Case Report

Revitalization Teeth: A Prospective Case Series

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Abstract: In addition to caries, dental trauma is one of the most common causes of damage to permanent teeth and pulp. Pulp necrosis or damage to Hertwig's epithelial root sheath (HERS) leads to arrested tooth root development in immature teeth. Pulp necrosis can be treated by revitalization, a biology-based treatment alternative to apexification. Induction of a blood clot inside the root canal can lead to healing of periapical lesions and increased root length and thickness. Traumatic impact as the cause of pulp necrosis may affect the treatment outcome negatively, depending on the severity of damage to HERS. Revitalization procedures in four teeth with pulp necrosis following dental trauma were performed using a standardized treatment protocol. Three teeth were dislocated, the fourth tooth was avulsed. Each patient exhibited at least two clinical signs of pulp necrosis as well as radiographic evidence of apical periodontitis. X-rays were taken using individualized film holders (IFH) to reliably assess the treatment outcome. Revitalization treatment was performed without instrumentation of the canal walls, but disinfection with sodium hypochlorite and intracanal dressing with triple antibiotic paste (TAP) for three weeks. Provocation of bleeding was induced in a second visit, the blood clot was covered with collagen followed by calcium silicate cement, and teeth were sealed with resin composite. Clinical and radiographic follow-ups were performed after 1, 3, 6 and 12 months. An increase of root length and thickness was evident in the three teeth with dislocation injuries. In one case, formation of mineralized tissue below the calcium silicate cement was observed. The tooth which had been avulsed and replanted showed resorption of the apical root area. The observations made in this study support the assumption that a separation of HERS and the cells that form pulp and dentin during tooth root development may negatively affect the outcome after a standardized revitalization procedure. The consistent implementation of standardized treatment protocols and the use of IFH are helpful receiving a reliable treatment outcome.

Keywords: Revascularization, Maturogenesis, Regeneration, Dental Trauma, Hertwig's Epithelial Root Sheath (HERS), Root Canal Treatment

1. Introduction

Tooth root development in humans begins after the completion of crown formation and is controlled by the cells of Hertwig's epithelial root sheath (HERS). Epithelial cells, which are derived from the cervical loop of the enamel organ, proliferate and extend in an apical direction to determine the root number, shape and length. Among other functions, HERS cells instruct the underlying mesenchymal stem cells of the apical papilla to differentiate into odontoblasts to form root

dentin and pulp [1]. HERS is a transient structure, it becomes fenestrated as root formation continues and is subsequently reduced to the epithelial rests of Malassez (ERM) [2-3]. If pulp necrosis occurs in a premature tooth with incomplete root formation, stem cells of the apical papilla will eventually perish and no longer be available to differentiate and form pulp cells and dentin. Pilot experiments by Huang et al. in minipigs have shown that surgical removal of the apical papilla at an early stage of root development led to arrested root development [4]. Similarly, odontoblast differentiation inevitably stops after a growth arrest of HERS. Radicular

dysplasia as a consequence occurs mainly due to trauma or in association with dentin disorders [5].

Revitalization procedures aim at the recruitment of stem cells of the apical papilla, which can repopulate the root canal, undergo differentiation and form dental pulp. After provocation of bleeding, stem cells of the apical papilla are flushed in with the blood stream and accumulate in the canal [6]. An increase of root length and thickness is described after revitalization in multiple case reports and case series [7-9]. Whereas the role of stem cells of the apical papilla in this process has been assessed and described, evidence on the influence of HERS cells is sparse. However, an intact HERS may be required in order to allow for continued root formation after a revitalization procedure. Traumatic impact, especially avulsion but possibly also severe dislocation injuries may cause a disruption of the epithelial cell layer and thus a separation of epithelial and mesenchymal cells. It can be speculated that this spatial disruption will abolish the signaling cues indispensable for mesenchymal cell differentiation and thus in an irreversible arrest of root development. Numerous case reports demonstrate the success of revitalization procedures in premolars with structural abnormalities, such as dentes evaginati. Interesting observations, which show the completion of root tip formation separated from the coronal part of the root after dislocation shed light on unfavorable conditions for a completion of root formation [10]. From a biological point of view, it appears feasible to assume that severe dislocation injuries with damage to HERS may result in impaired regeneration and failure to induce a completion of root formation.

