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Review of Current Concepts Associated with Pulp Revitalization in Teeth with Incomplete Rhizogenesis

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ABSTRACT

Pulp revitalization can be defined as the differentiation of progenitor cells from the apical portion of young teeth that start to colonize the root canal, resulting in the deposition of mineralized tissue in the dentinal walls. This procedure is currently indicated for young teeth with incomplete rhizogenesis as an alternative to the traditional treatment of apexification. The objective of this study was to review the literature about the knowledge about pulp revitalization, knowing its mechanism of cell differentiation, advantages and disadvantages, making a comparison with the apexification and exploring in general the studies related to a future regenerative trend through the scaffolds. Currently, there is a constant search for new treatment alternatives that allow the complete development of the roots without becoming fragile. Studies show that scaffolds have the function of providing support for cell growth. Revitalization emerged as a new option for the treatment of young teeth with pulp necrosis, using therapeutic techniques such as inducing clot formation inside the root canal and applying polyantibiotic pastes, always aiming at the new formation of pulp tissue, which would take in addition to root complementation in length, also increasing its thickness, providing greater resistance.

INTRODUCTION

The endodontic treatment of teeth with incomplete rhizogenesis and pulp necrosis has been a challenge for endodontic therapy, both because of its technical and biological difficulties, with this, the desire to create biological alternatives for the treatment of canals has inspired the field of regenerative endodontics. , where damaged or necrotic pulp tissue is removed and replaced with regenerated pulp tissue to revitalize teeth[1]. In traditional endodontics, immature and necrotic teeth are treated with apicification, which consists of the application and exchange of calcium hydroxide paste in the root canal, for a variable period of time, in order to induce the formation of a mineralized barrier in the apical region for accommodation, filling material. The use of aggregated mineral trioxide (MTA) to make an apical barrier is also an alternative to the use of calcium hydroxide. However, regardless of the material used, both techniques have the same disadvantages, as they do not allow the continuation of root development, there is no increase in the thickness of the root canal walls, so the root remains with its fragility and susceptibility to fracture[2].

Recently, studies and case reports have presented an alternative for the treatment of immature teeth with pulp necrosis, the regeneration of this tissue. This revitalization process has received various names such as revascularization or maturation, and its protocol involves the use of a triantibiotic paste (metronidazole, minocycline and ciprofloxacin) and the induction of bleeding inside the root canal, thus forming a clot that would serve as a matrix for the growth of new vital pulp tissue. With pulp regeneration, normal formation of the injured tooth roots with strengthened walls and apical closure would be possible, as occurs in apicigenesis[3].

Revascularization is considered a treatment that restores the vitality of the teeth, allowing the repair and regeneration of periapical tissues, due to the following biological reasons: the open apex allows the migration of stem/progenitor cells into the root canals, apical papillae found in the immature apex region have greater tissue regeneration, stimulating root development and apical closure. Successful treatment is only possible when the root canal is clean and decontaminated, thus allowing cell growth for the future restoration of pulp vitality[4]. Root canal decontamination will occur through the association between instrumentation (mechanical preparation), irrigation with auxiliary chemicals and intracanal medication[5].

More recently, research has been carried out in regenerative endodontics on scaffolds, which are transient extracellular matrices that have the function of scaffolds serving as a support for cells and vessels to grow and develop their functions in the revascularization process[6]. Given the above, the objective of this work is to review the literature on the knowledge about pulp revitalization, knowing its mechanism of cell differentiation, advantages and disadvantages, making a comparison with the apexification and exploring in general the studies related to a future regenerative trend through of the scaffolds.

LITERATURE REVIEW

Considerations about endodontic treatment of immature teeth

Pulp necrosis in immature teeth usually occurs due to bacterial invasion or trauma. In these situations, the inflammation of the pulp tissue gradually occurs, and if it is not stopped, the same necrotism, leading to the death of odontoblasts, resulting in the interruption of root development. With that, many times these teeth are more prone to fracture. Pulp necrosis impairs the development of roots in permanent teeth with an open apex, and their treatment is still a challenge since there was no complete formation of the root apex, thus, both the modeling and the posterior filling of the canal will not be properly acceptable[7].

