

Maturogenesis

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Abstract: An immature permanent tooth with pulpal necrosis and apical periodontitis presents a unique challenge to endodontists. Possible treatment options consist of extraction, apexification Ca (OH)₂, MTA plug or more recently maturogenesis. Maturogenesis is based on principles of tissue engineering which is connected to the usage of stem cells, growth factors and scaffolds. The purpose of this review is to summarize the biological basis of maturogenesis and its practical application in an up-to-date clinical protocol.

Keywords: apexification, apical periodontitis, immature teeth, maturogenesis, revascularization, revitalization

I. Introduction

The treatment of immature necrotic teeth involves many obstacles. Due to the fact that the wall of the root canal is very thin, it is not possible to make drastic mechanical preparation and the tooth remains weakened even after successful treatment. In addition, it is very difficult to condense the filling materials in the root canal and to create a hermetic apical seal because of the widely open apical foramen. The result is then either extruded filling material into the apical region or inadequate apical seal of the root canal. Furthermore, the patient's uncooperation is another complication in the prepubertal age. Until recently, all these unfavorable factors put great demands on treating dentists and the success rate was quite low.

In 1964, Kaiser presented a clinical protocol using calcium hydroxide (Ca(OH)₂) by which the formation of an apical barrier could be achieved (1). Repeated changes of Ca(OH)₂ every 3 to 6 months showed that it was possible not only to achieve healing of periapical lesions but also to induce closure of widely open apices by inducing the formation of calcified tissue. Some of the teeth then continued to develop their roots. This technique was the gold standard for the treatment of immature necrotic teeth for a long time.

In 1993, Torabinejad and his colleagues published first articles about the new bioactive material, Mineral Trioxide Aggregate (MTA) (2, 3). At the turn of the millennium, the first case reports of apical plug using MTA appeared (4, 5). The main aim was to shorten the treatment period and to avoid increased fragility of the teeth which occurs after prolonged exposure of dentin to Ca(OH)₂ (6,7,8). The technique involving inner matrix facilitated the very difficult condensation of MTA in a widely open apex (9). This technique is done by using atelocollagen foam (pure lyophilized collagen type I) or calcium sulphate forced out of the root system into the periapical area. Consequently, it condenses to form solid matrix, on which MTA can be subsequently placed. This method can achieve a significant decrease in the length of the treatment.

In 2001, Iwaya published the first case report of modern regenerative endodontics on the basis of tissue engineering (10). In his clinical case he tried to modify the internal environment of the root system so that it could lead to pulpal tissue regeneration from blood clot. This was based on the concept of lesion sterilization and tissue reparation (LSTR) (11) and earlier attempts by Nygaard-Ostby with stimulated bleeding from the apical region (12, 13). Since then, a large number of case reports and consequently professional works with the intention to optimize the treatment protocol have been published. However, there are still uncertainties regarding the correct terminology, and several different terms are used for these procedures:

1.1 Revascularization

The term was first used by Iwaya (1). This term was subsequently defended by claiming that the nature of the tissue inside the root system was not predictable. The only certainty is the restoration of vasculature (14). However, the term "revascularization" better describes the restoration of blood supply to the damaged tissue (e.g. in the context of dental traumatology) (15).

1.2 Revitalization

This term describes the presence of nonspecific restoration of the vital tissue within the root system, without any relation to the formation of hard tissues and further development of the root (16).

1.3 Regenerative endodontic procedure (REP)

This is the official term used by the American Association of Endodontists (AAE), which includes all therapies using the principles of tissue engineering leading to the restoration of tissue resembling dental pulp. Unfortunately, even under sterile conditions in animal models, it was not possible to use the current protocol of maturogenesis to stimulate the regeneration of the dental pulp or similar tissue (17, 18, 19, 20).

