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POSSIBILITIES OF THERAPY IN THE DENTAL AREA WITHOUT THE USE OF DENTAL PROSTHESES - REVIEW

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Keywords: regenerative medicine, stem cells, regenerative therapy, dental stem cells, maxillofacial defects.
Abstract: Dental regeneration therapy opens up access to the provision of biocompatible and living functional tissues, in contrast to current therapies based on prostheses and implants. The identification of dental stem cells has supported research and the effectiveness of therapies for dental defects. For maxillofacial defects after various tooth extractions, traumas and other conditions caused by periodontal diseases, categorized grafts, such as autografts, allografts and xenografts, are also used to regenerate lost bone.

1 Introduction

Regenerative medicine (RM) is considered a rapidly evolving field of research that encompasses industries such as stem cells, tissue engineering and cell transplantation. Its goal is to replace damaged or lost tissue by acquiring stem cells and to provide new disease therapies. The main component of RM is tissue engineering, which uses knowledge from cell transplantation, materials science and biomedical engineering to develop biological replacements. The role of these replacements is to restore and maintain the normal function of damaged tissues and organs. RM uses several approaches to regenerate disrupted tissues, using injectable cell therapy to regenerate and restore tissue structure, or the use of biocompatible materials to create tissues and organs [1].

In the case of survival of serious trauma or illness, transplantation is in most cases considered necessary to save the patient. As efforts are currently being made to supplement classical transplantation with tissue and organ regeneration, research and application of RM is gaining prominence [2]. The regeneration process is based on the potentials of human stem cells, which arise from different cell types and are capable of self-renewal under appropriate conditions. Regeneration involves the replacement and reconstruction of missing or lost tissue with new ones that will take care of and ensure its proper biological functioning [3].

2 Stem cells

Stem cell (SC) research makes it possible to understand and explain the complex processes that take place in cells, as well as the development of organs themselves. Stem cells are undifferentiated cells that are capable of self-renewal and can become different types of specialized cells. Due to their properties, they are used in tissue regeneration, drug development, toxicology prediction and cell therapy. These cells are present in human body from the embryonic to the adult stage of life [4].

They represent the basis of organs, tissues, the whole organism and among their role during life is to repair and regenerate damaged tissues after an injury or illness. If SCs are genetically modified, they can provide faster spread and anchorage in the body [5].

The properties and behavior of these cells are largely influenced by the environment in which they are located, the so-called ”niche”. It is a specific microenvironment that is affected by a combination of growth factors, extracellular matrix and mechanical and chemical stress. It helps maintain and regulate the balance between cell differentiation and self-renewal. The process of differentiation is the gradual specialization of cells based on their structure, function and interrelationships during the development of the organism. Great efforts are currently being made to determine how the individual components of this environment affect stem cell function.

Innervation can play a major role in the functioning of these cells by affecting the regulatory sequence of events. Studies have shown that parasympathetic nerves regulate progenitor cells and are essential for salivary gland regeneration and development. The SC study itself will determine the direction in which regenerative medicine will go.

In dentistry, one of the most important experiments is the cultivation of a functional tooth with the help of stem cells. This process would be considered useful especially in congenital conditions where the tooth is completely missing or has not developed in part.

It will be able to be used as a regenerative therapy especially when technology and research progress to a state where the tooth can grow within months, not years. Stem cells should be useful and useful in the regeneration of dental pulp, periodontal ligament and bone [6].

Some research has shown that soft tissue regeneration and craniofacial muscle reconstruction are more likely to succeed in the future than tooth regeneration. These cells can be classified into several main groups, according to their differentiation potential and origin (Table 1).