For the situation mentioned above, the traditional treatment of choice was apexification, however, despite the success rates of this treatment, root development did not generally occur in its entirety, and the roots remained thin, fragile and prone to fracture. Thus, it was essential for researchers and clinicians to search for new treatment options. Currently, pulp revitalization has emerged as a possibility of treatment, based essentially on the proliferation of connective tissue cells to fill empty spaces left by pulp necrosis[8].

Apexification

Apexification is a treatment for teeth with an open apex, aimed at sealing the apical foramen through the neoformation of a mineralized tissue induced by the action of a calcium hydroxide-based medication used to fill the root canal[8]. The canal is filled with calcium hydroxide due to its ability to assist in disinfecting the root canal system and inducing an apical barrier. Hydroxyl ions damage the bacterial cell wall and its components, as well as lipopolysaccharides (LPS)[9].

The formation of the apical barrier is essential for subsequent filling so that there is no leakage of the filling material during condensation, and it has been shown that the high pH of calcium hydroxide can induce the formation of an apical barrier[10].

As an alternative to the use of calcium hydroxide-based pastes, MTA can be used, it has the ability to form a cement-like barrier when used adjacent to periradicular tissues due to its hydrophilic characteristic, which allows it to take prey in the presence of moisture and its biocompatibility. The MTA must be placed in the root canal in a similar way to calcium hydroxide, forming an apical barrier, and the rest of the canal must be immediately filled with filling material. This possibility reduces the treatment time, constituting an advantage over the use of calcium hydroxide, however, this technique does not desirably induce complete root formation. Currently, apexification has been indicated for cases of severe trauma, such as intrusion or avulsion, and are at risk of root resorption by replacement[11].

Pulpar revitalization

Pulp revitalization has been the main focus of regenerative endodontics, as it allows follow-up on root development. It is a procedure whose biological bases are aimed at replacing diseased, missing or damaged structures, among which we have the structures of the root, dentin and cells present in the dental pulp, thus restoring their normal physiological functions. This biological alternative should be developed to solve the case of immature teeth with pulp necrosis, because, despite the high success rates of apexification, conventional treatments solve only periapical problems, however, the teeth remain susceptible to fractures. The advantages of pulp revitalization are the possibility of root development and the strengthening of dentin walls by the deposition of hard tissue, making the tooth resistant to fractures[12].

For Thibodeau and Trope (2010)[5] the key factor for success in teeth with an open, necrotic and infected apex is in the disinfection of the root canal system, creating an environment that can lead to revascularization. After decontamination of the root canal systems, bleeding induces from the apical region, which will subsequently fill the canal with blood clot and undifferentiated cells that will induce the formation of new tissue. The tooth in question is sealed with MTA in the cervical portion of the root canal and with restorative materials such as glass ionomer cement or composite resin in the coronary portion. The revitalization process has demonstrated over the

years a considerable increase in the thickness of the dentinal walls, in the apical closure and in the root length, based on radiographic control. Porém, apesar das evidências clínicas favoráveis em curto prazo, a técnica ainda apresenta algumas limitações, como a dificuldade para se causar o sangramento apical e a formação do coágulo sanguíneo. Além de não se ter certeza sobre a natureza do tecido formado no interior do canal e dos resultados em longo prazo[1].

Triantibiotic paste

One of the objectives of endodontic treatment in teeth with pulp necrosis and periapical lesions is to promote the elimination of the infection found in the root canal system. This disinfection is achieved through instrumentation, irrigation and intracanal medication. The mixture of three antibiotics - minocycline, metronidazole and ciprofloxacin - has been shown to be effective in eliminating endodontic pathogens, both in vitro and in vivo. The authors maintain that vital pulp tissues have demonstrated good tolerance to this mixture.

The metronidazole has a wide bactericidal spectrum against mandatory oral anaerobes, but in isolation, even in high concentrations, it is not able to kill all the bacteria present in the infected dentin. The authors suggest that other drugs should be combined for sterilization of these channels to occur. The mixture of the three antibiotics already mentioned can achieve this goal, as it can penetrate the deeper layers of dentin. However, this union can present some disadvantages, such as the development of bacterial resistance, allergic reactions, and one of its components (minocycline) can produce discoloration of the crown. In an attempt to reduce these effects, some articles have suggested a decrease in the application time of the paste to prevent discoloration associated with its use considering that its antimicrobial action can take place within 24 to 48 hours[13].