2. Clinical Cases

Four teeth with pulp necrosis in three patients after dental trauma were treated with revitalization procedures. The medical history was without pathological findings in all cases, and tetanus shots had been given recently. After comprehensive discussion of the risks and possible outcomes of revitalization treatment, consent of the patients and parents was obtained.

Case 1

A 7-year-old boy experienced an accident at school and was referred to the Department of Conservative Dentistry at the University of Goettingen due to a luxation injury of both upper middle incisors. Both teeth 11 and 21 were dislocated 3 to 4 mm in a palatal direction and showed mobility grade II. The teeth were free of caries, tooth 21 presented with a dentin-enamel fracture without pulp exposure, which was sealed with a flowable composite. Diagnostic testing was inconclusive to cold, but the patient reported sensitivity to palpation and percussion. Periodontal probing depths were within normal limits (1 and 2 mm). The teeth were repositioned and splinted flexibly for 2 weeks.

Case 2

An 8-year-old girl was referred after an accident during swimming lessons at school. Tooth 11 was dislocated in a

palatal direction and presented with an enamel fracture at the distal edge, which was sealed with flowable composite resin. The tooth was splinted with a flexible splint for 2 weeks.

Case 3

An 11-year-old girl was referred after she had suffered a hit from a horse. Tooth 11 was avulsed, but promptly transferred to a dentosafe box (Medice, Iserlohn, Germany) and stored for approximately 30 minutes. Tooth 12 presented with a subluxation injury and mobility grade I. Tooth 11 was replanted and stabilized flexibly with a splint for 15 days. Additional lacerations of the gingiva were sutured.

3. Material and Methods

3.1. Diagnostic

For all patients, soft chow for one week and the use of chlorhexidine mouth rinse for oral hygiene were recommended. Follow-ups were conducted weekly during the first month post injury, including clinical and radiographic examinations. Additional diagnostics included electric pulp testing (EPT) or, if ankyloses was suspected, the use of a periotest device (Medizintechnik Gulden, Modautal, Germany). Endodontic treatment was initiated if at least two clinical signs or symptoms (e.g. negative response to vitality testing with cold or EPT, tenderness to percussion, pain) were present in combination with radiographic evidence of apical periodontitis.

3.2. Revitalization

After informed written consent, revitalization procedures were conducted in all cases and in accordance with the current guidelines [7, 11]. The procedural details were identical for all cases, following a standardized protocol (figure 1). All treatment steps were performed by only one experienced endodontic specialist. After rubber dam isolation, an access cavity was prepared and the diagnosis of pulp necrosis was clinically confirmed in all cases. Root canal length was determined electrometrically and additionally controlled under an operating microscope. The root canal walls were left intact, no mechanical instrumentation was performed, and canals were irrigated with 10 mL of 3% sodium hypochlorite for 60 seconds with a NaviTip cannula (G 30 Ultradent, Munich, Germany) 2 mm above the endometrically determined canal length. Patients did not experience pain throughout the treatment with exception of provocation of bleeding by instrumentation beyond the apex, which was dolorous despite local anesthesia in all cases. Provocation of bleeding was performed in the second visit two to three times before sufficient influx of blood into the canal was achieved. Insertion of a collagen matrix (PARASORB® Cone, RESORBA Medical GmbH, Nürnberg, Germany) provided a thrust block, which allowed for condensation of the tricalcium silicate cement (Biodentine, Septodont, France) against the blood clot.

Figure 1. Standardized treatment protocol according to the Clinical Considerations for a Regenerative Procedures of the AAE [11]. (IFH: individual film holder; WL: working length; TAP: triple-antibiotic-paste; CEJ: cemento-enamel junction).

