grafting, tissue engineering, molecular biology and the human genome project provide the basis for the introduction of new technologies into dentistry. Non-vital infected teeth have long been treated with root canal therapy (for mature root apex) and apexification (for immature root apex), or doomed to extraction. Although successful, current treatments fail to re-establish healthy pulp tissue in these teeth. But, what if the non-vital tooth could be made vital once again? That is the hope offered by regenerative endodontics, an emerging field focused on replacing traumatized and diseased pulp with functional pulp tissue. Restoration of vitality of non-vital tooth is based on tissue engineering and revascularization procedures. The purpose of this article is to review these biological procedures and the hurdles that must be overcome to develop regenerative endodontic procedures.

Key words: Growth factors, regenerative endodontics, revascularization, scaffolds, stem cells

Regenerative endodontic procedures can be defined as biologically based procedures designed to create and deliver tissues to replace diseased, missing and traumatized pulp–dentin complex. The science of regenerative endodontics has a long history dating back to 1952 when Dr. BW Hermann reported on the application of calcium hydroxide in a case report of vital pulp amputation.[1]

Presently, two concepts exist in regenerative endodontics to treat non-vital infected teeth - one is the active pursuit of pulp-dentine regeneration to implant or regrow pulp (tissue engineering technology), and the other in which new living tissue is expected to form from the tissue present in the teeth itself, allowing continued root development (revascularization).

Tissue engineering can be defined as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.[2]

The three key components for tissue engineering are:

- Stem cells – to respond to growth factors.
- Scaffold of extracellular matrix (ECM).
- Growth factors (signals for morphogenesis).

STEM CELLS

They are defined as clonogenic cells capable of both self-renewal and multilineage differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity.[3] They differentiate into one daughter stem cell and one progenitor cell. There are basically two types of stem cells:[4]

- Embryonic stem cells – located within the inner cell mass of the blastocyst stage of development.
- Postnatal stem cells – that have been isolated from various tissues including bone marrow, neural tissue, dental pulp and periodontal ligament.

Since the sourcing of embryonic stem cells is controversial and is surrounded by ethical and legal issues, many researchers are now focussing attention on developing stem cell therapy using postnatal stem cells donated by the patients themselves or their close relatives. Stem cells are often categorized by their source:

- Autologous stem cells – are obtained from the same individual to whom they will be implanted.
- Allogenic stem cells – originate from a donor of the same species.
- Xenogenic cells – are those isolated from individuals of another species.
For endodontic regeneration, the most promising cells are autologous postnatal dental stem cells because there are less chances of immune rejection. They show more striking odontogenic capability (typical tooth-shaped tissue with balanced amelogenesis and dentinogenesis) as compared to non-dental stem cell population like bone marrow stromal stem cell. Various sources for postnatal dental stem cells have been successfully studied:

- Permanent teeth – Dental pulp stem cells (DPSCs): derived from third molar.[2]
- Deciduous teeth – Stem cells from human-exfoliated deciduous teeth (SHED): stem cells are present within the pulp tissue of deciduous teeth.[7]
- Periodontal ligament - Periodontal ligament stem cells (PDLSC),[8]
- Stem Cells from apical papilla (SCAP).[9]
- Stem cells from supernumerary tooth – Mesiodens.[10]
- Stem cells from teeth extracted for orthodontic purposes.[11]
- Dental follicle progenitor cells.[12]
- Stem cells from human natal dental pulp- (hNDP).[13]

Stem cells from various sources and their features studied by various researchers[2,6-10,12-34] are shown in Table 1.

All types of postnatal dental stem cells studied have mesenchymal stem cell-like qualities, such as capacity for self-renewal and multilineage differentiation. Immunocytochemistry has proved the existence of stem cells in these cell populations using STRO-1 as a stem cell marker. These cells also express the mesenchymal stem-cell markers CD29 and CD44.[13] These stem cells are isolated from specialized tissue with potent capacities to differentiate into odontogenic cells; however, they also have the ability to give rise to other cell lineages similar to but different in potency from that of bone marrow stem cells.[24] From both DPSCs and SHED, tissue similar to normal dentin-pulp is reported to be regenerated which can be later on used for regenerative endodontics.[36] But SHED are retrieved from a tissue that is ‘disposable’ and readily accessible. The best candidates for SHED are moderately resorbed canine and incisors with the presence of healthy pulp. In children, other sources of easily accessible stem cells are supernumerary teeth, mesiodens, over-retained deciduous teeth associated with congenitally missing permanent teeth and prophylectically removed deciduous molars for orthodontic indications. SHED also show higher proliferation capability, abundant cell supply and painless stem-cell collection with minimal invasion, so SHED could be a desirable option as a cell source for regenerative endodontics.[37,38] However, in comparison, DPSCs show higher inclination towards neuronal lineage.[39] Stem cells are isolated from aging teeth, also but it is observed that number of cells and their proliferation rate decreases with age and it is maximum when only crown is formed (germ stage). SCAP have higher proliferation rate as compared to DPSCs. They appear to be the source of primary odontoblasts that are responsible for root dentin formation whereas DPSCs are the likely source of replacement odontoblast. SCAP represent early progenitor cells,[90] so whether SCAP are a more suitable stem-cell source than DPSCs and SHED require further investigation. For regeneration of periodontium, PDLSCs are better source as stem cell as compared to cells isolated from pulp. Viable periodontal ligament is reported to be generated from PDLSCs.[29] Instead of forming entire tooth, even a bio-root with periodontal ligament tissue has been generated by utilizing SCAP along with the PDLSCs. This bio-root is encircled with periodontal ligament tissue and has natural relationship with the surrounding bone.[40]

