The Application of Tissue Engineering to Regeneration of Pulp and Dentin in Endodontics

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Abstract

Caries, pulpitis, and apical periodontitis increase health care costs and attendant loss of economic productivity. They ultimately result in premature tooth loss and therefore diminishing the quality of life. Advances in vital pulp therapy with pulp stem/progenitor cells might give impetus to regenerate dentin-pulp complex without the removal of the whole pulp. Tissue engineering is the science of design and manufacture of new tissues to replace lost parts because of diseases including cancer and trauma. The three key ingredients for tissue engineering are signals for morphogenesis, stem cells for responding to morphogens and the scaffold of extracellular matrix. In preclinical studies cell therapy and gene therapy have been developed for many tissues and organs such as bone, heart, liver, and kidney as a means of delivering growth factors, cytokines, or morphogens with stem/progenitor cells in a scaffold to the sites of tissue injury to accelerate and/or induce a natural biological regeneration. The pulp tissue contains stem/progenitor cells that potentially differentiate into odontoblasts in response to bone morphogenetic proteins (BMPs). There are two strategies to regenerate dentin. First, is in vivo therapy, where BMP proteins or *BMP* genes are directly applied to the exposed or amputated pulp. Second is ex vivo therapy and consists of isolation of stem/progenitor cells from pulp tissue, differentiation into odontoblasts with recombinant BMPs or *BMP* genes and finally transplanted autogenously to regenerate dentin. This review is focused on the recent progress in this area and discusses the barriers and challenges for clinical utility in endodontics.

Key Words

Odontoblasts, gene therapy, dental pulp capping, reparative dentin, bone morphogenetic proteins (BMPs), pulp stem cells, tubular dentin

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There is a high rate of success in retention of teeth by endodontic therapy. A recent study of more than 1.4 million cases indicate that about 97% of treated teeth remain functional over an 8-yr follow-up period (1). However, many teeth are not restorable because of apical resorption and fracture, incompletely formed roots, or carious destruction of coronal structures. In addition, vital pulp therapy is not always predictable. One novel approach to restore tooth structure is based on biology: regenerative endodontic procedures by application of tissue engineering. Over the last two decades, tissue engineering has evolved from science fiction to science. Indeed, isolated clinical case reports are consistent with the concept that certain clinical treatments might evolve into regenerative endodontic procedures (2). However, additional translational research is needed to develop predictable clinical regenerative procedures. The purpose of this article is to review the biological principles of tissue engineering and the hurdles that must be overcome to develop regenerative endodontic procedures.

Tissue Engineering

Tissue engineering is the field of functional restoration of tissue structure and physiology for impaired or damaged tissues because of cancer, diseases, and trauma (3, 4). The key elements of tissue engineering are stem cells, morphogens, and a scaffold of extracellular matrix (3, 4) (Table 1).

Adult Stem/Progenitor Cells

Adult stem/progenitor cells reside in a variety of tissues. There is an explosion of interest in the utility of cell and gene therapy for regeneration (5, 6). Adult stem cells have unique characteristics (7); (a) they exist as undifferentiated cells and maintain this phenotype by the environment and/or the adjacent cell populations until they are exposed to and respond to the appropriate signals, (b) they have an ability to self-replicate for prolonged periods, (c) they maintain their multiple differentiation potential throughout the life of the organism (7). Progenitor cells retain the differentiation potential and high proliferation capability, but have lost the self-replication property unlike stem cells. Recent data suggests that the capacity and potential for adult stem cells to differentiate into a wider spectrum of phenotypes, 'stem cell plasticity', is caused by fusion of stem cells with endogenous tissue-specific cells (5, 8, 9).

Scaffold

The scaffold provides a physicochemical and biological three-dimensional microenvironment for cell growth and differentiation, promoting cell adhesion, and migration. The scaffold serves as a carrier for morphogen in protein therapy and for cells in cell therapy. Scaffold should be effective for transport of nutrients, oxygen, and waste. It should be gradually degraded and replaced by regenerative tissue, retaining the feature of the final tissue structure. They should have biocompatibility, nontoxicity, and proper physical and mechanical strength (10). Natural polymers such as collagen and glycosaminoglycan offer good biocompatibility and bioactivity, and synthetic polymers can elaborate physicochemical features such as degradation rate, microstructure, and mechanical strength. Commonly used synthetic materials are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers, poly(lactic-co-glycolic acid) (PLGA). Synthetic hydrogels include poly(ethylene glycol) (PEG) based polymers, and those modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acid (RGD), can improve cell adhesion and matrix synthesis within the three-dimensional network (11). Scaffolds containing inorganic compounds such as hydroxyapatite and calcium phosphate are used to enhance bone conductivity (12).