3.3. Evaluation of the Treatment Outcome

In order to assess the progress after treatment with a series of radiographs, each patient received an individually adapted film holder (IFH). The position of the front teeth was retained using silicone impressions, which allowed for reproducible imaging with identical angulation (figure 2). X-rays were taken after adhesive seal of the cavity in order to avoid the risk of contamination of the root canal.

Figure 2. Radiographs were taken using individualized film holders (IFH).

Consecutive radiographs were superimposed using a special software (GIMP; Version 2.8.16) such that a standardized evaluation of root development was possible. To superimpose images, the horizontal plane was aligned at the

enamel-cementum junction, the vertical plane at the anatomically prominent structure of the *sutura palatina media*. The superimposed image created from 3 radiographs (figures 3D, 4D, 5D) shows the root tip in red (directly after revitalization), orange (first follow-up) and blue (second follow up), respectively, in order to visualize changes.

3.4. Follow up

Case 1

Figure 3. A: Preoperative radiograph. B: One month after revitalization treatment. C: 6-month follow-up. D: Evaluation after 6 months after superimposition of three radiographic images. An increase of root length is visible as well as a narrowing of the canal in the apical area. E: Clinical image after 6 months.

Case 2

Figure 4. A: Postoperative radiograph. B: 6 months after revitalization treatment. C: 12-month follow-up. D: Evaluation after 6 months after superimposition of three radiographic images. An increase of root length is visible as well as a narrowing of the canal in the apical area. E: Clinical image after 12 months.

Case 3

Figure 5. A: Postoperative radiograph. B: One month after revitalization treatment. C: 6-month follow-up. D: Evaluation after 6 months after superimposition of three radiographic images. The root is distinctly shortened compared to the initial situation. E: Clinical image after 6 months.

Figure 6. In case 1, a possible formation of a mineralized barrier (bridging) below the tricalcium silicate cement is visible after 6 months.

During follow-ups, no pain, swelling, sinus tracts or tenderness to percussion or palpation were observed in any case. Probing depths were physiological, and apical periodontitis diminished in all cases. In neither case, ankylosis or discoloration was observed. A slight increase in root length was detectable along with a narrowing of the apical part of the canal after 6 months (Case 1, figure 3) and after 12 months (Case 2, figure 4). The radiograph in Case 1 taken after 6 months showed dentin bridging 2 mm below the tricalcium silicate cement (figure 6) and in the apical region (figure 3c). Case 3 presents with resorption of the root tip (figure 5). Whether an increase of root thickness has occurred remains speculative.

4. Discussion

Approximately one third of all adolescents suffer from dental trauma until their twentieth year [12-14]. Dislocation injuries account for 73.4%, avulsion for 8-9.2% [15]. In more than half of all damaged teeth, fractures of enamel or dentin can be observed. Teeth which present with combined injuries of dislocation and fractures to enamel and dentin, which are observed in 31.6% of dental trauma cases, appear to have a higher risk of developing pulp necrosis, even without pulp exposure [15]. The risk of pulp necrosis increases from 4.8% in isolated injuries to 56.5% for combined injuries [16]. As a cause, compromised blood circulation is suspected, which leads to impaired immunoresponse of the pulpal tissue after traumatic impact. Both case 1 and 2 presented with a combination of dislocation and enamel-dentin fractures. Exposed dentin was sealed with dentin bonding during attachment of the splint. Whether pulp necrosis occurred as a consequence of the dislocation injury or the enamel-dentin fracture remains uncertain. Since cold testing after dental trauma has limited informative value regarding pulp vitality [17-18], at least two clinical symptoms in combination with radiographic findings of apical periodontitis were required in this study to validate the diagnosis of pulp necrosis. The suspected diagnosis was confirmed after accessing the root canal. Since root development was not complete in either case, revitalization procedures were chosen as the best treatment option. After informed consent, the treatment protocol was identical for all three patients. It complies with the guidelines and consideration of the AAE and ESE [7, 11]. After preparation of the access cavity, necrotic tissue was found in all cases. Sufficient disinfection is a pre-requisite for successful regeneration [19], therefore, the removal of debris and smear layer and a disruption of the biofilm inside the root canal is essential. Instrumentation of the root canal walls, however, weakens the thin dentin walls of immature teeth even further. Therefore, mechanical debridement was deferred, but passive ultrasonic activation of the disinfection irrigant was considered indispensable. Ultrasonic activation in teeth with open apices may cause undesirable effects in the population of stem cells of the apical papilla and thus affect the outcome negatively. Histological studies also show that despite pulp necrosis and apical periodontitis, vital tissue