1.4 Maturogenesis

The term was first used in connection with direct capping of the pulp of immature permanent teeth (21). This term describes the physiological continued development of the entire root, and not only the apical segment (15, 21, 22). However, there is an overlap with apexogenesis, which has been used in vital immature permanent teeth (23). According to the authors' opinion, maturogenesis best reflects the current goals of treatment of immature necrotic permanent teeth (more is explained later in Evaluation of success of the therapy).

II. Tissue Engineering

Tissue engineering is a multidisciplinary field combining medicine, biology, and engineering. Its aim is to restore the function of damaged or completely destroyed tissues or organs(24). The key components for using the principles of tissue engineering in endodontics are stem cells, tissue factors, and internal matrix.

2.1 Stem cells

Stem cells that play a role in maturogenesis belong to the group of multipotent mesenchymal stem cells (MSC). These cells are present and regulated in small defined areas called niches. The niches of odontogenic stem cells are found mostly perivascularly (25) and are characterized by three basic features (26, 27, 28):

- They form an anatomical area where the number of stem cells is regulated.
- They affect the mobility of stem cells.
- Stem cells are maintained in a quiet mode and, if necessary, their differentiation is started and their regenerative capacity is set.

Stem cells can survive under certain conditions in the root canal. They may also be actively transported to the root system by blood, or they may be attracted by tissue factors on the "cell homing" principle.

2.1.1 Stem cells of apical papilla (SCAP)

These stem cells are likely to play a major role in maturogenesis. They are less differentiated mesenchymal stem cells (26, 29) that are likely the precursors of radicular pulp (30, 31) and primary odontoblasts involved in the formation of radicular dentin (32). It is probably due to the proximity of the apical papilla to the apex that much higher concentrations of mesenchymal stem cells occur in blood from periapical region than in the bloodstream (33).

2.1.2 Dental pulp stem cells (DPSC)

Their role in maturogenesis assumes the presence of residual vital pulp. This possibility occurs when necrosis extends coronopically, wherein the apical portion may be vital pulp. There have been cases reporting that apical residual pulp was probably present in an immature tooth with an extensive periapical finding (10, 34, 35, 36). It was also shown that the DPSC were present unchanged in teeth with irreversible pulpitis (37, 38, 39).

2.1.3 Periodontal ligament stem cells (PDLSC)

The ability of these cells to form calcified tissues is significantly lower compared to the two previously mentioned types of cells (40, 41, 42).

2.1.4 Inflammatory periapical stem cells (iPAPSC)

In recent years, stem cells have been described in periapical granulomas or periapical cysts (43, 44, 45). It is unlikely that these stem cell lines could play a big role in maturogenesis.

2.1.5 Bone marrow stem cells (BMSC)

These are very heterogeneous but well-researched mesenchymal stem cells. Their role in maturogenesis is limited.

2.2 Growth

Factors Tissue factors that are encased in the collagen matrix of dentin during dentinogenesis play the greatest role in maturogenesis. Another possibility is to supply these factors by means of plasma rich in growth factors (PRGF). The most important growth factors and their activity and sources are summarized in Table 1.

Table 1. The most important growth factors and their activity and sources

Abbreviation	Name	Source	Activity	Usability
TGF-beta	Tumor Growth Factor Beta	odontoblasts dentin matrix	antiinflammatory stimulates healing processes	promote mineralization
bFGF	basic Fibroblast Growth Factor	dentin matrix	stimulates proliferation	stimulation of stem cell proliferation stimulation of reparative dentin
VEGF	Vascular Endothelial Growth Factor	endothelial cells	increases angiogenesis	increase blood supply
PDGF	Platelet-derived Growth Factor	platelets endothelial cells	increases cell proliferation mesenchymal origin	stimulation of stem cell proliferation
BMP	Bone Matrix Proteins	bone matrix	induces differentiation of odontoblasts mineralization of bone matrix	differentiation of stem cells stimulation of mineralization of the newly formed matrix

