Clinical Perspectives of Pulp Regeneration

ABSTRACT

Introduction: A sound and vital pulp is an essential prerequisite for long-term tooth survival and preservation. However, current endodontic treatment concepts are based on the removal of inflamed or necrotic pulp tissue and the replacement by a synthetic biomaterial. Recently, total or partial pulp regeneration has been proposed as an alternative treatment concept. The aim of this review was to evaluate the current options of pulp treatment and regenerative approaches, both for immature and mature teeth, in a clinical context. Methods: Clinical success rates of classic treatment options such as pulpotomy or root canal filling after pulpectomy or the removal of necrotic tissue are compared with recent reports on regenerative approaches like revitalization or partial and total pulp regeneration. Results: Revitalization in immature teeth with pulp necrosis is an additional treatment option besides placing an apical plug, leading to clinically acceptable outcomes, although with low predictability regarding the completion of root formation. Coronal regeneration of the amputated pulp in immature teeth constitutes a promising scientific approach, but data from clinical studies are missing. Mature teeth display a reduced potential for regeneration. Regenerative procedures using cell transplantation or cell homing are mainly in the experimental phase with only 2 clinical studies on cell transplantation. In parallel to the further development of regenerative therapies, the classification of pulp diseases should be revised, and the diagnostic tools need improvement. Conclusions: The rethinking of current concepts for biology-based treatments and improved diagnostic concepts might postpone the point of root canal filling depending on the clinical situation. (J Endod 2020;46:S161–S174.)

KEY WORDS

Dental pulp necrosis; pulpectomy; pulpitis; pulpotomy; regenerative endodontics; root canal obturation

Over the past decade, the number of studies describing different treatment methods for dental pulp regeneration has significantly increased. These methods include the treatment of immature and mature teeth with so-called revitalization procedures; partial and total pulp regeneration procedures have also been proposed. However, considerable controversy still exists as to whether these methods may already be considered a suitable alternative or even a replacement for conventional endodontic treatment procedures.

In this review, innovative pulp regeneration procedures are compared with classic treatment modalities, each in the context of specific clinical situations. Classic vital pulp therapies like pulp capping or pulpotomy applying bioactive materials on the vital pulp may lead to the generation of new hard tissue. Therefore, these therapies also show a regenerative or reparative aspect; however, in this review, we focus on pulp regeneration and used the term for any induced new formation of pulp or pulplike tissue.

Furthermore, the question of what a desirable, or at least a clinically acceptable, outcome of these regenerative procedures is is addressed. Thus, this review focuses on different clinical situations, such as immature and mature permanent teeth, as well as different stages from early dental pulp inflammation to the loss of vitality (pulp necrosis), with or without apical periodontitis (AP), as a basis for the assessment of the suitability of present regenerative endodontic procedures.

IMMATURE TEETH

Endodontic treatment of immature teeth is a challenge, mainly because of the incompletely developed roots, both in length and thickness, and the wide open apical foramen. Clinical situations that may
necessitate endodontic intervention in immature teeth are pulp necrosis, with and without AP, pulp exposure without loss of vitality, and irreversible pulpitis/coronal pulpitis.

Pulp Necrosis with and without AP
The main function of dental pulp is primarily the secretion of dentin (formative function). In contrast to the deposition of primary and secondary dentin, tertiary dentinogenesis represents a defensive function of the pulp because it is stimulus dependent. Other defensive functions are executed by the humoral and cellular components of the immune system, which eliminate invading pathogens. The pulp tissue also has a nutritive function because arteriolar and subodontoblastic capillaries supply the odontoblasts and other cells with oxygen and metabolites. Primarily afferent neurons of the trigeminal ganglion enable noxious stimuli to be perceived, however, efferent neurons modulate the microcirculation as well as the immune reaction in the pulp (Fig. 1). When pulp necrosis occurs, the entire pulp tissue is degraded and loses all functions. As a result, bacteria colonize the empty root canal and form a biofilm that has to be removed by thorough disinfection.

Conventional/Classic Procedures
Although previously in such cases an apexification procedure with repeated applications of calcium hydroxide pastes was favored, today hydraulic calcium silicate cements (HCSCs), like mineral trioxide aggregate (MTA) or Biodontine (Septodont, Saint-Maur-des-Fossés, France), are commonly used to place an apical plug because of the superior sealing properties as well as the lower solubility and improved biocompatibility of these setting materials. Furthermore, multiple applications of calcium hydroxide pastes, which weaken the root dentin and lead to an increased risk of fractures, are thus avoided. Clinically, success rates >90% after the application of HCSC plugs have been reported. Nevertheless, a disadvantage of using HCSCs as an apical plug material is mainly related to the loss of the dental pulp. One consequence is an arrested root growth with no further root lengthening or thickening. It is well established that the risk of root fracture is increased in immature teeth. Although this is a transitional stage during physiological tooth development, this high-risk situation is preserved after HCSC treatment. Recently, it was demonstrated by finite element analysis that the apposition of dentin to increase root length and thickness significantly reduces stress.

The human dental pulp is a highly innervated tissue. A consequence of pulp loss is the deficiency of neurologic pulp functions with the result of pulp nerve deafferentation on the brainstem (Fig. 2), an effect that has been intensively investigated by Kwan et al., Shortland et al, and Sessle. In animal experiments, it has been shown that pulp nerve deafferentation alters the mechanoreceptive field properties of many low-threshold mechanoreceptive neurons in the trigeminal brainstem nuclear complex. Although the clinical relevance of these findings is a matter of discussion, it was reported that 3%–12% of cases with apparent successful root canal treatments show chronic pain. After unilateral dental pulp injury, the authors observed an increased expression of activating transcription factor 3 and neuropeptide Y messenger RNA and a decreased tachykinin precursor 1 gene expression in the ipsilateral trigeminal ganglion. Significant neuroplasticity after pulp injury may contribute to persistent pain and functional changes in the trigeminal primary afferent endings. These data show that the loss of neurologic pulp function through pulp loss may have clinical implications.