Autologous stem cells are relatively easy to harvest and easy to inject by syringe (injectable postnatal stem-cell therapy). But in this technique, cells have low survival rate and they might migrate to different locations within the body possibly leading to aberrant patterns of mineralization.[41] A solution to the above problem is to apply the cells together with scaffold material – the second component of tissue engineering.

SCAFFOLD

A scaffold used for regeneration should provide the framework for cell growth, differentiation and organization at a local site. A scaffold should be porous to allow for placement of cells and also be biocompatible with host tissue.[42] It should be biodegradable and should degrade gradually so that it is replaced by regenerative tissue.[43] It should be effective for transport of nutrients and waste.[44] Most tissue engineering efforts use biomaterials for scaffolds already approved by the FDA. They can be natural (collagen, dentin, fibrin, silk, alginate) or synthetic (various polymers like PLA, PGA, etc.). Synthetic polymers are generally degraded by simple hydrolysis while natural polymers are mainly degraded enzymatically. Various scaffolds studied by different researchers[36,45-58] are shown in Table 2.

Collagen is the most widely studied natural scaffold. The most widely used synthetic scaffolds are polymers of lactide and glycolide. In regenerative endodontics, a tissue-engineered pulp is not required to provide structural support to the tooth. So, engineered pulp tissue can be administered in a soft three-dimensional scaffold matrix, such as polymer hydrogel,[95] which can be injected at the site (injectable scaffold delivery). Hydrogels have similar physical properties as that of living tissue, which is due to their high water content, soft and rubbery consistency and low interfacial tension with water or biological fluids. Research is focusing on making hydrogels photo-polymerizable[60] or self-hardening e.g., silanized hydroxyl-propyl-methyl cellulose,[61] so that they form rigid structures once they are implanted into the tissue sites. Another injectable scaffold studied is β-tricalcium phosphate.[62] It is alginate in gel phase and forms beads in solid phase. Treated dentin matrix also provides suitable environment for regeneration.
Results of the study

DPSCs exhibit differentiation potential toward adipogenic, neurogenic, myogenic and chondrogenic lineages. DPSCs are capable of forming ectopic dentin and associated pulp tissue in vivo.

Histomorphometric analysis of pulpal cell population with ageing indicate that some reduction in pulpal cell numbers occur including the subodontoblastic cells which may be the prime candidate for participation in regeneration.

DPSCs express dentin-sialo-phospho-protein (DSPP)-dentin marker in Xenogenic transplant and constitute an ideal source of odontoblast and mineralized tissue.

Stem cells can also be derived from human adult [aged 30-45 yrs] dental pulp; newly formed tissue constitu tes odontoblast and mineralized tissue.

DPSCs maintain their stem cell properties even after cryopreservation.

DPSCs exhibit differentiation potential toward adipogenic, neurogenic, myogenic and chondrogenic lineages under the influence of inductive media.

SHED were reported to have higher proliferation rate and higher population doublings.

Characterized the self-renewal capability, multilineage differentiation capacity and clonogenic efficiency of DPSCs.

DPSCs are located predominantly in perivascular area of pulpal cavity.

DPSCs have more striking odontogenic capability than BMMS cells.

DPSCs culture contains multipotent neural crest stem cells.

DPSCs from human tooth germ (crown completed stage) show higher proliferation rate than those isolated at a later stage. But in long term, these DPSCs underwent a change in morphology and lost their differentiation ability.

DPSCs from pulp horn were mature than pulp core cells. Cervical loop epithelial cells combined with pulp horn cells mainly reconstitute dentin-cementum structure. By contrast, cervical loop epithelial cells combined with pulp core cells reconstituted enamel-dentin structures.

SHED were reported to have higher proliferation rate and higher population doublings.

SHED are identified to be capable of differentiating into a variety of cell types including neural cells, adipocytes and odontoblasts.