TABLE 1. Key elements of tissue engineering

Adult Stem Cells
Capable of differentiating into specialized cells
Able to respond to morphogens by dividing or specializing
Morphogens
Biological factors that regulate stem cells to form the desirable cell type
5 major families (BMPs, FGFs, Wnts, Hhs, TNF)
BMPs are major morphogen family for tooth regeneration
Scaffold
Provides a biocompatible 3-dimensional structure for cell adhesion and migration
Biological scaffolds (eg., collagen, glycosaminoglycan)
Artificial scaffolds (eg., PLA, PGA, PLGA)

TABLE 2. Current examples of tissue engineering/stem cells research fronts in medicine

Bone marrow stem cells
Bone marrow transplant (eg., leukemia, certain other cancers)
Direct injection into heart after infarction to produce cardiomyocytes
Regeneration of liver, bone, kidney, neurons (CNS)
Muscle-derived stem cells
Regenerate muscle, bone, blood cells
Mesenchymal stem cells
Accumulates at sites of injury (eg., bone fracture, MI, stroke)
Skin grafts
Vascular grafts

Morphogens

Morphogens are extracellularly secreted signals governing morphogenesis during epithelial-mesenchymal interactions. The morphogenetic signaling networks include the five major classes of evolutionarily conserved genes: bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), wingless- and int-related proteins (Wnts), Hedgehog proteins (Hhs), and tumor necrotic factor (TNF) families (13, 14). These families exhibit redundant and reiterative signaling, each with distinct temporal and spatial expression during initiation, patterning formation and morphogenesis, and cytodifferentiation (15). Although five distinct families of morphogens are involved in embryonic tooth development, BMPs appear to be sufficient for tooth regeneration in adults (4).

BMP family members are sequentially and repeatedly involved in embryonic tooth development. Six different *Bmps* (*Bmp2* to *Bmp7*) are co-expressed temporally and spatially (16). Ten BMP members [*Bmp2*, *Bmp4*, *Bmp6*, *Bmp7*, *Bmp8*, *Growth/differentiation factor* (*Gdf*)*1*, *Gdf5*, *Gdf6*, *Gdf7*, *Gdf11*, and *glial cell line-derived neurotrophic factor* (*GDNF*)] were cloned from rat incisor pulp (17, 18). The interactions between epithelium and mesenchyme are important in tooth development. BMP4 from the epithelium induces the mesenchyme to be odontogenic. *Bmp2*, *Bmp4*, and *Bmp7* signals expressed in the enamel knot influence both epithelial and mesenchymal cells and are responsible for the maintenance of the enamel knot and the subsequent morphogenesis of epithelium (15). These signals also regulate the patterning of the tooth crown by influencing the initiation of the secondary knots together with mesenchymal signals such as BMP4 (13). *Bmp2*, *Bmp4*, *Bmp6*, *Bmp7*, and *Gdf11* are also expressed during odontoblast differentiation and *Bmp4* and *Bmp5* during ameloblast differentiation (16, 18). The BMP signaling networks are complex and regulated at three levels at least. They are extracellular sites, cell membrane site, and intracellular domains (19). BMP antagonists such as noggin, chordin, and follistatin modulate the bioavailability of the morphogens (19). Two transmembrane receptors, type I and type II with serine-threonine kinase activity are expressed in dental pulp (20–22). BMP signals are transduced from the plasma membrane to the nucleus through a limited number of Smad proteins, receptor-activated Smads (R-Smads), common mediator Smads (co-Smads), and inhibitory Smads (I-Smads). Many Smad-interacting proteins have been detected and determine the outcome of the signaling (23). The BMP signals are reiteratively used for communication and signaling between epithelium and mesenchyme. The same signals in different tissues and at different times result in the various cellular responses because of the histories of the cells determining their competence to respond to the signals (15).