Another consequence of pulp loss is the altered pulp defense. This relates to both innate and acquired immunity. As a reaction to invading caries bacteria, the dental pulp secretes a large number of substances like neutrophil elastase, and neurogenic inflammation is initiated to counteract further bacterial invasion. Furthermore, classic immunocompetent cells like immature dendritic cells, natural killer cells, and T cells but also odontoblasts and fibroblasts are involved in the innate pulp immune defense system and secretion of cytokines such as proinflammatory interleukin (IL)-1 and IL-6, chemokines, or human beta-defensins (human beta-defensin-2). This may even be linked to the induction of tertiary dentin formation. The adaptive/acquired immune system in the dental pulp includes antigen-specific lymphocytes (T and B cells) and their products. Although a number of cytokines from these cells also play a role in the innate immune system, IL-2, IL-4, IL-5, or IL-13 are only induced by activated T cells. Therefore, if no vital pulp is present, defense lines against invading bacteria are lost. These are all important reasons for the failure of root canal treatments.

In summary, although the use of HCSC plugs can be considered to yield more favorable clinical outcomes compared with the repeated application of calcium hydroxide pastes, mechanical and biological problems still exist that call for better treatment options.

Regenerative Procedures
The disadvantages of using HCSC plugs may be avoided by applying revitalization methods as described by the American Association of Endodontists or the European Society of Endodontology. These are essentially based on the following:

1. Careful disinfection of the root canal with sodium hypochlorite
2. Removal of loose or necrotic pulp tissue using suitable endodontic instruments and avoid mechanical instrumentation of the root canal walls
3. Intracanal dressing with local antibiotics or calcium hydroxide pastes
4. Removal of dressing and EDTA rinsing

FIGURE 1 – A histological image of the pulp-dentin interface. The dental pulp serves a formative, defensive, sensory, and nutritive function. Kluver-Barrera staining, scale bar = 50 µm.
5. Stimulation of bleeding and coverage of the intracanal blood clot with collagen
6. Closure with HCSC and adhesive restoration

The new formation of vascularized tissue inside the root canal has been demonstrated, and, furthermore, reinnervation (positive response to pulp sensibility testing) was reported in 50%–60% of published cases of revitalization\(^{19,20}\). Interestingly, histologic studies showed the presence of immunocompetent cells and lymphatic vessels, even when osteoid/cementum is formed inside the root canal as a response to bacterial contamination\(^ {19} \). Relevant signaling molecules, like different neurotrophins, are encapsulated in dentin and can be released after EDTA treatment (Fig. 3A and B)\(^ {21} \). The neurogenic potential of such neurotrophins from the dentin matrix could be shown in vitro by the formation of trigeminal neurites as well as in an in vivo model\(^ {21} \). These results show that it is possible to stimulate the formation of repair tissue, even under more complicated conditions like bacterial invasion, and ingrowth of neuronal structures induced by dentin-derived proteins.

A large number of case reports, prospective and retrospective cohort studies, randomized clinical trials, and meta-analyses have been published on the clinical outcome of revitalization procedures under these clinical situations\(^ {22-29} \). Clearly, a broad scientific basis exists for the assessment of revitalization treatments. In a more recent meta-analysis, the survival and success rates for apical plug and revitalization treatment were compared\(^ {23} \). The mean follow-up time ranged from 1.6–120 months in the apical plug group and 5.5–72 months in the revitalization group. Survival rates (retained tooth in the oral cavity at follow-up) were 97.1% for apical plug therapy and 97.8% for revitalization. Success rates (lack of clinical symptoms and complete radiographic healing of the periapical lesion) were 94.6% for apical plugs and 91.3% for revitalization. No statistical difference between the clinical outcomes of these treatment methods was found. However, after revitalization, further root development occurred in 79% of the cases. On the other hand, data on long-term outcomes are limited\(^ {23} \). In some cases, histologic studies have shown connective tissue with cementum deposition on the root canal walls as well as ectopic hard tissue formation\(^ {30} \) associated with residual bacteria\(^ {31} \). The rate of root development varied from 21%–100%, which shows the low predictability of the outcome after revitalization\(^ {23} \). Furthermore, bacteria in the canal may impede root development\(^ {31} \).
Conclusions
No difference in clinical outcomes between conventional/classic methods and revitalization could be shown for immature teeth and loss of pulp vitality, but the revitalization treatment showed a tendency toward further hard tissue deposition. Therefore, the proof of principle for revitalization is positive, but the predictability is low. A main problem is the risk of reparative tissue formation and cementum deposition inside the root canal. The question is whether this risk is acceptable. The main risk factors are bacterial invasion, total pulp necrosis, and the presence of apical inflammation. Interestingly, in a recent animal study, it was shown that pulpal regeneration was possible when 1–4 mm of the uninfamed apical pulp remained intact in immature teeth. Additional risk factors for classic root canal treatment are invading bacteria and existing periapical lesions. Evidently, an efficient but nondestructive disinfection regimen needs to be developed.