2.3 Scaffold

It serves as a physico-chemical and biological 3D microenvironment where cells may divide, differentiate, and where they can migrate. The scaffold can serve as a carrier for growth factors and should also be efficient in the transport of nutrients and oxygen and removing waste products. It should gradually degrade and be replaced by regenerated tissue (24). In most publications, the blood clot formed after the stimulation of bleeding of the periapical area was used as scaffold. In a small number of articles, PRGF were utilized as an alternative to the blood clot (22, 46, 47). When examining the quality of the resulting tissue using blood clot or PRGF in in vivo tests on dogs, it was found out that there was no difference between the two matrices in terms of success of the apical closure, the amount of the newly formed tissue and its quality (48).

III. Disinfection Of The Root System

Given the extent of the root system and the residual thickness of the root wall, mechanical preparation must be limited. Immediately after making an adequate access cavity, we can achieve adequate fluid hydrodynamics. The mechanical instrumentation is probably necessary in the presence of a microbial biofilm (49).

3.1 Bacterial profile

In vivo, it was found that the microbial profile in infected root systems with incomplete development is similar to the microbial profile in primarily infected root systems with completed development of the root (50).

3.2 Irrigation protocol

3.2.1 Sodium hypochlorite

In many published case reports, sodium hypochlorite was used as the main irrigating solution (51). Its antimicrobial action is very well documented in multiple in vitro and in vivo experiments (52). At a concentration above 1 %, the antimicrobial effect of sodium hypochlorite increases only slightly (53, 54). On the other hand the higher concentration of hypochlorite, the faster is the dissolution of the tissue because of increase proteolytic action (55). Closely related to the proteolytic properties of sodium hypochlorite is activity against extracellular matrix of the biofilm. From the point of view of molecular biology, it seems more advantageous to use lower concentrations of sodium hypochlorite for suppressing the proteolytic properties. Overall, it appears that sodium hypochlorite causes lower survival of stem cells as a result of alterations in the chemical composition of dentin including the denaturation of incorporated tissue factors and collagen fibers that serve as attachment of stem cells (56). However, it must be emphasized that with the use of 1% sodium hypochlorite solution, there is no significant change in the composition of dentin nor is there change in its mechanical properties (57).

3.2.2 Ethylenediaminetetraacetic acid (EDTA)

It is a chelating agent which is used together with sodium hypochlorite under classical protocols to remove the smear layer and to open the dentinal tubules, which leads to better disinfection of the root system (58). EDTA treatment affects the inorganic component of hard dental tissues and therefore exposes the collagen fibers and growth factors. Its effects depend mainly on the time of exposure (59). Another positive effect of EDTA is the inactivation of sodium hypochlorite (60). All these properties of EDTA lead to better adhesion of SCAP to dentin and their increased survival (51, 61).

3.2.3 Chlorhexidine

From the point of view of molecular biology, the ability of chlorhexidine to bind to exposed collagen fibers of dentin matrix plays the greatest role. This binding not only inactivates the antimicrobial action of chlorhexidine, but also prevents the adherence of SCAP to dentin (61), which leads to minimal survival of SCAP, although chlorhexidine is one of the least toxic antiseptics (62). From this perspective, the usage of chlorhexidine is not appropriate in maturogenesis.

3.3 Medication within the canal

Sometimes we are not able to suppress bacterial infection and create conditions for the repair or regeneration with irrigating solutions only. In these cases, we tend to use intracanal medication, either by conventional Ca(OH)₂ or the various combinations of antibiotics according the concept of LSTR.