Stem Cell Therapy

Adult stem cells are prime candidates for cell therapy. A substantial body of early evidence points to the therapeutic potential of these cells in preclinical studies (Table 2). Hematopoietic stem cell transplantation has long been used in the treatment of leukemia and other cancers (24). Bone marrow stem cells are under investigation for the potential clinical use for production of cardiomyocytes following infarction by direct injection into heart or by mobilization through the circulation (25, 26). They have been also applied in the regeneration of liver (27), bone (28), kidney (29, 30), and central nervous system (31). There has been also enthusiastic interest in neural stem cells as therapeutic agents to repair/regenerate the brain and spinal cord (31). The concept of regeneration of central nervous system that recapitulates normal neural development includes: (a) re-growth of the damaged neuronal axons, (b) replenishment of neural (or neuronal) cells, and (c) reconstruction of neural functions (31). Muscle-derived stem cells have properties of both hematopoietic stem cells and myoblasts and enhance regeneration of blood, muscle, and bone (32). Multipotential adult stem cells differentiate into endothelium, neuroectoderm, and endoderm in vitro under defined culture conditions. Injection of multipotential adult stem cells into the nonobese diabetic/severe combined immunodeficient (NOD/SCID) model resulted in engraftment in stem cell niches including bone marrow, spleen, intestine, lung epithelium, and the blood (33). The mesoangioblast, a vessel-associated stem cell restores function in dystrophic mice lacking the δ-sarcoglycan protein (34).

Mesenchymal stem cells are capable of specific migration to sites of injury, for example, in bone fractures (35, 36), myocardial infarction (37), and ischemic cerebral injury (38) and knee joint injury (39). The mechanisms that guide homing of injected or implanted cells, however, remain unclear.

The choice of the tissue source is governed by availability, as well as by the degree of characterization of the stem/progenitor cells in terms of surface markers and differentiation pathway and the consistency of the preparations. The generally weak immunogenicity of mesenchymal stem cells has broad advantages and implications for allogeneic cell therapy.

Kidney transplantation can provide full renal function in the patients with renal failure, but has risks of opportunistic infection and development of malignancies because of the requirement of long-term immunosuppression. The availability of organs for transplantation is limited. For more complete renal replacement therapy, bioartificial kidney consisting of synthetic hemofilter in series with a renal tubule assist device (RAD) containing human renal tubule cells has been developed (40). For treatment of heart failure, engineered heart tissue has been demonstrated using collagen type I and extracellular matrix proteins mixed with freshly isolated heart cells (41). Another potential approach is therapeutic cloning. Nuclei from skin cells are injected into an enucleated donor oocyte to form embryoid bodies. After differentiation into cardiomyocytes with a specific cocktail of growth factors, one can reinject into a patient or use with a biodegradable scaffold to gen-

TABLE 3. Gene therapy: Making "Designer Cells" for therapy

In vivo methods
Add gene in vivo to induce new function
examples: angiogenesis
Ex Vivo methods
Culture cells ex vivo, add gene, transplant back to host
examples: re-growth of cartilage and bone

erate an artificial patch for heart (42). However, a fully functional artificial heart has a long road ahead.

Although early preclinical data demonstrate the safety and efficacy of mesenchymal stem cell therapy, many questions still remain, such as pharmacokinetics of transplanted cells, the mechanisms of engraftment, homing, and in vivo differentiation. Clinical medicine is entering an exciting new era, and that new therapeutic approaches will provide opportunities for the use of mesenchymal stem cells in a broader variety of applications (8).

Gene Therapy

Gene therapy is recently used as a means of delivering genes for growth factors, morphogens, transcription factors, extracellular matrix molecules locally to somatic cells of individuals with a resulting therapeutic effect. The gene can stimulate or induce a natural biological process by expressing a molecules involved in regenerative response for the tissue of interest (43). Precise delivery and efficient transfer of genes into target tissue cells, prompt assessment of gene expression at required times and appropriate levels and the minimization of undesirable systemic toxicity are essential for successful gene therapy. Both an in vivo and an ex vivo approach can be used for gene therapy (Table 3). In the in vivo approach, the gene is delivered systemically into the bloodstream or locally to target tissues by injection or inhalation (44). For example, many genes such as vascular endothelial growth factors (VEGF), angiopoietin, fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) have been directly transfected in tissues. The efficacy for stimulating angiogenesis to improve cardiac and limb vascular insufficiency has been demonstrated preclinically (45). The ex vivo approach involves genetic manipulation of cells in vitro, which are subsequently transplanted to the regeneration site. For example, the delivery of a pure population of mesenchymal cells transduced with morphogens or growth factors such as BMP, Sonic hedgehog (Shh), insulin-like growth factor (IGF) genes are implanted into the defect site of cartilage and bone (44, 46, 47). The cells play a role not only in the repair process but also in secretion of growth factors locally to stimulate host cells (48). The choice of in vivo or ex vivo approach depends on morphological and physiological characteristics of target tissue, the vector used, nature of affected disease and the safety of the procedure (44).