If revitalization leads to unspecific connective tissue and cementum/osteoid hard tissue deposition instead of pulplike tissue and dentin inside the root canal, the root will not gain mechanical strength because of weak mechanical properties of cementum/osteoid hard tissue compared with dentin apposition. However, reduced mechanical strength can also occur after HCSC plug treatment. Furthermore, failed revitalization may result in a completely obturated root canal because of uncontrolled cementum/osteoid hard tissue apposition. This may complicate the following classic root canal treatment. Therefore, it can be concluded that revitalization treatment in immature teeth with loss of vitality (necrosis), with and without AP, is presently an additional method to be offered to the patient. However, because of the low predictability, providing comprehensive information to the patient is necessary. The presence of AP may be considered a risk factor here, and regular clinical and radiographic controls (every 12 months) are necessary to detect the emergence or increase of AP at an early stage.

Total or partial root canal obliteration, which is often seen in traumatized teeth, can also occur after revitalization. After obliteration, teeth tend to present a negative or lower response to sensibility testing. Another problem with such teeth is crown discoloration to a yellowish or, more seldom, gray hue. The development of pulp necrosis is a late complication after pulp obliteration, but the response to pulp sensibility tests and tooth discoloration are not reliable criteria for defining pulp necrosis in this context. Thus, pulp obliteration after revascularization without a periapical lesion may be considered as an acceptable, if not optimal, outcome. However, continuous re-examination of such teeth is necessary.

Pulp Exposure without Loss of Vitality (with and without Reversible Pulpitis)
Such a clinical situation for immature teeth may occur after trauma, in the course of tooth preparation or during caries removal. Special attention should be given to teeth immediately after trauma. Here, the lack of response to pulp sensibility tests is frequently observed during post-traumatic pulpal healing and cannot be associated with later development of pulp necrosis. This diagnostic uncertainty may lead to unnecessarily invasive treatment resulting in a root canal filling. In such cases, or if the pulp is exposed during caries removal, it must be kept in mind that the inflammatory reaction is still compartmentalized and limited to the coronal part of the pulp.

Conventional/Classic Procedures
The classic/conventional treatment methods for pulp exposure without loss of vitality are pulp capping or partial pulpotomy (amputation of 2–3 mm of the coronal pulp). The latter is often performed when there is not enough space for the capping material (ongoing preparation, present restoraution, or fragment reattachment) or if the dental pulp is considered to be partially affected. In the past, calcium hydroxide pastes were used, but recently developed HCSCs show better clinical results. Here, we concentrate on the partial pulpotomy.

In a recent systematic review and meta-analysis including mature and immature teeth, partial pulpotomy in treating permanent posterior teeth with carious vital pulp exposure showed a clinical success rate of 98%, 96%, and 92% after 6 months, 1 year, and 2 years, respectively. Clinical success was defined by the absence of spontaneous pain, tenderness of percussion, swelling, fistulation, pathologic mobility, fuction radiolucency, periodontal ligament space widening, or internal or external root resorption. Interestingly, no difference in the outcome was seen between immature and mature teeth. However, the literature is not conclusive regarding a difference in the outcome of partial pulpotomies in teeth with open or closed apices; it appears that teeth with open apices have a better prognosis. The only significant factor was the presumptive diagnosis of irreversible pulpitis indicating a 75% success rate. Recently, partial pulpotomies were also performed in cases in which pulp exposures were accompanied by symptoms of irreversible pulpitis. Six- to 18-year-old patients displayed an overall success rate of 90% after a mean follow-up of 32.2 ± 17.9 months.

Regenerative Procedures
The biological conditions for pulp regeneration in cases of a partial pulpotomy are excellent because the cell-rich coronal pulp tissue including multipotent pulp stem cells is preserved. Furthermore, generally no or only few bacteria are present that might interfere with regeneration. Even in the case of bacteria being present, pulp tissue is able to proliferate as in pulp polyps. Studies using signaling molecules for partial pulpotomies have been performed with little success, although the application of bone marrow stem cells (BMSCs) on pulpotomy sites in dogs was reported to successfully induce tertiary dentin formation.

Thus far, no studies have been available supporting regenerative procedures as an alternative to partial pulpotomy. One animal study refers in the title to “partial pulpotomy,” however, the coronal pulp parenchyma of the experimental animals was eliminated with a rotary instrument; thus, this study refers rather to a full pulpotomy.

Conclusions
There are no studies supporting regenerative procedures as an alternative to partial pulpotomy. Because of the high success rate reported for partial pulpotomy, the clinical benefits of regeneration in this context recede into the background. Furthermore, it would be technically difficult to apply a scaffold, with or without cells, covered by layers of different materials to regenerate 2–3 mm of pulp tissue. Therefore, to our knowledge and because of technical problems, regeneration procedures as an alternative to partial pulpotomy do not seem to provide any clinical advantage. Furthermore, dentin regeneration may form the basis for further concepts in this clinical context, but this is beyond the scope of this article.

Irreversible Pulpitis/Coronal Pulpitis
Traditionally, the diagnosis of irreversible pulpitis is based on clinical signs and symptoms like increased sensibility, spontaneous and long-lasting pain, or pain
the case of irreversible pulpitis.

Therefore, for permanent teeth to maintain pulpectomy in general has recently been challenged for permanent teeth to maintain vitality (see later)\textsuperscript{48}. Therefore, for challenged for permanent teeth to maintain pulpectomy in general has recently been challenged for permanent teeth to maintain vitality (see later)\textsuperscript{48}. Therefore, for permanent teeth to maintain vitality (see later)\textsuperscript{48}. Therefore, for permanent teeth to maintain pulpectomy in general has recently been challenged.