Table 1 Classification of stem cells according to potential and the possibility of their differentiation

Cell type	Differentation
Totipotent	Create any other cell type (zygote during fertilization)
Pluripotent	Source of almost every cell type (derived from three germ layers)
Multipotent	They form several cells in the body
Oligopotent	Produce more cell types (lymphoid and myeloid)
Unipotent	Create one cell type (muscle cells)

2.1 Dental stem cells

Dental stem cells (DSCs) are most similar to mesenchymal stem cells (MSCs) in their nature, i.e. in their ability to self-renew and differentiate. Dental pulp stem cells (DPSCs) were first isolated, and other cells were later discovered. These include human exfoliated tooth stem cells (SHED), periodontal ligament stem cells (PDLSC), apical papilla stem cells (SCAP) and dental follicle stem cells (DFSC) (Figure 1) [7].

DSCs can be isolated from the dental pulp of deciduous and permanent teeth, as well as from the periodontal ligament and apical region of the teeth, as well as various other structures of the healthy tooth.

According to their origin, they originate from mesenchymal cells and neural crest cells. The odontoblasts that line the inner surface of the tooth come from MSCs and are involved in the production of dentin. Enamel-forming ameloblasts are responsible for the formation of dental tissue. These ameloblasts belong to the epithelial cells of the dental lamina [8].

The SHED and DPSC studies found a significantly higher rate of proliferation and gene expression in SHED. Given the demonstrated capabilities, this demonstrates that SHED could be an option for therapeutic applications [9].

An important factor is also the study of innervation in tooth regeneration and the impact on tooth stem cell populations. Another aspect of regeneration is to ensure the vascularization of the teeth, because the dental pulp is richly vascularized and the teeth are dependent on the supply of nutrients and oxygen to the blood. While blood vessels are found in the pulp of the tooth germs in the early stages of development, innervation is formed only in the late stages of odontogenesis. Innervation and vascularization are the basis of the physiology and pathology of the teeth, identification of the mechanisms that regulate these processes will be essential in the partial regeneration of the marrow, or complete regeneration of the teeth.

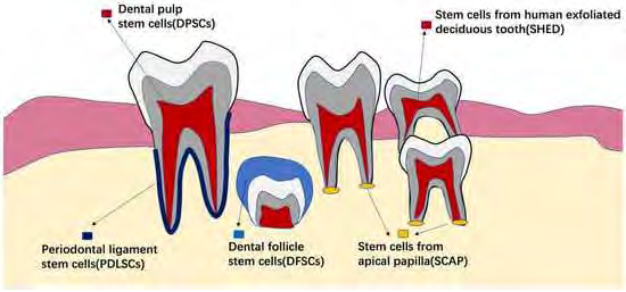

Figure 1 Schematic picture of dental stem cells [10]

3 Possibilities of therapy without the use of dental surgeries

Current dental prosthesis procedures are based on implants and dental replacements, which, however, fail to form a natural root structure, which in turn leads to the loss of supporting bone due to inflammation of the gums and bones around the dental implants. New strategies and therapies for dental regeneration *in vitro* using stem cells, biomaterials, 3D culture conditions that can faithfully mimic the environment in which dental stem cells are located are being explored [11].

3.1 Tooth regeneration

Regeneration of the whole tooth, which is based on similarities to the natural development of teeth, is a complex set of complex processes. It requires precise interactions and repeated molecular signaling between dental epithelium and dental mesenchyme (DM). This mesenchyme originates from neural crest cells, condensing around the dental epithelium to form a dental structure called the dental bud (root) [12].

These dental buds are highly specialized dental organs that consist of dental epithelial and mesenchymal cells that develop into adult teeth. Several approaches to tooth regeneration have been studied and tested, focusing on individual tooth components such as dentin, dental pulp, enamel, cement and periodontium. The combination of

these studies shows promising results, but does not guarantee successful regeneration of a viable tooth [11].

Research has shown that early stages of embryonic dental epithelium can induce tooth development. In the later stage of tooth development, odontogenic capacity is transferred to the dental mesenchyme, and this induces tooth formation when combined with the epithelium. The ability to regenerate healthy teeth in humans is partially lost because dental epithelium is no longer present in these teeth. Therefore, two approaches have been proposed to restore human teeth. One of these approaches is the formation of tooth root *in vitro* (Fig. 4) and its subsequent transplantation to the defect site. The second possibility is the formation of a bioengineered tooth root at the defect site *in vivo* [12].