Either viral or nonviral vectors are used to enable the cellular uptake and expression of genes. Viral vectors are genetically altered to eliminate ability of causing disease without losing infectious capacity to the cell. The viruses can replicate genes of interest together with their own genome through the use of the host cell genetic machinery. At present, adenoviral, retroviral, adenoassociated virus, herpes simplex virus, lentivirus are being developed (44, 49). Nonviral delivery systems of plasmids, peptides, cationic liposomes, DNA-ligand complex, gene gun, electroporation, and sonoporation have been developed to address safety concerns such as immunogenicity and insertional mutagenesis (4, 44). Most of the risks of gene therapy may arise from the vector system rather than the gene expressed (44). Widespread clinical application still awaits the development of vectors that are safe, affordable, efficient, simple for application, and that have ability to express the required level of transgene for the sufficient long term (47).

Pulp Cells and Regenerative Dentinogenesis

The pulp is an organ known to have tremendous reparative/regenerative abilities (4, 50–52). The bridge of reparative/regenerative dentin that directly bonds to reactionary and primary dentin around the exposed site of the pulp can be more useful protection from physicochemical and bacterial irritation than any restorative material (53). Regenerative endodontics endeavors to use a tissue engineering approach.

Pulp Stem/Progenitor Cells

During wound healing process after the exposure of pulp, the pulp cells and the undifferentiated mesenchymal cells that have de-differentiated from pulp cells, endothelial cells, and pericytes, migrate to the exposed site from the deeper region of the pulp and replace degenerated odontoblasts (54). Human pulp stem cells have self-renewal capability and multi-lineage differentiation capacity in vitro (55, 56). Tubular dentin is formed after transplantation of the human pulp stem cells with hydroxyapatite/tricalcium phosphate powder into immunocompromised mice (55, 56). A recent study using several markers of the microvasculature networks has suggested that pulp stem cells are intimately associated with the blood vessels of pulp tissue, especially pericytes and smooth muscle cells (57). The demonstration of specific markers in pulp stem cell differentiation pathways is, however, still in a rudimentary state. The key stem cell characteristics of extensive self-renewal capacity and maintenance throughout the life of an organism, can be exploited in culturing large numbers of pulp stem cells in vitro for therapeutic use of regenerative endodontics. The success of clinical applications of pulp stem cells is limited by the culture conditions and the nature of microenvironment in which the primitive multipotential pulp stem cells are maintained and expanded.

Scaffold

Pulp cells adhere to osteodentin before differentiation into odontoblasts to form tubular dentin in calcium hydroxide pulp capping (58). Tubular dentin formation is also observed surrounding the implanted demineralized or native dentin matrix in the pulp tissue (59–62). Recombinant human BMP2 induces tubular dentin formation only when implanted on the amputated pulp with 4M-guanidine choloride extracted inactivated demineralized dentin matrix. On the other hand, only osteodentin formed with type I collagen as the scaffold (63, 64). These findings suggest that there is prerequisite for a physicochemical surface for odontoblast differentiation. However, mechanisms underlying this are unclear (4). Fibronectin mediates the binding of signaling molecules and plays a role in interactions between extracellular matrix and cells to reorganize the cytoskeleton of polarizing preodontoblasts in pulpal wound healing process (65). The RGD site in fibronectin is critical for cell attachment (66). Collagen allows arrangement of preodontoblasts and bind newly formed odontoblasts to pulp tissue, supporting a reparative dentinogenesis framework (67). Bone sialoprotein has RGD cell attachment sequence and polyglutamic acid stretches involved in binding to hydroxyapatite. When bone sialoprotein is implanted in the pulp tissue, up-regulation of secretory activity of extracellular matrix of newly generated odontoblasts and a thick reparative dentin formation are observed (68). Synthetic extracellular matrix may also be a potential scaffold for reparative dentinogenesis (69). Alginate hydrogel facilitates pulpal wound healing with hydration properties and tethering growth factors (70). Mineral trioxide aggregate (MTA), powder consisting of fine hydrophilic particles of tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide has recently investigated as a potential alternative restorative materials. MTA sets in the presence of moisture, prevents microleakage, is biocompatible, and