Both DPSCs and SHED are capable of regenerating pulp and dentin, if proper biochemical stimuli are provided. DPSCs and SHED are pluripotent cells. These cells expanded in vitro and differentiated into osteoblasts, chondrocytes and adipocytes.

Periodontium contains progenitor cells that can migrate and differentiate into either cementoblasts or osteoblasts.

PDLCs expressed mesenchymal stem cell markers STRO-1 and CD146/MUC18. PDLCs differentiated into cementoblast like cells, adipocytes and collagen-forming cells. In mice they generated cementum/periodontal like structure. They form sparse calcified nodules as compared to DPSCs.

PDLCs possess self-renewal capability and multilineage differentiation quality.

PDLCs differentiate into cementoblast like cells and formation of cementum/periodontal ligament like tissues.

Discovered mesenchymal stem cells from apical papilla of human immature permanent teeth. Cells in apical papilla proliferated 2-3-fold greater than those in the pulp in organ culture. Apical papilla is distinctive to the pulp in terms of containing less cellular and vascular components than those in pulp.

SCAP exhibited adipogenic, neurogenic differentiation capability.

Dental papilla derived stem cells have osteogenic potential and could be used as additional source of cells.

DPSCs derived from Mesiodens expressed stem cell-like qualities.

hNDP showed adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential.

DPSCs proliferated significantly more in hypoxia (3% oxygen tension) than in normoxia (20% oxygen tension)

The differentiation capacity of DPSCs into odontoblasts, osteoblasts and chondrocytes changes during cell passaging and is restricted to osteoblast lineage only at the ninth passage.

Table 1: Features of stem cells studied by various researchers

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Results of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gronthos et al.[6]</td>
<td>DPSCs have the potential to form dense calcified nodules</td>
</tr>
<tr>
<td>Shi et al.[14]</td>
<td>DPSCs are located predominantly in perivascular area of pulpal cavity</td>
</tr>
<tr>
<td>Gronthos et al.[21]</td>
<td>Characterized the self-renewal capability, multilineage differentiation capacity and clonogenic efficiency of DPSCs</td>
</tr>
<tr>
<td>Murray et al.[15]</td>
<td>DPSCs are capable of forming ectopic dentin and associated pulp tissue in vivo</td>
</tr>
<tr>
<td>Laino et al.[17]</td>
<td>Histomorphometric analysis of pulpal cell population with ageing indicate that some reduction in pulpal cell numbers occur including the subodontoblastic cells which may be the prime candidate for participation in regeneration</td>
</tr>
<tr>
<td>Zhang et al.[18]</td>
<td>DPSCs express dentin-sialo-phospho-protein (DSPP)-dentin marker in Xenogenic transplant and that this expression is not present in bone formed by bone marrow stromal cells suggesting their undifferentiated preodontogenic phenotype</td>
</tr>
<tr>
<td>Yang et al.[19]</td>
<td>Stem cells can also be derived from human adult [aged 30-45 yrs] dental pulp; newly formed tissue constitutes an ideal source of odontoblast and mineralized tissue</td>
</tr>
<tr>
<td>Yu et al.[20] and Huang et al.[21]</td>
<td>DPSCs maintain their stem cell properties even after cryopreservation</td>
</tr>
<tr>
<td>Stevens et al.[21]</td>
<td>DPSCs exhibit differentiation potential toward adipogenic, neurogenic, myogenic and chondrogenic lineages under the influence of inductive media</td>
</tr>
<tr>
<td>Takeda et al.[22]</td>
<td>SHED were reported to have higher proliferation rate and higher population doublings</td>
</tr>
<tr>
<td>Sumita et al.[23]</td>
<td>SHED are identified to be capable of differentiating into a variety of cell types including neural cells, adipocytes and odontoblasts</td>
</tr>
<tr>
<td>Miura et al.[24]</td>
<td>Both DPSCs and SHED are capable of regenerating pulp and dentin, if proper biochemical stimuli are provided</td>
</tr>
<tr>
<td>Cordiero[25]</td>
<td>DPSCs and SHED are pluripotent cells. These cells expanded in vitro and differentiated into osteoblasts, chondrocytes and adipocytes</td>
</tr>
<tr>
<td>Koyama et al.[26]</td>
<td>Periodontium contains progenitor cells that can migrate and differentiate into either cementoblasts or osteoblasts</td>
</tr>
<tr>
<td>Gould et al.[27]</td>
<td>PDLCs expressed mesenchymal stem cell markers STRO-1 and CD146/MUC18. PDLCs differentiated into cementoblast like cells, adipocytes and collagen-forming cells. In mice they generated cementum/periodontal like structure. They form sparse calcified nodules as compared to DPSCs</td>
</tr>
<tr>
<td>Seo[28]</td>
<td>PDLCs possess self-renewal capability and multilineage differentiation quality</td>
</tr>
<tr>
<td>Ballini et al.[29]</td>
<td>PDLCs differentiate into cementoblast like cells and formation of cementum/periodontal ligament like tissues</td>
</tr>
<tr>
<td>Yang et al.[30]</td>
<td>Discovered mesenchymal stem cells from apical papilla of human immature permanent teeth</td>
</tr>
<tr>
<td>Sonoyama et al.[30]</td>
<td>Cells in apical papilla proliferated 2-3-fold greater than those in the pulp in organ culture</td>
</tr>
<tr>
<td>Huang et al.[30]</td>
<td>Apical papilla is distinctive to the pulp in terms of containing less cellular and vascular components than those in pulp</td>
</tr>
<tr>
<td>Park et al.[31]</td>
<td>SCAP exhibited adipogenic, neurogenic differentiation capability</td>
</tr>
<tr>
<td>Huong et al.[30]</td>
<td>Dental papilla derived stem cells have osteogenic potential and could be used as additional source of cells</td>
</tr>
<tr>
<td>Karaz et al.[32]</td>
<td>DPSCs derived from Mesiodens expressed stem cell-like qualities</td>
</tr>
<tr>
<td>Sakdee et al.[33]</td>
<td>hNDP showed adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential</td>
</tr>
<tr>
<td>Arthur et al.[34]</td>
<td>DPSCs proliferated significantly more in hypoxia (3% oxygen tension) than in normoxia (20% oxygen tension)</td>
</tr>
<tr>
<td>Yu et al.[34]</td>
<td>The differentiation capacity of DPSCs into odontoblasts, osteoblasts and chondrocytes changes during cell passaging and is restricted to osteoblast lineage only at the ninth passage</td>
</tr>
</tbody>
</table>