For a full pulpotomy, coronal pulp tissue is completely removed. After hemostasis, a dressing agent is applied to the surface of the radicular pulp. Calcium hydroxide preparations and HCSCs are generally used as dressing agents. In a recent meta-analysis, the success rates of complete pulpotomies in immature permanent teeth were compiled\textsuperscript{46}. The criteria for success were preserved sensibility, symptom-free status, absence of radiographic abnormality besides bridge formation, and continued root development. A success rate between 86% and 100% for calcium hydroxide or MTA at an observation period of up to 24 months was found. There was no statistically significant difference found in either the clinical or radiographic success rates between MTA and calcium hydroxide after 6- and 12-month follow-ups. Few cases of marginal discoloration were observed, of which MTA accounted for the majority\textsuperscript{46}.

In a prospective clinical study in patients with irreversible pulpitis and pulp exposure in carious dentin, immature teeth showed a 100% success rate and mature teeth a 90% success rate after a total pulpotomy\textsuperscript{28}. In a further prospective study with patients 9–17 years old (12.3 ± 2.7 years) and clinical signs and symptoms suggestive of irreversible pulpitis, an overall success rate of 95% (19/20) was observed\textsuperscript{49}. This was confirmed in another study conducting partial pulpotomy in permanent teeth with pulp exposure and irreversible pulpitis of 6- to 18-year-old patients displaying an overall success rate of 90% after a mean follow-up of 32.2 ± 17.9 months\textsuperscript{39}. Total pulpotomy in permanent molars of 7.6- to 13.6-year-old patients after a follow-up period of up to 73 months revealed 100% clinical success; radiographically, a hard tissue barrier was noticed in 13 teeth (57%)\textsuperscript{50}.

The disadvantages of pulpectomy and root canal treatment in immature teeth with irreversible pulpitis are virtually the same as with the loss of vital pulp tissue. Of course, one drawback of a full pulpotomy is the loss of all coronal pulp tissue or at least a large part of it. If tertiary dentin is formed at the root canal entries as a reaction to the treatment procedure, this may impede root canal treatment in the case of necrosis, and there may be no response to sensibility testing despite the vitality of the remaining root canal pulp\textsuperscript{51}. Therefore, regenerative procedures may offer clinical advantages.

**Regenerative Procedures**

Dental pulp regeneration can be performed according to the tissue engineering concept using stem cells, a scaffold, and signaling molecules. The necessary cells can be delivered either by cell transplantation or through attraction of the body’s own stem cells (cell homing)\textsuperscript{52,53}. In a study on immature animal teeth mentioned previously, it was shown that the presence of 1–4 mm unirradiated tissue was beneficial for successful pulp regeneration after the revascularization procedure\textsuperscript{52}.

In 2009, Iohara et al\textsuperscript{54} published the first study on the regeneration of canine pulp after autogenous in vivo transplantation of selected dental pulp stem cells in a collagen I/III scaffold on the amputated pulp. They found complete regeneration of pulp tissue with capillaries and neuronal cells within 14 days. However, there was “no engraftment at the pulpotomy site after transplantation of the scaffold alone”\textsuperscript{54}.

Similar experiments have been performed by Souron et al\textsuperscript{55} transplanting rat pulp cells in a type I rat collagen as a scaffold on amputated coronal pulps of rat molars. The occlusal cavity was sealed with an HCSC and a light-cured resin composite. Controls were scaffolds with lysed cells. One month after implantation, living and mitotically active fibroblasts, as well as new vessels and nervous fibers, were present.

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**FIGURE 4** – Endodontic intervention in an immature (left) and a mature tooth (right). Depending on whether the dental pulp is only exposed, inflamed irreversibly (coronally) or even necrotic, different conventional/classic (c) and regenerative (r) treatment options are possible. Because of biological and mechanical aspects, the treatment decision is based conditionally on the maturity of the tooth. With emerging evidence in the literature, the range of indications might expand and provide future alternatives to current options. However, for pulpal exposures, partial pulp regeneration does not constitute a practical option marked as (Ø).
in the pulp equivalents seeded with entire cells, whereas pulp equivalents prepared from lysed cells were devoid of cell colonization; micro-computed tomographic imaging showed no pulp or root canal obliteration by mineral deposition in both groups. In a further study on dog teeth with open apices, simvastatin, an inhibitor of the competitive 3-hydroxy-3-methylglutaryl coenzyme A reductase that stimulates the expression of bone morphogenetic protein, was placed together with dental pulpal stem cells using a gelatin sponge as a scaffold on amputated pulps. The cavities were sealed with a glass ionomer cement and an adhesive resin composite. After 10 weeks, canine dental pulp stem cells stimulated by simvastatin enhanced the mineralization process and regeneration of the pulp and dentin after pulpotomy of dogs’ teeth more than the scaffold alone. In another study using immunosuppressed rats, bone marrow mesenchymal stem cells in a poly-L-lactic acid/Matrigel (Corning Inc., Corning, NY) scaffold were placed on the amputated pulp. The pulp chamber had been rinsed with 1.5% sodium hypochlorite followed by 15% EDTA. After 7 days, pulp tissue regeneration was observed in almost the entire implanted region. At day 14, pulp tissue regeneration further progressed throughout the implanted region, and nestin-expressing odontoblastlike cells beneath the dentin were found. In contrast, when acellular constructs were implanted into the pulpoticized space, minimally regenerated and poorly organized tissue was observed.