3.3.1 Calcium hydroxide

It is considered the gold standard for the disinfection of the root system and stimulating the formation of hard tissues in apexification of immature permanent teeth (7). Thanks to this reputation, it also received attention in connection with maturogenesis. It is important that calcium hydroxide should be applied to the coronal half of the root (63). It was shown that, when applied to the coronal half of the root, wall thickness was increased by 53.8 %, while the classic application to whole root canal system increased wall thickness only by 3.3 % (64). Freshly mixed calcium hydroxide has a pH of approximately 12.5 and is potentially toxic to human cells including SCAP. Applications further from the apical region are likely to reduce toxicity, while they still retain their positive characteristics (35, 63, 64). Calcium hydroxide has the ability to dissolve hard dental tissues and expose the dentin collagen matrix with growth factors from the dentinogenesis period. In vitro, it was found that the SCAP shows greater survival rate and proliferation in the dentin that was under the effect of calcium hydroxide than the dentin which was exposed to various concentrations of antibiotic pastes (65).

3.3.2 Antibiotics

The use of antibiotics in maturogenesis is based on the concept of LSTR, which was developed in Japan in the late 1980s and during the 1990s for use in pediatric dentistry. A mixture of antibiotics mainly targets obligate anaerobes (66, 67, 68). The combination of antibiotics can achieve the formation of a bacteria-free environment at much lower concentrations than using one kind of antibiotics only (69, 70). The most frequently used protocol is the triple antibiotic paste (TAP), sometimes referred to as 3Mix. It is based on the classic Hoshino triple antibiotics – metronidazole, ciprofloxacin and minocycline. In vitro experiments revealed that sterilization of the root system requires mixtures, wherein each sub-antibiotic has a concentration of 25 µg / ml. Thus, the total concentration of antibiotics in the composition is 75 µg / ml. It must be emphasized that these concentrations were not applied in the first case reports. The aim was not to create a very specific concentration, but “creamy to pasty consistency” (34, 10). This procedure obtains antibiotic paste with antibiotic concentration exceeding 1 g / ml. Such a high value has a negative effect on the survival of the SCAP (71) and modifies the dentin to the extent that the SCAP in vitro are not able to settle and proliferate (65). The disadvantage of using TAP is tooth discoloration caused by minocycline. This can be avoided by adhesive sealing of pulp chamber dentin (72) or substitution of minocycline by amoxicillin (73) and cefaclor (74). Another option is the omission of the third antibiotic (minocycline) and the use of a double antibiotic paste (DAP) (10).

IV. Clinical Protocol

The workflow is based on the recommendations of the AAE that established the Regenerative endodontics committee in 2006. The latest version, which was published in April 2015, is strongly influenced by the latest findings about the concentration of irrigating solutions and medications within the root canal. This standard protocol recommended therapy in two visits, although there are successful case studies with one-visit therapies only.

4.1 Indications and contraindications

The general indications and contraindications for maturogenesis are identical with the indications and contraindications for endodontic treatment. Specific indications include the status of tooth development and diagnosis. Maturogenesis can be used as the method of choice for permanent immature necrotic teeth where repeated access to the root system can be achieved. The specific contraindications include allergy to antibiotics used in the TAP / DAP, and tooth destruction where it is necessary to use the pulp chamber and the root of the tooth to ensure its stability and resistance. Maturogenesis is preferred when the root is in early stage of development as stage 1 to stage 3 of Cvek classification. In later stages of root development as stage 4 and stage of Cvek classification the one step MTA apexification is more favorable.

4.2 Visit 1

- a) Application of local anesthesia, rubber dam and preparation of the access cavity (Fig. 3, 4).
- b) Determination of the working length by an apex locator, verification using a measuring radiograph (Fig. 5, 6).
- c) Rinse 20 ml of 1.5% NaOCl solution for five minutes. The tip of the irrigating cannula should be introduced approximately 1 mm from the end of the root canal to minimize the cytotoxic effect on the apical tissues. Subsequently, the root system is flushed with 20 ml of saline solution for five minutes.

- d) Drying the canal with paper points.
- e) Application of medication into the canal (Fig. 7, 8). There are two possible alternatives:
 1. Ca(OH)₂ is applied via a cannula into the coronal third of the root (63, 64).
 2. A mixture of antibiotics is administered via a cannula (20 G diameter) 2 mm from the end of the root canal (73). Alternatively, condensation of the mixture can be achieved by the other end of a paper point, in order to be positioned below the CEJ.
- f) Temporary closure. It is necessary to apply a sterile spacer, usually in the form of a Teflon tape. Subsequently, application of a 3-4 mm thick GIC is recommended, or a sandwich-type filling material using calcium sulfate material and an adhesively fixed flow composite (Fig. 9, 10).