In addition, in order for microorganisms to be completely eliminated, a minimum concentration of 25g / ml is required, however, antibiotics alone do not have a sufficient spectrum of action against the various types of bacteria present in root canal systems. Therefore, it is necessary to combine different antibiotics for a greater spectrum of action. The increase in the spectrum of the triple antibiotic paste is due to the fact that each component acts on different types of microorganisms: Minocycline (Gram + and Gram); Ciprofloxacin (Gram + and Gram -) and Metronidazole (Anaerobic and protozoan bacteria)[14].

The acidic pH of minocycline and ciprofloxacin may not be favorable for stem cells since they have cytotoxicity. Metronidazole among the three antibiotics is the only one that has a neutral pH, causing no damage to stem cells. Metronidazole and ciprofloxacin induce the formation of fibroblasts. Another point in favor of the use of triple antibiotic paste is that it is more effective when compared to medication with calcium hydroxide because it achieves an almost total elimination of microorganisms in the root canal[9].

The tri-antibiotic paste or also known as Hoshino's paste, has been shown to be effective in several cases. Trope (2010)[3] describes its composition as a mixture of 200 mg ciprofloxacin, 500 mg metronidazole and 100 mg minocycline. The infection of the canal system is considered a polymicrobial infection, and that these bacteria can be in the entire canal system and even in the cement of the periapex. Due to all these details, the infection becomes too complex for only one medication to be effective in sterilization of the channels. Thus, a combination of drugs is needed to combat the diversity of microorganisms found.

Induction of artificial match/matrix

According to Trope (2010)[3] the necrotic pulp itself, although not infected (cases of avulsion), can be used as a matrix for the development of new tissue similar to the pulp. In the case of teeth with necrotic and infected pulp, the author finds that the induction of a blood clot inside the canal can minimize the problem, as it serves as a matrix for stem cells and growth factors. And there is still the possibility that, in future studies, synthetic matrices will be developed to facilitate this process. According to Saber (2009)[15] a matrix must be porous, allow the transport of nutrients and oxygen, be biodegradable, be replaced by a regeneration tissue, be biocompatible and have adequate physical and mechanical resistance. They can be natural, like collagen, or artificial, which are synthetic polymers. The authors report that pulp cells can be isolated, multiplied in culture and sown in a matrix, forming a new tissue similar to the original pulp tissue.

Stem cells

Stem cells are considered to be the most valuable cells in regenerative medicine. The studies have provided advanced knowledge about how the organism develops from a cell, and how healthy cells can replace damaged ones in adult organisms. Stem cells have the ability to divide

continuously to replicate them (self-replication), or to produce specialized cells that can differentiate into many others. Stem cells are classified into two groups: multipotent and pluripotent. Multipotent cells are cells capable of specializing in any cell that originates from the same embryonic tissue, and pluripotent cells are cells capable of specializing in any other cell regardless of the tissue of origin. These are cells found in the embryonic period and may be of mesenchymal or ectomesenchymal origin. Its main objective is to replace, repair and improve damaged organs and tissues[16].

Dental pulp stem cells have a high proliferative capacity, self-renewal property and differentiation potential in multiple strains. Several studies of this type of cell have shown that they are multipotent and proliferate extensively, can be safely cryopreserved, are applicable with different matrices, have a long life, have immunosuppressive properties, and are capable of forming mineralized tissue similar to dentin. Regarding cell differentiation, this refers to the progressive specialization of cell morphology and function that leads to the formation of specialized cells, tissues and organs, following the differential expressions of specific genes. It usually causes variations in cell volume, the appearance of new surface markers, changes in enzyme activity, and even changes in protein cell composition[14].

The success of regenerative endodontic procedures in the face of pulp necrosis depends on the ability of the stem cells in that area to survive. At the level of the periapical region and with interest for regenerative procedures, there are five types of stem cells: stem cells from the apical papilla (CEPA); dental pulp stem cells (CEPD); stem cells of the periodontal ligament (CELP); bone marrow stem cells (CEMO) and progenitor cells of periapical inflammation (CPIP)[9].