Less positive results were reported in a study by Mangione et al. after the application of pulp-derived porcine cells in a self-assembling peptide scaffold (BD PuraMatrix, BD Biosciences, San Jose, CA, USA) into pulp chambers after removal of the coronal pulp. As controls, the scaffold material alone was inserted, and the constructs were covered with an HCSC. The study showed no coronal pulp regeneration, both with and without cells, after 21 days. However, the HCSC was in direct contact with the cell/scaffold construct in this study. This material releases a significant amount of calcium hydroxide after mixing, which is dependent on the exposed surface. This may be the reason for the difference between the pulp capping or pulpotomy situation with a rather small contact area and the situation with comparably large teeth of miniature pigs. Interestingly, the formation of a dentin barrier at the entrance of the root canals was observed, which may be considered to be the result of the calcium hydroxide.

However, Torabinejad et al. widely removed the pulps in ferrets and performed classic revitalization by stimulation of bleeding from the residual pulp tissue in the apical region. New dental pulplike tissue formed in contact with the uninfamed remaining pulp in contrast to controls in which no vital pulp tissue had been left in the canals.

Cell transplantation to date is not without technical limitations, therefore, it has been attempted to select suitable scaffolds together with signaling molecules that support cell homing. As signaling molecules, either single human recombined proteins like bone morphogenetic proteins or transforming growth factor beta can be applied or combinations of such molecules. An alternative would be to use a mixture of such molecules that are released from dentin after EDTA treatment. In a recent in vivo study, immunodeficient mice received human root implants filled with different scaffolds, with or without dentin-derived proteins, together with a dental pulp stem cell pellet at the entry of the root. After 4 weeks, cell migration into the roots and the formation of a pulplike tissue were observed for custom-made fibrin and a commercially available fibrin sealant with dentin matrix proteins, whereas the peptide-based scaffold appeared less suitable.

Conclusions
For an amputated pulp situation in immature vital teeth, the biological condition for coronal pulp regeneration is comparatively good, and animal studies in mice, rats, and ferrets have shown promising results. However, results in miniature pigs were disappointing, as were results using scaffolds without cells or with lysed cells. Details in the technical procedures as well as the use of suitable scaffolds and the type of signaling molecules are all important factors that need to be taken into consideration. Fibrin or similar scaffolds have shown promising results together with protein mixtures derived from dentin after EDTA treatment. However, clinical studies are lacking in this area; thus, coronal regeneration of the amputated pulp cannot currently be regarded as an alternative to conventional/classic treatment modalities. On the other hand, this is a promising field for further preclinical and clinical research in the area of coronal pulp regeneration (Fig. 4).

MATURE TEETH
The term immature tooth describes various stages of tooth development in children and adolescents but can at least be somewhat narrowed down by the patient’s age and the dimension of the apical foramen. However, a “mature tooth” is characterized by complete apexogenesis and a fully developed tooth root and in the best case scenario fulfills its function over many decades. Compared with immature teeth, mature teeth have a smaller pulp chamber, a narrow apical foramen, and multiple calcifications within the pulp tissue.

Cellular changes include the increasing formation of fibrous bundles and the reduction of fibroblast density. Stem cells in aged pulps exhibit a decrease in autophagy and a lower differentiation potential. Although little is known about the mechanisms underlying senescence-related phenomena, oxidative stress may be one cause of senescence. Odontoblasts in aging pulps decrease in number and show lipofuscin accumulation as well as a reduced fitness, eventually impairing their dentin maintenance capacity. These lipofuscin deposits are considered inherently toxic and could affect cell function through the inhibition of lysosomal-degradative capacities, leading to even faster lipofuscin accumulation. Finally, Couve et al. described that the dental pulp displays age-related changes within the glial network in association with a reduction of coronal dental pulp innervation in old adult versus young adult teeth. This all leads to a reduced capacity of regeneration and makes the situation for regenerative therapies more challenging.

Pulp Exposure without Loss of Vitality (with and without Reversible Pulpitis)
Pulp exposure may occur during the course of excavating deep dental caries, after trauma or during tooth preparation. It may be a symptom-free state or accompanied by clinical signs indicating a reversible pulpitis.

Conventional/Classic Treatment
Standard treatment methods in this clinical situation aim to preserve pulp vitality by direct pulp capping or partial pulpotomy with calcium hydroxide pastes or even more successfully with HCSCs. Direct pulp capping is associated with very high success rates (93%–100%) after artificial and mechanical exposure, and in all other cases, the 5-year success rate with calcium hydroxide pastes in permanent teeth was about 59%–69%, and for HCSC, it was 78%–98%. For partial pulpotomy, high success rates have been observed independent of age, but other studies have reported less successful outcomes in mature teeth.

Regenerative Procedures
There are no preclinical or clinical studies available for pulp regeneration procedures as an alternative to direct pulp capping or partial pulpotomy.
Conclusions
What was delineated previously for immature teeth is also valid for mature teeth; because of the lack of advantages and technical problems, regenerative procedures for pulp exposure, with or without reversible pulps, as an alternative or replacement of direct pulp capping or partial pulpotomy are presently not indicated (Fig. 4).

Irreversible Pulpitis/Coronal Pulpitis
So far, the diagnosis of an irreversible pulpitis means that the pulp, which is still reacting to sensibility testing, is incapable of healing. The diagnosis is mainly based on clinical symptoms like intense, lingering pain to temperature changes (often to warm stimuli), spontaneous pain, and radiating pain. The therapeutic consequence of this diagnosis is pulpectomy and root canal treatment.