remnants can still be present inside the root canal [20], and it might be beneficial to preserve these islands of cells. The goal is to achieve maximum reduction of microorganisms, but at the same time prevent damage to the cells and tissues that are involved in regeneration and repair. As an intracanal medicament, triple antibiotic paste consisting of ciprofloxacin, metronidazole and cefuroxime was used. Calcium hydroxide, the alternative intracanal medicament, disinfects due to its high pH, which stays stable for up to two weeks [21]. It induces a mineralized barrier, but can cause cell necrosis to a depth of 1.5 mm, as shown in histologic studies after direct pulp capping [22]. Whereas calcium hydroxide has been found to be uncritical with stem cells of the apical papilla [23], triple antibiotic paste was used in the first published report by Banchs and Trope [24] and therefore the medicament of choice for most case reports published so far. The reported clinical success rates with TAP ranged between 91% [25] and 93% [26]. However, antibiotic pastes have cytotoxic effects as well [27-29]. Therefore, low concentrations between 0.39 mg/mL [28] and 0.1 mg/mL [23] are recommended to date. For triple antibiotic pastes, minocycline has been replaced with cefuroxime to avoid tooth discoloration [30-32]. In the described cases, triple antibiotic paste was prepared in a liquid consistency and inserted into the canal by use of a syringe in a controlled manner under an operating microscope. The clinical images document that tooth discolorations were not observed in any of the cases. Mineral trioxide aggregate, which has been commonly used after revitalization procedures, can cause discolorations after contact with blood. The tricalcium silicate cement used in this study contains zirconium dioxide as a radiopacifier, which does not lead to discoloration, and may therefore be a suitable material to cover the blood clot during revitalization procedures.

Whereas a slight increase in root length and thickness were observed in cases 1 and 2, case 3 shows apical resorption of the treated tooth at the 6-month follow-up. This might be the consequence of disruption of apical papilla and HERS caused by avulsion of the tooth. Without instruction and control by the epithelial cells of HERS, the stem cells of the apical papilla might not be able to differentiate and form pulp cells, odontoblasts and root dentin, which could explain why there was no increase in root length. It can be speculated that an ingrowth of clastic cells caused the resorption at the apex. However, periosteal results do not provide any indication of replacement resorption and ankylosis. In both cases 1 and 2, an increase in root length was observed, along with internal mineralized barrier formation in case 1. This mineralized tissue formation was also observed in a recently published case report [33]. The teeth did not respond to cold testing and EPT after revitalization. This might be due to formation of ectopic tissue inside the root canal [30, 34-35], or, more likely, due to the large distance between the location where the stimulus was placed and the tissue. Thus, the nociceptive stimulus was not triggered. An increase in root thickness cannot be proven explicitly in this study, which might be due to the short time frame of follow-ups. It should be mentioned that comparable studies report an increase of thickness rather

than root length with similar follow-up periods of 12 months [36-37]. The prognosis for the cases presented here is estimated to be good, all teeth are without signs and symptoms of inflammation and apical radiolucency's are decreasing. Survival rates after revitalization amount to 95%, success rates are stated to be 78% [36]. Comparing revitalization and apexification, revitalization appears to entail more unfavorable side effects (discoloration, pain, reinfection), but can induce an increase of root length and thickness, which do not take place after apexification [38].

5. Conclusion

The assumption that the outcome after revitalization procedures may be compromised after severe dislocation injuries and avulsion due to a disruption of HERS and SCAP seems plausible. Nevertheless, this has to be confirmed in clinical studies with sufficient sample sizes. The consistent implementation of standardized treatment protocols and the use of individual film holders are helpful receiving a reliable treatment outcome.

Conflict of Interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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