How revitalization happens

The teeth with incomplete rhizogenesis have multipotent cells in their periapical space, with great capacity for differentiation, being able to form new odontoblasts, cementoblasts and fibroblasts. According to studies in the literature, a possible hypothesis would be the migration of cells that remained vital in the apical region to the newly formed framework and then differentiate into odontoblasts under the organization influenced by the cells of the hertwig epithelial sheath. The new odontoblasts help to elongate the root due to the deposition of peritubular dentin at the apical end, causing the root to be strengthened and strengthened.

Another hypothesis of root development can be explained due to the possibility that after inducing bleeding in the periapical region, stem cells from the bone marrow or apical papilla enter the root canal, and they have a high capacity for proliferation. A third possibility would be in the proliferation and growth of stem cells from the periodontal ligament at the apex and within the root canal, thus having deposition of hard tissue both at the apical end and at the root walls. The presence of cement and sharpey fibers in the formed fabric evidence this hypothesis. Another hypothesis may be related to the growth factors present in the blood clot, thus presenting an important role in regeneration. Finally, another mechanism for the continuation of the root can be explained due to the abundance of multipotent stem cells in the pulp of immature teeth, and they can adhere to the canal walls, transforming into odontoblasts that will deposit dentin, increasing the thickness of dentinal walls and ending root development[17].

The current scenario of regenerative therapy with the use of scaffolds

The field of regenerative endodontics for the treatment of necrotic teeth with immature apex relies on the development of pulp tissue from the combination of three key elements: stem cells, bioactive molecules/growth factors and scaffolds. Although revitalization is already clinically employed with the use of calcium hydroxide and tri-antibiotic paste medications, there is currently another device used in endodontics, but which is still in the universe of in vitro and animal research, which is the introduction of the scaffolds inside the root canals. Scaffolds are factors used in regeneration processes, which serve as a “framework” or “skeleton” for stem cells. Studies for the inclusion of cell growth factors, as well as for the creation of an appropriate structure for these matrices have been carried out in order to be successful. This material serves as a support for cells and vessels to grow and develop[18].

The structure of scaffolds can vary between nanofibers and macropores, which can be manufactured by different routes, such as electrospinning, self-assembly and phase-separation. There is a variety of scaffolds synthesized based on both synthetic and natural polymers, which are developed to assist the proliferation and differentiation of dental pulp stem cells, for future regeneration of the dentin-pulp complex. The scaffold works as a transient extracellular matrix composed of a porous three-dimensional structure responsible for providing mechanical and regular support to cellular functions[6].

A scaffold must contain growth factors to assist in the proliferation and differentiation of stem cells, leading to better and faster tissue development, it can also contain nutrients to promote cell survival and growth, or even antibiotics to prevent any bacterial growth in the root canals. To achieve the goal of pulp tissue reconstruction, scaffolds must meet some specific requirements. Biodegradability is essential, as scaffolds need to be absorbed by the surrounding tissues, without the need for surgical removal. High porosity and adequate pore size are essential to facilitate cell fixation and subsequent diffusion throughout the structure of both cells and nutrients. The rate of degradation that occurs has to match, as much as possible, the rate of tissue formation, which means that at the same time that the cells are making their own structural matrices around themselves, the scaffold has to be able to ensure its structural integrity within the body, so that it is subsequently dismembered, leaving the newly formed tissue that will assume the mechanical load[19].

These scaffolds have been used successfully for tissue engineering applications, as they are degradable fibrous structures with the ability to support the growth of several different types of stem cells. The main disadvantages are related to the difficulty in obtaining high porosity and regular pore size. It is for this reason that scaffold engineering is carried out at the nanostructural level, to improve cellular interactions. Although the initial results are promising in terms of promoting cell functions and survival, some immune reactions to these types of materials may threaten their future use in regenerative medicine[20].