A mouse model of embryonic dental buds was studied for tooth regeneration. The layers of mouse dental epithelial (DE) and dental mesenchymal (DM) cells were joined to form an artificial tooth bud. Tooth formation was observed in combination with DE with DM cells, embryonic stem cells from the spinal cord and bone marrow.

In 2007, Nakao et al. has seen the successful regeneration of fully functional teeth using artificial embryonic cell embryos. In the following years, this method proved to be functional and is an advanced model for the functional regeneration of teeth from embryonic tissue. Single-cell DE and DM were harvested from embryonic embryos of mouse teeth and cultured *in vitro* to develop into an early stage. They were then transplanted into the mouse jaw. This study reports full-size tooth formation [12,13].

The scheme below (Figure 2) shows cells taken from embryonic enamel and pulp, the tissues are subsequently recombined in a collagen gel drop and cultured *in vitro*. The restored dental embryo is then transplanted and grown in the jaw bone of the host adult animal.

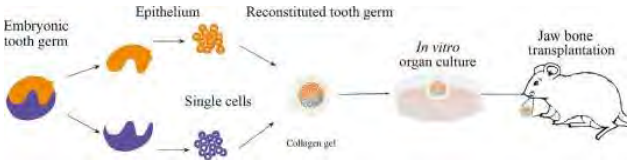

Figure 2 Method of whole tooth regeneration by in vitro culture and subsequent transplantation to the defect site [12]

Another possible therapy for tooth regeneration is the possibility of using induced pluripotent stem cells (iPSC). The researchers differentiated mouse iPSCs into cells similar to the neural crest and odontogenic mesenchmal cells. They further reprogrammed the patient's somatic cells to a mesenchymal line derived from the ectodermal epithelium and neural crest. This recombination of the cells and the subsequent transplantation allowed the formation of a dental embryo and a functional adult tooth. Many factors need to be taken into account in the complete regeneration of a functional tooth, but the use of iPSC seems to be a promising path. These cells have optimal proliferation and higher potential in autologous transplantation than other cell types. When combined with biomaterials and different types of scaffolds, iPSCs can induce and promote tooth development [11].

3.1.1 Usage of scaffolds and biomaterials

Dental tissue engineering relies on a combination of elements such as cell, scaffold and regulatory mechanisms (biological environment) for functional and proper tissue regeneration. Scaffolds and biomaterials are an important part because they support this regeneration. In order to function properly, they must meet certain requirements for the *in vitro* physiological environment necessary for cell growth, expansion, and differentiation. Scaffolds must meet biocompatibility conditions, have porous structures that allow cells to penetrate and must also ensure nutrient diffusion. They should be able to prevent chronic inflammatory diseases and provide regulatory signals to promote healing. Biocompatible and biodegradable materials have been developed and optimized over the years to support the regeneration process. Natural biomaterials, also referred to as nature polymers (collagen, keratin, chitosan, platelet-rich plasma, etc.), have low toxicity, are environmentally friendly and are a cheaper choice than synthetic biomaterials. Their advantage is that they promote cell adhesion, proper cell signaling and degradation without immune rejection. Synthetic materials such as polylactic acid, polyglycolic and polycaprolactone are more elastic and flexible than natural ones, but they are not as favorable for remodeling as natural ones. Composite materials are most preferred. Thanks to the development of these biomaterials for scaffolds, tissue engineering in dentistry has made significant progress [11].

3.2 Gene therapy

Gene therapy currently appears to be a promising therapy option in regenerative medicine and tissue engineering. It presents options such as circumventing the limitation of the short half-life of growth factors *in vivo* and controlling protein distribution. It uses genetically modified cells to deliver specific doses of bioactive protein over a long period of time. Gene therapy involves several steps, such as consideration of tissue growth factor, route of administration, target cells, and local effect. Non-viral plasmids, small circular DNA structures, can replicate in the cell independently of chromosomes. They are considered safer for the host than viral vectors because they are not incorporated into chromosomes. They provide a protein expression transition and improve bone formation with various carriers [14].