promotes reparative dentin formation (71). Further progress in the scaffolds for regenerative endodontics depends on a three-dimensional microenvironment that promotes cell behavior including cell polarization and optimal delivery of morphogens such as BMPs.

Morphogen

Morphogens are inductive signals that function as growth/differentiation factors in odontoblast differentiation. Bone morphogenetic proteins (BMPs) were originally isolated from demineralized bone matrix (3). Recombinant human BMP2 stimulates differentiation of adult pulp cells into odontoblasts in monolayer cultures (72, 73) and in three dimensional pellet cultures (74). Recombinant human BMP2, and BMP4 and GDF11 soaked in agarose beads (75, 76), and TGFβ1 associated with inactive total EDTA-soluble fraction (77) also stimulate odontoblast differentiation in organ cultures of dental papillae cells (75, 76). The similar effects of TGFβ1–3 and BMP7 have been demonstrated in cultured tooth slices (78, 79). Recombinant human BMP2, BMP4, and BMP7 induce reparative/regenerative dentin formation in vivo (63, 64, 68, 80–87). Intrapulpal implantation of Millipore filters containing human TGFβ1 induced odontoblast differentiation and reparative dentin formation in close proximity to the implants (88), suggesting the effective nature of this signal for odontoblast differentiation within the pulp (52). Recombinant human insulin-like growth factor-I with collagen membrane induces complete dentin bridging and tubular dentin formation (89). It is still unclear what regulates the abrupt transition of stem/progenitor cells from quiescent to active state in terms of proliferation, migration, differentiation, and matrix secretion following pulp tissue injury. The molecular control mechanisms underlying morphogen release requires to be elucidated for therapeutic uses in regenerative endodontics.

In Vivo and Ex Vivo Gene Therapy by BMPs

In Vivo Gene Therapy

The half-life of BMPs as recombinant proteins is limiting and the high concentrations are required in addition to an optimal scaffold during the local application to the exposed pulp for reparative dentin formation (64). Gene therapy is a potential alternative to conquer these disadvantages of the protein therapy. The successful bone induction has been reported after application of the BMP family members, *Bmp2*, *Bmp4*, *Bmp7*, and *Bmp9* by gene therapy using viral vectors (90–93). A recombinant adenovirus containing *Bmp7* gene induced only a small amount of poorly organized dentin after direct transduction in experimentally inflamed pulp (94). BMP7 protein therapy is effective to induce reparative/regenerative dentin, but not in the experimentally inflamed pulp (85). Nonviral approaches have been used in pulp tissue, taking the advantages into consideration, such as stable production of cDNA plasmids with a high level of purity, easy manipulation, minimal risk of replication or incorporation (76, 93) and weakly immunogenic (95). The *Gdf11* cDNA plasmid was transduced efficiently by electroporation into pulp cells in vitro and induced the expression of *dentin sialophosphoprotein* (*Dspp*), a differentiation marker for odontoblasts. However, *Gdf11* gene transfer in vivo in the amputated pulp induced incomplete and not homogeneous reparative dentin formation because of thermal damage and tissue invasiveness of electrode (76). On the other hand, the ultrasound-mediated *Gdf11* gene transfer together with microbubbles also induced differentiation of pulp stem cells into odontoblasts in vitro and complete reparative dentin formation in vivo (93) (Fig. 1*A*).