DISCUSSION

In my review, I agree with the studies by Soares et al. (2013)[11]when they state that currently great attention has been given to regenerative endodontics in the context of pulp revitalization, since despite being consecrated in dentistry, apexification tends to generate thin and fragile roots, which can lead to a fracture. Revascularization involves the invagination of undifferentiated cells from the periodontium to the apical region of immature teeth, restoration of the odontoblastic layer, replacement of damaged structures, including dentin and root structures, as well as cells of the dentin-pulp complex. It is indicated for immature teeth with pulp necrosis due to the presence of deep caries or trauma that result in paralysis of root development treatment aims to restore the compromised root canal system complex, through decontamination, followed by the induction of

bleeding in the periapical region, in which it will fill the root canal with the blood clot for the development of new vascular tissue. This improves the prognosis of the teeth by restoring a functional pulp tissue that will promote continuous root development and immunological competence[20].

In agreement with Albuquerque et al (2014)[1] state that during preservation, a thorough clinical and radiographic evaluation of the tooth should be performed, where the success of pulp revascularization therapy is evidenced based on requirements such as absence pain or edema and positive response to the pulp sensitivity test. It also states that the radiographic examination must demonstrate an increase in the length of the root, an increase in the thickness of the dentinal walls, a decrease in the apical diameter and a change in periapical bone density.

According to Garcia-Godoy and Murray (2012)[8] pulp regeneration treatment should only be performed if some requirements are met: (1) the tooth must be necrotic and not suitable for treatments such as apexification, partial pulpotomy or treatments that aim at filling the root canals; (2) the tooth must be permanent and appear immature with an open apex and pulp exposure; (3) the dentinal walls must be thin; (4) the patient must be between 7 and 16 years old; (5) an anesthetic without vasoconstrictor should be used in an attempt to prevent ischemia, that is, stimulating bleeding into the root canal; (6) an intracanal medication must be positioned above the blood clot.

According to Vijayaraghavanet al. (2012)[21] the success of revitalization or revascularization depends on the disinfection of the root canal, the presence of the blood clot and an airtight filling of the coronary region. The revascularization of an immature necrotic tooth was once considered impossible, due to the extreme difficulty in disinfecting the root canals. Albuquerque et al (2014)[1]agree that when the environment becomes free of bacterial infection and the cell growth medium is favorable, pulp regeneration may occur. However Nagy et al (2014)[22] states that the eradication of bacteria inside the root canal is mandatory in successful self-regenerating endodontic procedures. With regard to the triantibiotic paste, Albuquerque et al (2014)[1]states that it has an action on bacteria in the root canal systems, inhibiting bacterial growth and allowing root growth. In addition, it must be used for a balance between less stem cell cytotoxicity and maximum bacterial disinfection.

Li et al (2005)[23]believe that in the future scaffolds can be used as an alternative to the use of medications, being used in order to provide support for cells and vessels to grow and develop their functions, in addition to promoting the diffusion of medication, promoting disinfection of the root canal system. Most regenerative therapies use the host's own pulp or vascular cells for regeneration, but other types of therapies from dental stem cells are under development. Currently, it is certain that the pulp space returns to a vital state after revascularization, but based on research on avulsed teeth, it has been proven that the tissue in the regenerated pulp space is more similar to the tissue of the periodontal ligament than to the pulp tissue itself[3].

I agree in my review, with the statements by Hargreaves et al (2014)[24] when they say in their studies that revascularization is a relatively new subject in endodontic therapy, that there is a variety of divergent treatment protocols in the literature due to low scientific evidence, and that regardless of the type of therapeutic approach, the goal is to preserve any living pulp cells that still exist at the apex.

CONCLUSION

After reviewing the literature, it is concluded that: 1- Regenerative endodontics is one of the most promising areas in the field of dentistry, with rapid advances and intense experiments; 2- That pulp revitalization is in fact a valid treatment for teeth with incomplete risogenesis and pulp necrosis, presenting clear advantages when compared to the apexification technique, however, according to research carried out, there is still no protocol established and considered ideal; 3- That the period of preservation after the procedure is very important and must be carefully monitored to confirm or not the success of the procedure; 4- although the regenerative techniques are currently only directed at immature permanent teeth, it is possible that the application of the obtained knowledge can also be directed to mature permanent teeth; 5- Because revitalization is a recent treatment, there are still many questions to be clarified through further studies, such as the nature of the tissue that fills the root canal, the possible development of resistant bacterial strains, possible discoloration of the crown by use of minocycline, the possibility of treatment in a single session and knowledge of the post-revascularization effects.

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