4.3 Visit 2

The second visit takes place 1–4 weeks after the first visit. There should be an absence of clinical symptoms. If not, repeat the first phase.

- a) Application of local anesthesia without epinephrine (3% mepivacaine), use a rubber dam.
- b) Removing the temporary fillings and repeated lavage with 30 ml of EDTA solution for 10 minutes (75).
- c) Drying with sterile paper points.
- d) Creating a suitable environment in the root system, using one of the two possible techniques:

1. Induction of bleeding and clot stabilization (Fig. 11)

It is important not to damage the Hertwig's epithelial sheath during the induction of bleeding, because it plays an important role in the further development of the root. It is recommended to use sterile K-file instruments of size ISO 20-25, which should be pre-curved by about 30° in order to induce greater irritation of the apical tissues and substantial bleeding. This instrument is inserted 2 mm beyond the physiological apical foramen and then rotated (34, 36, 73, 74, 75). Because of the rich blood supply to the apical papilla, this immediately leads to bleeding. Attempts to stabilize the clot at roughly the level of CEJ are done with the opposite end of a sterile paper point soaked in physiological saline. Coagulum is fully stable after about 15 minutes (34, 36, 73, 74). Another possibility is the application of collagen foam to the unstable clot at roughly the level of CEJ. It Collagen subsequently acts as matrix for the application of MTA (36, 75) (Fig. 12).

2. Application of PRGF – PRGF is administered via a cannula into the root system. PRGF is fully stable after 5 minutes (46).

- e) Application of MTA. MTA should be applied coronally at about the level of CEJ. In frontal region it is recommended to place the MTA rather below the CEJ because of the possibility of discoloration. Another possibility is to use a nonstaining alternative to MTA, such as calcium silicate materials also known as bioceramics – we have mentioned the use of the second (e.g. Biodentin, SEPTODONT, France) or third generation (e.g. BC RRM Fast Set Putty, Brasseler, Canada).
- f) Completion of 3–4mm thick layers using GIC or adhesive filling (Fig. 15).

4.4 Evaluation of success of the therapy

Therapy can be considered successful if there is no pain and the tooth is clinically asymptomatic. Control radiographs are made 3, 6, 9, 12, 18, and 24 months after the therapy and then at yearly intervals for as long as 5 years. From the radiological point of view, the reduction or loss of periapical translucency (primary goal) is considered. In the long term we are primarily interested in the increase in the thickness of the root wall and the root length and the degree of closure of the apical region (secondary goal). Finally, we expect to restore the function of the pulp, particularly the sensitive and immunologic function (tertiary goal) (75). If the tooth is clinically calm after maturation, it is recommended not to carry out subsequent endodontic treatment (74). This is based on the recommendations for treatment of teeth with a calcified or obliterated root system after trauma (76, 77, 78).

V. Conclusion

Treatment of immature permanent teeth with necrotic dental pulp is very demanding due to weak cooperation of the patient and the need for a highly skilled treating dentist. Furthermore, it should be emphasized that this treatment method is very sensitive and has to be followed strictly. Treatment using maturogenesis brings a significant thickening of the root wall, more than apexification using Ca(OH)₂ and MTA (64). In addition, it must be emphasized it is very unlikely that this would lead to a complete recovery of the pulpodentinal tissue in the real sense (49, 79). However, it can be assumed that the reparative apposition of hard tissues on the inner surface of the tooth improves its mechanical properties. From today's perspective, it is a very promising method of treatment, which could provide better long-term prognosis for immature teeth with necrotic dental pulp, especially with an early stage of root development (64, 79).

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