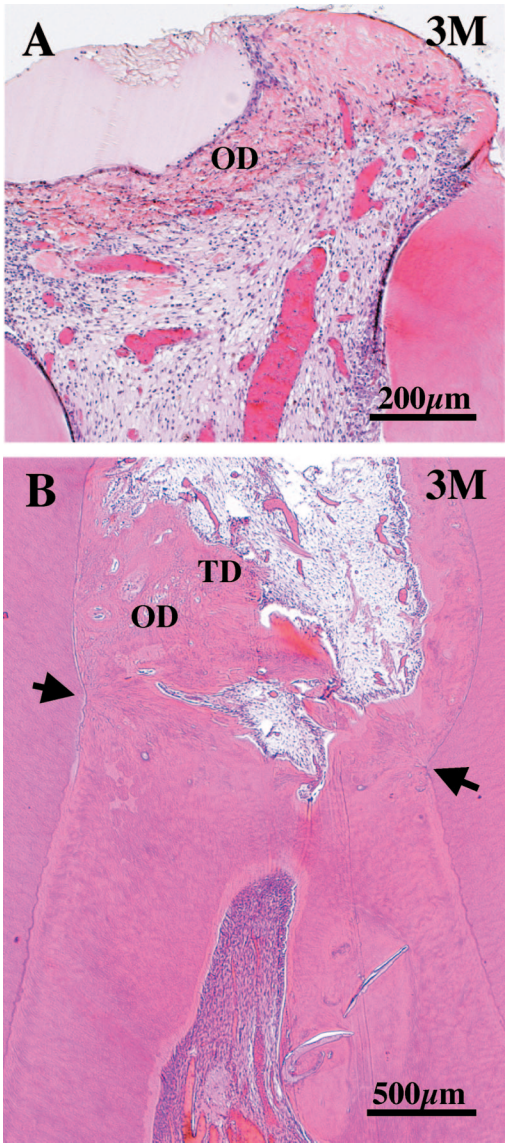

Figure 1. Regenerative dentin formation by two main strategies. (*A*) In vivo method. Induction of osteodentin formation by ultrasound-mediated *Growth/differentiation factor* (*Gdf11*) gene transfer on the amputated pulp in canine teeth after one month. (*B*) Ex vivo method. Pulp stem/progenitor cells are first isolated, transduced with *Gdf11* gene by electroporation to differentiate into odontoblasts and autogenously transplanted on the amputated pulp. A large amount of osteodentin and tubular dentin formation after 3 months.

Ex Vivo Gene Therapy

The in vivo gene therapy does not have much effect on reparative dentin formation in case of severe inflammation and few stem/progenitor cells in the pulp tissue (94). An alternative ex vivo approach, autogenous transplantation of *BMP* transfected cells into the exposed pulp might be useful. Ex vivo gene therapy stimulated initially osteodentin and followed by lasting tubular dentin formation in the exposed pulp by both *Gdf11*-electrotransfected (Fig. 1*B*) and *Bmp2*-electrotransfected pulp stem/progenitor cells (96, Iohara et al., unpublished data). The transplantation of cultured dermal fibroblasts transduced with *Bmp7* using a recombinant adenovirus also induced reparative dentin formation in the exposed pulp with reversible pulpitis (94). *Bmp*-transduced cells directly contributed to reparative/regenerative dentin formation. In addition the various growth/differentiation factors retained in the matrix of

the transplantation might be gradually released and induce host pulp cells differentiation. The great potency of *Bmp* genes to provoke differentiation of pulp cells, even if in reversible pulpitis, demonstrates the utility of ex vivo gene therapy in reparative/regenerative dentin formation for clinical endodontic treatment. The main challenges for gene therapy in the next decade will be the requirements to demonstrate that gene therapy can provide cost-effective and safe long-term treatments for conditions that would otherwise lead to significant pulp necrosis.

Challenges and Future Direction

The pulp tissue repair/regeneration recapitulates tooth development. Despite the impressive progress in tissue engineering approaches to regenerative pulp therapy, numerous challenges remain. The associated broad spectrum of responses in pulp includes neural and vascular regeneration.

Nerve Regeneration

Dental pulp is richly innervated. The main nerve supply enters the pulp through the apical foramen along with the vascular elements. Nerves proceed to the coronal area and form a plexus in proximity to the odontoblasts and finally enter the dentinal tubules. They include both sensory and sympathetic nerves. There are three types each of the A and C sensory nerves. Their functions, locations and interactions with pulp, dentin, vasculature, and immune cells are different (97). In general, the A type fibers are myelinated and the C type are nonmyelinated. The cytochemical localization of neuropeptides, calcitonin-gene-related peptide (CGRP), nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), and neurofilaments vary with the type of nerve fiber (97). The temporal sequence of dental pulp innervation is dependent on gradients of neurotrophic growth factors emanating from the pulp cells. NGF, brain-rerived neurotrophic factor (BDNF) and GDNF are expressed in dental pulp (98). GDNF is further transported retrogradely to trigeminal neuron cell bodies from dental pulp. Dental pulp cells acquire morphology of neurons (98). Pulpal nerves play a key role in regulation of blood flow, dentinal fluid flow, and pressure (99–101). In addition there is evidence for neural regulation of pulpal fibroblasts, inflammation and immunity (99, 102–104).