However, more recently, it was found that in cases of irreversible pulpitis according to the definition provided previously, morphologic changes indicating inflammation or necrosis were principally occurring in the coronal pulp, whereas the radicular pulp was still viable. The pulp is more resilient to significant microbial attacks than previously thought. This has consequences for therapy because according to this concept it would be possible to remove the coronal part of the pulp, which is inflamed, and cover the remaining radicular pulp with a material that induces healing and dentin bridge formation, like HCSC (pulpotomy). The details of when to perform a pulpectomy or a pulpotomy is beyond the scope of this article, but both treatments methods can be regarded today as classic/conventional methods.

Conventional/Classic Treatment
Pulpectomy and root canal treatment are a standard treatment method for teeth with irreversible pulps in order to prevent extraction. Success rates of 92% have been observed in large clinical studies. However, failure rates of standard root canal treatments performed by general dentists are significantly higher than expected.

Pulpotomy, which was formerly only indicated in primary teeth, has today also been used in immature permanent teeth. Some data show that pulpotomy is even a treatment option in mature teeth for teeth with irreversible pulps. In a study including 55 permanent teeth with irreversible pulps and AP, a success rate of 87.3% was observed at follow-up periods up to 62 months: when root development was complete, the rate was 84%. In another study, teeth with a closed apex received a total pulpotomy with one third showing symptoms of irreversible pulpitis. After a 1-year follow-up, over 91% were clinically successful. This renaissance of the pulpotomy may also have an influence on new approaches for pulp regeneration.

Regenerative Procedures
Pulp regeneration is discussed here as an alternative for both total and coronal removal of the dental pulp (pulpotomy or pulpectomy). After pulpotomy, there is an empty coronal and radical pulp chamber, perhaps containing bacteria or residual pulp tissue at the apical end. In contrast, after pulpotomy, pulpal tissue is present throughout the orifices of the root canals. In both situations, the regenerative approach is based on the concept of tissue engineering using (stem) cells, signaling molecules, and a suitable scaffold. Cells can be explanted, expanded, and then transplanted back, or cells may be attracted from local tissues (cell homing).

For in vivo analyses, cleaned and shaped roots were filled with pulp- or apical papilla–derived cells, different scaffolds, and signaling molecules and then implanted ectopically. Here, the generation of pulplike tissue with an odontoblastlike layer on the root canal walls was confirmed.

The formation of a dental pulp tissue was shown in situ after removal of the pulp in mature teeth of dogs with complete apical closure. The empty pulp chamber was filled with a scaffold containing pulp-derived stem cells and signaling molecules (stromal cell–derived factor-1 [SDF-1]). After 14 days, a newly formed tissue contained nerves, vasculature, and odontoblastlike cells attached to the dentinal walls. After 90 days, most of the pulp spaces were filled with new pulpal tissue, and after 120 days all were filled. In the same dog model, the influence of aging on dental pulp regeneration was studied. Dental pulp stem cells from young dogs (6–10 months) and older dogs (5–6 years) were compared and showed similar expression of trophic factors, migration, and antiapoptotic effects. However, pulp regeneration was significantly retarded when cells from older dogs were used, which indicates an age-specific decline in regenerative capacity.

Another variable is the diameter of the apex. In a tooth transplantation study in dogs, the tissue ingrowth for teeth with an apical diameter of 0.24–1.09 mm was investigated. The 6 most successful teeth showing vital tissue in the entire pulp chamber had an apical diameter between 0.32 and 0.65 mm. This indicates that even comparatively small apical diameters allow for tissue ingrowth into the de defined area.

The first clinical trial using cell transplantation was performed by Nakashima et al. Five patients with irreversible pulps and pulpectomy received autologous mobilized dental pulp stem cells with granulocyte colony-stimulating factor in atelocollagen. No adverse events or toxicity were observed, and after 4 weeks the teeth of 4 patients reacted positively to the electric pulp test and only 1 patient negatively. Magnetic resonance imaging results of the regenerated tissue in the root canal after 24 weeks were similar to that of normal dental pulp in the untreated control. Cone-beam computed tomographic imaging demonstrated functional dentin formation in 3 of the 5 patients.

However, further prospective clinical studies with a larger patient collective are necessary. Because of technical, ethical, and economic problems associated with cell transplantation, cell homing has been proposed as an alternative to cell transplantation. The idea is that endogenous (resident) stem cells are attracted by specific signaling molecules or by mixtures of such molecules in a suitable scaffold. Interestingly, Iohara et al. used transplanted cells for pulp regeneration and speculated that the transplanted cells mainly induced regeneration by the release of suitable signaling molecules. The previously mentioned revitalization procedure used for immature teeth can also be applied to mature teeth.

A large number of in vitro studies have been published showing that migration and differentiation of various stem cells (mainly pulp-derived or BMSCs) can be stimulated by a variety of signaling molecules aimed at pulp regeneration. Particularly, EDTA extracts of dentin are a potent source of such molecules. Furthermore, various in vivo ectopic implantation studies using different scaffolds with signaling molecules and no cells have been performed. Suzuki et al. reported on recellularization and revascularization of an implanted human tooth root into the dorsum of rats. After cleaning and shaping, the canal was filled with collagen and basic fibroblast growth factor. Similar results were reported by Kim et al. after implantation of roots with collagen gel and different signaling molecules into the dorsum of mice. The chemotaxis-based approach had potent cell homing effects in mice. A similar model was used by Zhang et al., implanting human roots filled with collagen loaded with SDF-1 into mice, along with additionally labeled BMSCs injected into the tail vein of the animals. Systemic BMSCs could home to the root canal and participate in dental pulp–like tissue regeneration and SDF-1 enhanced this process. In a different model, pretreated roots were placed subcutaneously.
on top of the calvarial bone of rats, and the addition of stem cell factor was beneficial in terms of regeneration\textsuperscript{34,35}.