Silk scaffolds may be used for mineralized osteo-dentin formation. The size and shape of silk scaffold pores guide mineralized tissue. Enamel matrix derivatives (Emdogain), whose major component is amelogenins, have also been used as potential scaffolds.

Growth factors are quite versatile, stimulating cellular participation in regeneration.

GROWTH FACTORS

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

of dental tissue. Silk scaffolds may be used for mineralized osteo-dentin formation. The size and shape of silk scaffold pores guide mineralized tissue. Enamel matrix derivatives (Emdogain), whose major component is amelogenins, have also been used as potential scaffolds.

The seeding of cells on tissue engineering scaffolds is known as 'creating a tissue construct'. To promote the formation of higher order tissue structures, tissue constructs are maintained in cell culture in the presence of bioactive molecules called growth factors—the third component of tissue engineering.

GROWTH FACTORS

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. Various growth factors studied in regeneration of pulp-dentin complex are depicted in Table 3.

Growth factors play a role in signalling many events in pulp-dentine regeneration. Two important families of growth factor that play a vital role are transforming growth factor (TGF) and bone morphogenetic protein (BMP). TGF-β1 and β2 are important in cellular signalling for odontoblast differentiation and stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and are deposited within the dentin matrix, where they remain protected in an active form through interaction with other components of the dentin matrix. The addition of purified dentin protein fractions stimulates an increase in tertiary dentin matrix secretion suggesting that TGF-β1 is involved in injury signalling and tooth-healing reaction. BMPs induce higher quantity and more homogeneous reparatory dentin with the presence of many tubes with defined odontoblastic process as compared to that with calcium hydroxide. BMP-2, BMP-4 and BMP-7 have been shown to direct stem cell differentiation into odontoblasts and result in dentin formation making the BMP family the most likely candidate as growth factors. Some natural materials like dentin are also used because they release bio-active molecules. Enamel matrix derivative is also capable of inducing dentin formation when applied to dentin pulp complex.

Poor angiogenesis is a major roadblock for tissue regeneration. Following approaches are currently being studied for the development of vasculature to support the metabolic needs of engineered tissue:

- Transplanted endothelial cells can increase the vasculature in polymer scaffolds and integrate with growing host capillaries.
- Localized delivery of inductive angiogenic factors (VEGF, PDGF, EGF) at the site of the engineered tissue.
- Co-transplantation of hematopoietic and mesenchymal stem cells.