The innervation of the pulp has a critical role in the homeostasis of the dental pulp. Invasion of immune and inflammatory cells into sites of injury in the pulp is stimulated by sensory nerves (105). Sensory denervation results in rapid necrosis of the exposed pulp because of impaired blood flow, extravasation of immune cells (105–108). Reinnervation leads to recovery in the coronal dentin (105). Schwann cells appear to release neurotrophic growth factors and play a role in recruitment of sensory and sympathetic nerves during reinnervation. Thus, the pulpal nerve fibers contribute to angiogenesis, extravasation of immune cells and regulate inflammation to minimize initial damage, maintain pulp tissue, and strengthen pulpal defense mechanisms.

BMPs have a role in reparative/regenerative dentin formation as described in the previous section. It is noteworthy that members of the BMP family have pronounced effects on neurogenesis (109–112). Thus, it is likely BMPs can be used for regenerative pulpal therapy and dentinogenesis may have concurrent beneficial effects on nerve regeneration.

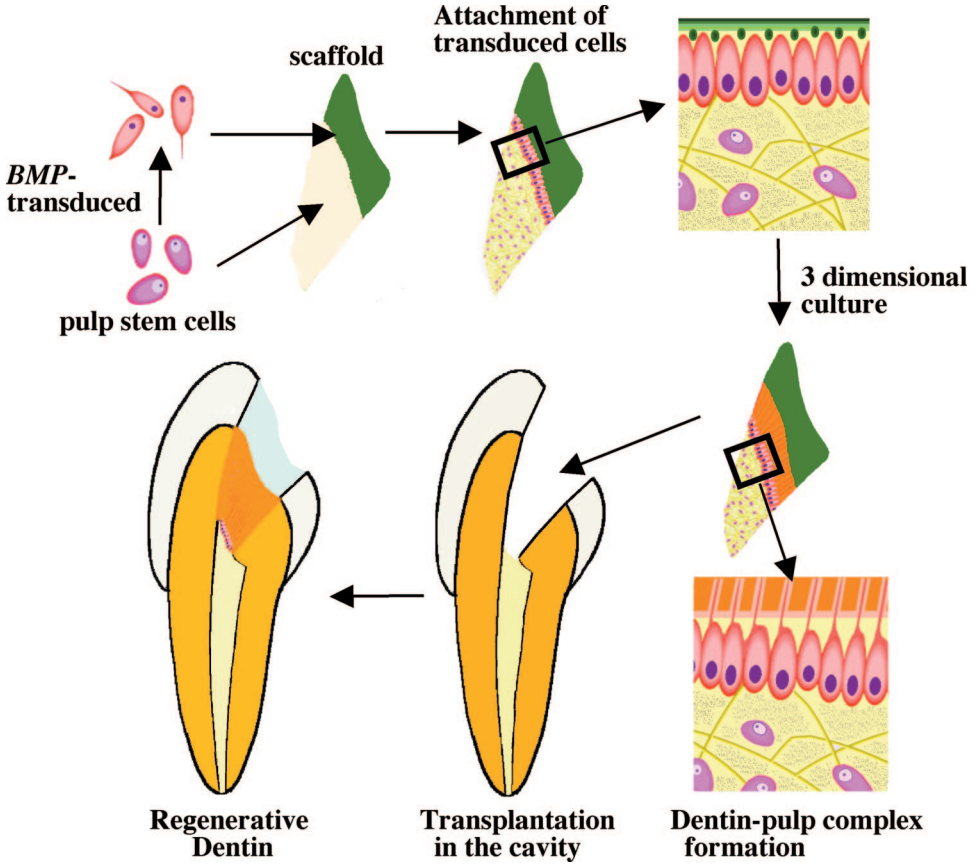

Figure 2. Dentin-pulp complex formation with optimal orientation for clinical application of regenerative therapy. The pulp stem cells are transduced with *BMP* gene and attached to a defined scaffold to differentiate into odontoblasts. The tubular dentin-pulp complex can be transplanted on the exposed or amputated pulp in the cavity.