Scaffolds play an important role in pulp regeneration, although commonly used scaffolds alone without any special bioactive functions do not induce cell homing\textsuperscript{33,36}. Recently, investigations of a series of natural (collagen and fibrin) and synthetic (PEG derivatives and self-assembling peptides) scaffolds in various in vitro experiments and ectopic transplantation in mice revealed that cell viability was significantly higher in natural versus synthetic materials. In vivo experiments showed the potential of fibrin to facilitate pulplike tissue formation and differentiation of cells into odontoblasts at the dentin interface\textsuperscript{37}. Finally, different scaffolds together with or without dentin-derived proteins were injected into human roots, and dental pulp stem cells in collagen were placed at the root tip. These constructs were subcutaneously implanted into the dorsum of mice\textsuperscript{38}. Best results of tissue ingrowth and the formation of a pulplike tissue were observed for custom-made fibrin and fibrin sealant with dentin matrix proteins after 4 weeks. With dentin matrix proteins and EDTA conditioning of dentin, differentiated odontoblast-like cells extended cellular processes into the dentinal tubules\textsuperscript{39}. However, no clinical studies applying the cell homing approach in mature teeth are available thus far, and studies in large animals simulating pulpotomy situations are also currently lacking.

Conclusions

Pulpectomy followed by root canal treatment is a generally recognized method with high success rates. However, the diagnosis irreversible pulpitis is currently being challenged as well as the strict indication of removing the entire pulp (pulpotomy). There is presently an increasing tendency to only remove the coronal part of the pulp (pulpotomy) and apply an HCSC (Fig. 4). A large number of in vitro and in vivo studies are available focusing on pulp regeneration after pulpectomy as an alternative to root canal treatment in mature teeth. They suggest that with suitable scaffolds, signaling molecules, and pulp-derived stem cells, pulplike tissue can be regenerated. However, the first clinical trials show that cell transplantation is a very demanding procedure with respect to cost, safety, availability of suitable stem cells, extracorporeal cell expansion, and transplantation\textsuperscript{33,38,39}. Therefore, these procedures are still in the experimental phase and are currently not a feasible clinical alternative to the presently used root canal treatment. Methods using cell homing are definitively less demanding, and in vitro as well as in vivo data are promising, but because no clinical data have been available until now, this method cannot yet be regarded as an alternative treatment.

The situation after pulpotomy seems to be more suitable for full pulp regeneration in mature teeth. However, again, no data for this approach are presently available but are urgently warranted because this seems to be a very promising clinical option.

Pulp Necrosis with and without Apical Periodontitis

This condition, which generally involves affliction with apical radiolucency, is characterized by loss of sensibility, presence of necrotic tissue, and a massive bacterial invasion of the root canal system.

Conventional/Classic Treatment

Root canal treatment together with extensive chemomechanical disinfection of the root canal system is the standard procedure. Special emphasis is placed on disinfection using irrigation protocols and antibacterial dressings. Success rates are reported to be 68%–85\%\textsuperscript{72}. However, the patient’s systemic health status, such as diabetes, is significantly associated with a higher prevalence of periapical radiolucencies in endodontically treated teeth\textsuperscript{30}. Tooth extraction and implant placement is also a treatment alternative with success rates (implant still in situ) of up to 90% over 10 years under optimal conditions\textsuperscript{100}.

Regenerative Procedures

In addition to the problem of pulp regeneration after pulpotomy, special problems involving heavy infection reaching into the dentin and periapical area arise. Therefore, not only tissue regeneration protocols have to be developed but also suitable disinfection regimens\textsuperscript{31,101}. Yang et al\textsuperscript{102} investigated pulp regeneration after the induction of apical periodontitis in dogs. Standard treatment was performed, and the apical constriction was widened up to the size of an #80 K-file. A triple antibiotic paste was used for disinfection. In 1 group, bleeding into canals was induced, leading to blood clot formation. In the second group, SDF-1α-loaded silk fibroin was mixed with blood induced from the apex and placed into the canal. Regenerated intracanal tissues were evident in both groups. In the clot-only group, extensive mineral deposition along the dentinal wall and in the middle of the canal as well as connective tissue with few blood vessels were found. The addition of SDF-1α to a silk fibroin scaffold led to a connective tissue that was similar to normal canine pulp. In contrast, in a dog model with infected dental pulps, Fahmy et al\textsuperscript{103} found tissue ingrowth after the application of ciprofloxacin and double antibiotic paste, vascularization, cementum formation, and little inflammation after 12–14 months. However, no pulplike tissue was present. This corresponds to results found for immature teeth in which infection was associated with cementum formation. Case reports in which mature necrotic teeth with periapical lesions underwent regenerative procedures\textsuperscript{104,105} showed radiographic evidence of periapical healing and regression of clinical signs and symptoms. The pulp cavity decreased in size and the apex closed. The pulp cavity appeared to be obliterated by mineralized tissue. These data indicate ingrowth of new vital tissue into the chemomechanically debrided canals. However, none of the treated teeth responded to thermal or electric stimuli.