Although we are aware of the role played by these growth factors, for tissue engineering to be successful it is critical to deliver appropriate growth factors to the desired site at the appropriate dose and for appropriate time for which further research is required. Many of these proteins have

**Table 2: Various scaffolds studied by different researchers**

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Scaffold studied</th>
<th>Results of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feng et al.</td>
<td>Collagen (natural)</td>
<td>Collagen provides great tensile strength in tissues. It allows for easy placement of cells and growth factors and allows for replacement with natural tissues after undergoing degradation.</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>Collagen (natural), PLA, PGA (Synthetic)</td>
<td>Natural scaffold provide good biocompatibility and bioavailability, synthetic scaffolds offer more control over the degradation rate and mechanical properties.</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>Spongeous collagen, porous ceramic, fibrous titanium mesh</td>
<td>All the three types supported attachment, growth and differentiation of DPSC in vitro and the cells organized into a well vascularized tissue that expressed DSPP, a marker of dentin in vivo.</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>Collagen and PLG</td>
<td>Polymer (PLG) scaffolds are contraction resistant as compared to collagen. Survival rate of DPSCs and PDLSCs was optimal in collagen and polymer scaffold as compared to calcium phosphate scaffold.</td>
</tr>
<tr>
<td>Gebhardt et al.</td>
<td>Polymer, collagen, calcium phosphate bioceramic scaffold</td>
<td>PGA act as suitable matrices for seeding of dental pulp fibroblasts, allowing their proliferation and development of a tissue with similar cellularity to normal pulp.</td>
</tr>
<tr>
<td>Mooney et al.</td>
<td>PGA</td>
<td>Culturing pulp cells grown on PGA in vitro resulted in high cell density tissue similar to native pulp.</td>
</tr>
<tr>
<td>Bohl et al.</td>
<td>PGA (polymer)</td>
<td>Polymer scaffolds hold the most promise for creating replacement tissue. Human dental pulp and gingival fibroblast adhere to non woven PGA scaffolds, proliferate and produce extracellular matrix in vitro.</td>
</tr>
<tr>
<td>Buurma et al.</td>
<td>PGA</td>
<td>Enhances attachment, proliferation and differentiation of PDLSCs.</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>Glycidyl metha acrylated dextran (Dex-GMA) gelatin hybrid hydrogel</td>
<td>When DPSCs was grafted on polyactic co-glycolic acid polymer scaffolds and implanted in rabbit, osteodentin matrix showing tubular structure developed.</td>
</tr>
<tr>
<td>El-Backly et al.</td>
<td>Poly lactic co glycolic acid polymeric porous scaffold</td>
<td>This is the first study to characterize bioengineered tissue generated from tooth bud cells seeded on silk scaffold.</td>
</tr>
<tr>
<td>Xu et al.</td>
<td>Silk scaffold</td>
<td>These scaffolds were able to create a lasting three-dimensional soft tissue augmentation and histologic analysis revealed revascularization of the area through the biomaterial.</td>
</tr>
<tr>
<td>Etienne et al.</td>
<td>Silk scaffold</td>
<td>TDM provided suitable scaffold and inductive micro-environment for growth of dental follicle cells (DFCs)</td>
</tr>
<tr>
<td>Guo et al.</td>
<td>Treated dentin matrix (TDM)</td>
<td>HA sponge has an appropriate structure, bio-compatibility and biodegradation for use as a scaffold</td>
</tr>
<tr>
<td>Inuyama et al.</td>
<td>Hyaluronic acid (HA) sponge</td>
<td>Amelogenins in Emdogain self-assemble into nanospheres that constitute an extracellular matrix. This matrix is slowly digested by extra cellular proteolytic enzymes to release bioactive peptides.</td>
</tr>
<tr>
<td>Lyngstadaas et al.</td>
<td>Enamel matrix derivative (Emdogain)</td>
<td></td>
</tr>
</tbody>
</table>

[Downloaded free from http://www.ijdr.in on Monday, July 30, 2012, IP: 125.16.60.178] || Click here to download free Android application for this journal
**Table 3: Growth factors studied by various researchers**