The increasing interest in tissue engineering of tooth must take into account neuro-pulpal interactions and nerve regeneration. The challenges include, but not limited to nociceptive mechanisms, altered thresholds to pain in inflamed teeth and dental pain. Thus, the life of teeth can be possibly prolonged by preservation of pulp and odontoblasts and promoting repair and regeneration by the study of neuropulpal interactions (106). The recent progress in dental stem/progenitor cells (55, 96) and mechanisms of neurotrophism of dental pulp cells (98) assures advances in regeneration of nerves based on neuropulpal interactions.

Vascular Regeneration

The vascular system in the dental pulp plays a role in nutrition and oxygen supply and as a conduit for removal of metabolic waste. The cellular elements of the blood vessels such as endothelial cells, pericytes, and associated cells contribute to pulpal homeostasis along with the nerves. Thus, the vascular contribution to regeneration of dentin-pulp complex is immense. Arterioles enter the pulp chamber through the apical foramen along with the nerve supply. The branching arterioles form a capillary plexus under the odontoblast layer. During development and regeneration there is increased vascular activity and blood flow. There is a common pathway of vascular reaction to varied stimuli such as chemical, physical including mechanical and thermal. This reaction includes a local inflammation and attendant dilation of blood vessels and increased blood flow. Extravasation of leukocytes and increased vascular permeability is a hallmark of early vascular response (113). Pulp vasculature plays an important role in regulating inflammation and subsequent repair and regeneration of dentin. There is an intimate association of the neural elements with vascular supply of the dental pulp (114), suggesting the interplay of neural and vascular elements and involvement in pulp homeostasis.

The critical importance of vasculature in tissue repair and regeneration is well known. Vascular endothelial growth factor (VEGF) is an excellent regulator of angiogenesis and is known to increase vascular permeability. VEGF induced chemotaxis, proliferation and differentiation of human dental pulp cells (115, 116). In addition human dentin matrix contains VEGF (117). The presence of VEGF in dentin and response of dental pulp cells to VEGF raises the possibility of the presence of endothelial progenitor cells in dental pulp alongside progenitors for odontoblasts and neuronal cells (56, 96, 118). In view of the role of endothelial progenitor cells in vascularization during tissue regeneration, it is likely VEGF and vascular endothelial cells are critical for dentin regeneration. The utility of gene therapy in stimulation of vascular growth (45) permits local stimulation of vascularization during regeneration. In fact gene therapy using members of BMP family including BMP7 and GDF11 successfully induced dentin-pulp regeneration (94, 96). Thus, the recent advances in vascular biology and VEGF and techniques of gene transfer and gene therapy will be of potential clinical utility in dentistry especially in endodontics.

Future Direction

There is no unanimity of opinion concerning the usefulness of dental pulp capping. Although reparative dentin provides a physical barrier and protects the pulp, it has some limitations in the integrity. When the pulp is exposed by caries, acute localized inflammation and liquefaction necrosis can be observed under the exposure site. It has been postulated that to preserve the remaining healthy pulp, this infected, necrotic, and disintegrated pulp tissue need to be removed (119). The complete restoration of the physiologic, structural, and mechanical integrity of the native dentin-pulp complex is the ultimate goal of endodontic treatment (Fig. 2). Regeneration of pulp tissue in a necrotic infected tooth with apical periodontitis might be possible if the apex shows opening of more than 1.1 mm or apicoectomized, and if the tooth is replanted within 45 min., and if tooth is soaked in doxycycline or minocycline before replantation to be effectively disinfected (2, 120). The blood clot created in the canal acts as a matrix for the growth of new tissue into the root canal space (2). An interesting question, the origin of the new pulp tissue still remains to be answered. Further systematic studies with stem/progenitor cells, morphogens, novel scaffolds, and effective disinfecting are required for regenerative therapy in apical periodontitis. The regenerative therapy will revolutionize the future endodontics with the synergistic confluence of advances in signaling pathways underlying morphogenesis and lineage of stem/progenitor cells by morphogens such as BMPs and synthetic scaffolds.

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