Conclusions

The basic problem of necrotic pulps, with or without AP, is the heavy infection and need for suitable disinfection regimens. This complicates both classic and regenerative treatments. Classic/conventional methods (root canal treatment) applied after pulp necrosis are successful; however, they are less effective as after pulpectomy. Results may even be worse when systemic diseases like diabetes are present. Tooth extraction and implant insertion are viable alternative approaches. Experimental and very limited data from clinical studies (case reports) show that by using a suitable disinfection regimen, tissue ingrowth and mineralized tissue formation in the pulp cavity are possible. However, only one study in dogs showed the formation of a pulplike tissue; others reported ectopic cementum and unspecific connective tissue formation. On the other hand, regression of periapical lesions and symptoms was seen to be associated with root canal obliteration. As mentioned, obliteration of the root canal could be considered an acceptable treatment outcome in certain clinical situations, even in mature teeth, if no periapical lesion or clinical symptoms appear and if classic treatment methods have limitations (Fig. 4). However, here again more clinical data are needed before regenerative procedures can be regarded as an alternative to conventional methods.

FUTURE PERSPECTIVES

It is evident that more scientific information is needed for a better basic understanding of pulp biology related to the following:
Neurogenesis
Cellular stress and stress reduction
Dentin matrix proteins
Differentiation of regenerated pulp and unspecific connective tissue with cementum formation

Furthermore, additional progress is necessary regarding such topics as pulp tissue engineering with respect to the following:

- New scaffolds
- Cells and cell equivalents, also serum-free culturing methods
- Signaling molecules
- Antimicrobial regimens
- Medicaments (eg, small molecules)

The existing classification for pulpal diseases and the link to treatment methods should be critically revised to combine the histologic background of the different stages of pulp inflammation with clinical symptoms. In addition, new approaches like “stepwise pulpotomy” should be further investigated.

Instrumental for ensuring clinical progress in this field is the development of new diagnostic tools. Such devices should be developed bearing in mind that inflammation is key in the regulation and maintenance of pulp vitality, but its current assessment relies on relatively weak clinical diagnostic and pain evaluation tools, with limited correlation to the pathologic state of the pulp. Correct diagnosis is not only the basis for optimal treatment decisions but also for monitoring treatment outcomes.

New approaches for such diagnostic tools may rely on the analysis of dentin, blood from the dental pulp, or periapical liquid. Potential indicators may be inflammatory markers like IL-6 or matrix metalloproteases. The determination of tooth vitality is presently based on the reaction of nerves to temperature changes or electric stimuli. The determination of pulpal blood flow might be a much better way to assess pulp vitality. Different methods for blood flow measurement in the dental pulp have been proposed, such as photoplethysmography, which uses light and quantifies the transmission. Pulse oximetry is based on the same technical principle but uses red and infrared light, allowing for the assessment of hemoglobin oxygen saturation. Laser Doppler flowmetry uses the Doppler frequency shift of laser light by reflection from moving erythrocytes to detect blood flow tissues. The main problem of these various methods are the lack of spatial resolution and the interference with blood signals from the surrounding (mainly periodontal) tissues. Recently, a promising method was proposed by which the pulsation of the blood can be assessed by different wavelengths. In an in vitro model in which blood flow through the dental pulp and through surrounding tissues was simulated and could be varied independently, signals mainly derived from the dental pulp could be recorded by a certain wavelength in the infrared area (Fig. 5A–C).

**CONCLUSIVE REMARKS**

The feasibility of dental regeneration has been shown for some clinical situations (proof of principle); however, the predictability is still low. Some scenarios seem to be more promising than others in the regeneration of dental pulp. Whenever remnants of pulp tissue are present, the probability of regenerating new pulplike tissue is higher than in situations in which this is not the case. Therefore, special impetus should be placed on such clinical scenarios to

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**FIGURE 5** – Assessment of pulpal blood flow by laser Doppler flowmetry. (A) A schematic drawing of the pulp model with (1) tooth, (2) glass pulp, (3) solid resin jaw, (4) soft silicone gingiva, (5) a silicone tube, (6) a light source, and (7) a signal detector. (B) Amplitudes of pulse synchronous voltage modulation signals (ΔU [V], medians with 25%–75% percentiles) were measured with pulsed circulation using the glass pulp model for a separate circulation reading in the gingiva or the tooth plus both combined at 2 wavelengths (red and infrared) and with 2 different erythrocyte concentrates (EC 3 and 4). Statistical analysis (Mann-Whitney U-test) was performed at a significance level of α = 0.05 separately for each wavelength and EC; all values were pairwise significantly different, except the pairs marked with equal letters. (C) Example recordings of the vascular pressure signals from pulp (blue) and gingiva (red) at 940 nm before and after cessation of the pulsed circulation in the glass pulp model at 10 seconds.
further develop more effective treatment methods.

The lack of remaining pulp tissue and heavy bacterial infection of the pulp chamber and periapical tissue both strongly impede regeneration. Stem cells from blood or inflamed periapical tissue may not lead to regeneration of pulplike tissue but rather to the formation of connective tissue resembling the periodontal tissue associated with cementum deposits. Obliteration of the dental pulp chamber would likely be the consequence and should thus be critically assessed. However, in certain clinical circumstances, this may be an acceptable clinical outcome (eg, for immature teeth or even for mature teeth if they are threatened by extraction).

Although the feasibility of cell transplantation has been shown in 2 studies, this approach is associated with numerous technical, economical, and ethical problems. The recruitment of resident stem cells may be a viable alternative. The question is whether such stem cells can be reprogrammed to act like pulp-derived stem cells. Iohara et al speculated that the transplanted cells mainly served as suppliers for signaling molecules, which may be a relevant clue for further development. Finally, from a clinical point of view, further development of dental pulp regeneration must be accompanied by an optimized diagnostic classification system and better diagnostic tools.

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REFERENCES