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Superfamily</th>
<th>Growth factors studied</th>
<th>Results of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith et al.[80]</td>
<td>Transforming growth factor (TGF)</td>
<td>TGF-β₁</td>
<td>They are secreted by odontoblasts and are deposited within the dentin matrix, where they remain protected in an active form through interaction with other components of the dentin matrix</td>
</tr>
<tr>
<td>Smith et al.[87]</td>
<td>-do-</td>
<td>TGF-β₂, TGF-β₃</td>
<td>Stimulated increase in tertiary dentin matrix secretion</td>
</tr>
<tr>
<td>Huoja et al.[74]</td>
<td>TGF</td>
<td>TGF-β₁</td>
<td>Indicated for the first time that TGF-β₁ induces ectopic mineralization through regulation of osteo-calcin and type 1 collagen expression in DPSC</td>
</tr>
<tr>
<td>Tai et al.[89]</td>
<td>-do-</td>
<td>TGF-β</td>
<td>TGF-β₁ may affect the growth and differentiation of dental pulp cells via an autocrine fashion by activation of the ALK/Smad 2/3 – signal transduction pathways</td>
</tr>
<tr>
<td>Begue-Kirn et al.[70]</td>
<td>-do-</td>
<td>TGF-β₁, BMP-2</td>
<td>Demonstrated that TGF-β₁, present in dentin could interact with some component which acts as a modulator of its activity on the initiation of the cytological and functional differentiation of odontoblasts.</td>
</tr>
<tr>
<td>Nakashima et al.[71]</td>
<td>-do-</td>
<td>TGF-β₁, BMP-2 BMP-4</td>
<td>Demonstrated regulatory role of TGF-β₁, BMP-2 and 4 on the gene expression of extracellular matrix proteins and the differentiation of pulp cells into preodontoblasts.</td>
</tr>
<tr>
<td>Sloan et al.[72]</td>
<td>-do-</td>
<td>BMP-7</td>
<td>BMP-7 when applied to freshly cut dentin in monkey teeth, stimulated tertiary dentin formation</td>
</tr>
<tr>
<td>Iohara et al.[73]</td>
<td>-do-</td>
<td>BMP-2</td>
<td>BMP-2 can direct pulp progenitor stem cell differentiation into odontoblasts and result in dentin formation.</td>
</tr>
<tr>
<td>Roberts-Clark et al.[74]</td>
<td>PDGF</td>
<td>VEGF, PDGF-AB EGF</td>
<td>Dentin matrix contains angiogenic growth factors</td>
</tr>
<tr>
<td>He et al.[70]</td>
<td>Others</td>
<td>PIGF, FGF₂</td>
<td>TGF-β₁ initiates odontoblasts like differentiation of DPSCs; FGF-2 exerts effect on cell proliferation and synergistically upregulates the effects of TGF-β₁</td>
</tr>
<tr>
<td>Ishimatsu et al.[76]</td>
<td>Others</td>
<td>FGF-2</td>
<td>Dentin regeneration on amputated pulp can be regulated by adjusting the dose of FGF-2.</td>
</tr>
<tr>
<td>Goncalves et al.[75]</td>
<td>Platelet derived growth factor (PDGF)</td>
<td>rhVEGF</td>
<td>Induced an angiogenic response in the pulp</td>
</tr>
<tr>
<td>Aranha et al.[78]</td>
<td>PDGF</td>
<td>VEGF</td>
<td>Findings suggest that cells of severed dental pulps are still capable of responding to the angiogenic stimuli mediated by VEGF</td>
</tr>
<tr>
<td>Li et al.[79]</td>
<td>Others</td>
<td>GH and IGF-1 rhlGF-1</td>
<td>VEGF could be useful in the treatment of dental pulp conditions that require revascularization (e.g. immediate replantation of avulsed tooth: this hypothesis is under investigation)</td>
</tr>
<tr>
<td>Lovschall et al.[80]</td>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>He et al.[81]</td>
<td></td>
<td>DMP</td>
<td>Hyoxia, consequent to trauma, enhances VEGF expression in DPSCs</td>
</tr>
<tr>
<td>Almushayt et al.[82]</td>
<td>DMP</td>
<td>DMP-1 DMP₁</td>
<td>TGF-β1 enhances reparative dentino-genesis in pulp capping of rat molars</td>
</tr>
<tr>
<td>Prescott et al.[83]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sobhani et al.[84]</td>
<td>Endochondral bone matrix gelatin (ec BMG) IL-6</td>
<td></td>
<td>GH and IGF-1 induce BMP-2 and 4</td>
</tr>
<tr>
<td>Iwasaki et al.[85]</td>
<td>Antimicrobial peptide LL 37</td>
<td></td>
<td>rhGF-1 enhances reparative dentino-genesis in pulp capping of rat molars</td>
</tr>
<tr>
<td>Kajiya et al.[86]</td>
<td>Heme-oxygenase (HO-1)</td>
<td></td>
<td>DMP, can induce differentiation of stem cells into odontoblast-like cells and stimulate the formation of mineralized tissues.</td>
</tr>
<tr>
<td>Kim et al.[87]</td>
<td></td>
<td></td>
<td>Found in dentin and bone and regulates mineralization.</td>
</tr>
</tbody>
</table>

**GENE THERAPY**

Genes can stimulate or induce a natural biological process by expressing a molecule involved in regenerative response for the tissue of interest. Precise delivery and efficient transfer of genes into target tissue cells, prompt assessment of gene expression at required times and appropriate levels and/or minimization of undesirable systemic toxicity are essential prerequisites for successful gene therapy. Either viral or non-viral vectors are used to enable the cellular uptake and expression of genes. Viral vectors are genetically altered to eliminate their disease-causing ability. The viruses can replicate genes of interest together with their own genome,
through the use of host cell genetic machinery. Various viral vectors studied\(^{(92-99)}\) are depicted in Table 4.

Non-viral techniques involve either electroporation or ultrasound method for gene delivery. Ultrasound-mediated gene delivery is found to be successful both in vivo and in vitro but electroporation method is found successful only in vitro. This may be because of the lack of erythrocytes in the plasma clot due to thermal changes during electroporation in vivo. In the in vivo approach, the gene is delivered systematically into the bloodstream or locally to target tissues by injection or inhalation. In this approach, the healing potential of pulp tissue is enhanced by genes inducing dentin directly applied on the exposed amputated dental pulp. The ex vivo approach involves genetic manipulation of cells in vitro, which are subsequently transplanted to the regeneration site. The ex vivo gene therapy stimulates reparative dentin formation more optimally and rapidly in comparison to in vivo gene therapy.\(^{(100)}\) From these very few available data there are certain challenges to the gene therapy:

- Need for establishment of isolation, identification and expansion protocol of pulp stem cells.
- Safe and efficient gene delivery system needs to be optimized.
- Potential serious health hazards exist with the use of gene therapy. These arise from the use of the vector (gene transfer) system, rather than the genes expressed.\(^{(101)}\) The FDA did approve research into gene therapy involving terminally ill humans, but approval was withdrawn in 2003 after a 9-year old boy receiving gene therapy was found to have developed tumors in different parts of his body.\(^{(102)}\)
- Researchers must learn how to accurately control gene therapy and make it very cell specific so that it is safe to use clinically.
- Requirements to demonstrate that gene therapy can provide cost-effective and safe long-term treatment for conditions that would otherwise lead to significant pulp necrosis.

Numerous regenerative studies have demonstrated that stem cells can attach to and grow on tissue-engineered scaffolds but there are few studies on potential of stem cells to create dental pulp constructs within human cleaned and shaped root canals.\(^{(103)}\) Recent study has reported for the first time regeneration of dental pulp-like tissue in endodontically treated root canals of real-size, native human teeth. This newly formed tissue appeared dense with disconnected cells surrounded by extracellular matrix. Erythrocyte filled blood vessels were formed with endothelial-like cell lining. There was complete fill of dental pulp-like tissue in entire root canal from root apex to pulp chamber with tissue integration to dental walls.\(^{(104)}\) Dental pulp construct growing in root canal without functional connection is meaningless; hence, further research is required to regenerate a replacement vital pulp attached to the circulatory system and the old dentin as well as produce new dentin matrix.

### REVASCULARIZATION

Regeneration of tissue from cells in teeth itself.

- Basically, body tissue is composed of two components: cells and the surrounding environment. The latter includes the ECM for cell proliferation and differentiation (natural scaffold). Revascularization approach in young permanent infected teeth with immature root apex and apical periodontitis was first attempted in 1971,\(^{(105)}\) but it was not successful due to limitations in technologies, material and instruments available in those times. But with the currently available technologies, several case reports\(^{(106-107)}\) have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over-instrumentation. The revascularization method assumes that the root canal space has been disinfected and that the formation of blood clot yields a matrix (e.g., fibrin) that traps cells capable of initiating new tissue formation. It is different from apexification because not only the apex is closed but the canal walls are thicker as well. It is also different from apexogenesis which also accomplishes a

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Viral vector studied</th>
<th>Results of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naldini et al.(^{(92)})</td>
<td>Retrovirus</td>
<td>Demonstrated in vivo gene delivery and stable transduction of non-dividing cells. Effective method for inducing dentin regeneration in teeth with reversible pulps. Bone marrow stromal cells infected with this vector showed stable expression of BMP-2</td>
</tr>
<tr>
<td>Rutherford et al.(^{(92)})</td>
<td>Recombinant virus encoding BMP-7</td>
<td>Showed enhanced odontogenic differentiation</td>
</tr>
<tr>
<td>Sugiyama et al.(^{(94)})</td>
<td>Lentivirus vector encoding BMP-2</td>
<td>Bone formation in these scaffolds was greater. Modulate proliferation activity of PDLSCs; high expression of collagen Type I</td>
</tr>
<tr>
<td>Yang et al.(^{(95)})</td>
<td>Adenovirus encoding BMP-2 gene</td>
<td>Over-expression of β-catenin suppresses the differentiation and mineralization of DPSCs (negatively regulate odontoblast-like differentiation of DPSCs)</td>
</tr>
<tr>
<td>Zhang et al.(^{(96)})</td>
<td>Adenovirus encoding BMP-7</td>
<td>All the three scaffolds revealed increase in bone formation (highest increase in combination)</td>
</tr>
<tr>
<td>Shang et al.(^{(97)})</td>
<td>Adenovirus encoding PDGF-B</td>
<td></td>
</tr>
<tr>
<td>Scheller et al.(^{(98)})</td>
<td>Retrovirus encoding active form of β-catenin</td>
<td></td>
</tr>
<tr>
<td>Zhang et al.(^{(99)})</td>
<td>Adenovirus encoding BMP-7, PDGF-B or combination of both</td>
<td></td>
</tr>
</tbody>
</table>
Advantages of root canal revascularization:

- Revascularization occurs most predictably in teeth with open apices and necrotic pulp secondary to trauma.
- Apex open > 1.5 mm.
- Bacteria should be removed from canal by any of the following methods:
  - '3 mix-MP' triple antibiotic paste consisting of ciprofloxacin, metronidazole and minocycline.\(^{106}\)
  - Calcium hydroxide,\(^{108}\) formocresol.\(^{109}\)
  - Effective coronal seal.
- Matrix into which new tissue can grow.
- Patients should be young.
- Use of anaesthetic without a vasoconstrictor when trying to induce bleeding.\(^{110}\)
- No instrumentation of the canals.
- Sodium hypochlorite is used as an irritant.
- Formation of a blood clot probably serves as a protein scaffold permitting 3-dimensional ingrowth of tissue.

All the studies report continued thickening of the dentinal walls and subsequent apical closure. The root length is increased by the growth of cementum. Connective tissue similar to periodontal ligament was also present in the canal space.\(^{111}\)

The success of root canal revascularization is mainly due to the following facts: firstly, the immature avulsed tooth has an open apex, short root and intact but necrotic pulp tissue. Therefore, the new tissue has easy access to the root canal system and a relatively short distance for proliferation to reach the coronal pulp horn. The speed with which the tissue completely revascularizes the pulp space is important because bacteria from outside are continually attempting to enter the pulp space. The ischemically necrotic pulp acts as a scaffold into which the new tissue grows, and the fact that the crown is usually intact slows bacterial penetration because their only access to the pulp is through cracks or enamel defects. Thus, the race between proliferation of new tissue and infection of the pulp space favors the new tissue. Secondly, minimum instrumentation preserves viable pulp tissue which contributes to further development of open apex root. Thirdly, young patients have greater healing capacity and more stem cell regenerative potential.

Further studies required in root canal revascularization:

- Radiographical findings of continued dentinal wall thickening do not address the cellular nature of this calcified material. In contrast, source of cells regenerating the replacement pulp tissue in implanting dental pulp construct is endodontic in origin.
- Although these case reports primarily involve treating the immature permanent teeth, it is quite possible that knowledge gained from this clinical application will have value in developing regenerative endodontic procedures for the fully developed permanent teeth.
- It is more likely that the tissue in pulp space is more similar to periodontal ligament than to pulp tissue.\(^{53}\)

CONCLUSIONS

Future regenerative endodontics may involve the cleaning and shaping of root canals followed by the implantation of vital dental pulp tissue constructs created in laboratory. The success of regenerative endodontic therapy is dependent on the ability of researchers to create a technique that will allow clinicians to create a functional pulp tissue within cleaned and shaped root canal systems. The source of pulp tissue may be from root canal revascularization, stem-cell therapy and pulp implantation.

Clinical success of regenerative endodontic therapy will depend on the following clinical outcomes:

- Vascular blood flow
- Mineralizing odontoblastoid cells
- Intact afferent innervations
- Lack of signs or symptoms

Limitations (Concern for researchers)

- Although the replacement pulp has the potential to revitalize teeth, it may also become susceptible to further pulp disease and may require retreatment; the implantation of engineered tissue also requires enhanced microbiological control methods required for adequate tissue regeneration.
- The success of clinical applications of pulp stem cells is limited by the culture conditions and the nature of microenvironment in which the primitive multipotent pulp stem cells are maintained and expanded.
- To improve the ability of dental pulp constructs to adhere to root canal walls, it seems that the ideal scaffold design is in the same shape as gutta-percha cones. Researchers had used single–canal teeth and cylindrical scaffolds in an attempt to simplify the transplantation process. A more complex root canal anatomy will require more complex scaffolds or the use of more flexible scaffolds to perform regenerative endodontics.
- Dental pulp tissue constructs adhered more completely to the coronal aspects of the root canal and less completely to the middle and apical aspects. This likely was caused by the increasing complexity of root canal

Advantages of root canal revascularization:

- The greatest benefit of these biological approaches for dental tissue restoration over many conventional dental materials is that the reparative matrices become an integral part of the tooth, overcoming any of the problems of retention of a restoration and possible marginal bacterial microleakage.
- This treatment approach strengthens the root walls of immature teeth.
anatomy toward the apex and the physical constraints of the scaffold materials, as well as the placement method.

• Since most of the tissue-engineered parts have been developed using very potent signal molecules to induce the transformation the growth of the stem cells, a way has to be found to insure that these transformation and growth will not continue beyond control when implanted.

• Matching the aging of the implanted tissue-engineered parts with that of the surrounding tissues and organs is a great obstacle too.

REFERENCES