In a study by Dunn et al. recombinant adenoviral vectors encoding the bone morphogenetic protein BMP 7 were delivered to the bone defects using a collagen matrix. The results revealed that this combination led to improved alveolar bone defect filling and new bone contact with the implant, and thus *in vivo* BMP-7 gene therapy offers potential for alveolar bone engineering applications [15].

Combination gene therapy using AdBMP7 alone or with an adenovirus with a mineralization protein gene (LMP3) promoted bone formation *in vivo* through progenitor cells. Adeno-associated virus (AAV) has a single-stranded DNA genome and requires the host DNA polymerase to form a complete strand. In periodontitis, AAV inhibited disease progression and prevented alveolar bone loss. Gene therapy is performed by various techniques. MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of a target gene. They affect osteogenesis, or the regulation of genes involved in direct bone formation. MiRNA research in bone development is based on *in vitro* cell culture experiments, but there are *in vivo* studies that demonstrate promising results in bone repair [14].

3.3 Dental autotransplantation

Dental autotransplantation is a surgical procedure consisting in the replacement of missing teeth, especially in minors and young adults, where implants or fixed prosthetics are not a suitable solution for various reasons. This treatment can be defined as the transplantation of a tooth from its original position to a surgically prepared recipient site in the same patient. An autotransplanted tooth, unlike an implant, allows alveolar growth along with adjacent teeth and periodontal ligament. It has the potential to induce new bone, gingiva and periodontal ligament formation at the recipient site. An important factor influencing the success of this transplant is the presence of the periodontium and its support. There must be sufficient bone and adequate width of keratized tissue at the recipient site. The problem of achieving periodontal and marrow healing of the transplanted tooth is proving to be a biological limitation. Transplantation should be performed when they have formed from the root of the donor tooth and this statement limits the age range of the transplant. High survival rates are reported in studies mainly in the autotransplantation of premolars, ie the front chairs. Before planning surgery, the patient must meet certain indicators of how to fall into the younger age category and must have the presence of a suitable donor tooth for transplantation. Missing tooth therapy is performed by implantation and prosthetic treatment, but this option is not suitable for young patients who are still developing the body. Here, autotransplantation, which is also referred to as a biological prosthesis, is a suitable therapy [16,17].

Case studies have been published in a review article by Tsukiboshi and colleagues on the long-term outcomes of dental autotransplantation. In a study in a 17-year-old patient, resorption (hard tissue loss) of the mandibular left second molar was observed (Figure 3A). This molar was replaced by autotransplantation of the adjacent left third immature molar, which was considered the best option in this situation. After 12 years of follow-up, successful healing of the periodontal ligament and pulp was noted, and root development was likely to be arrested.

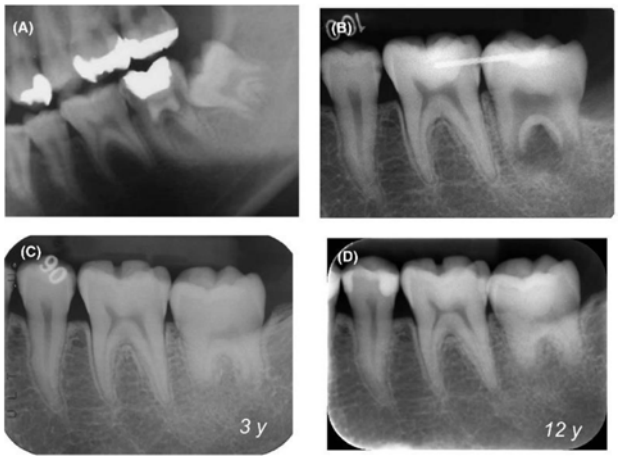

Figure 3 External root resorption (patient 17 years). (A) - view around the site of the procedure; (B) - X-ray after transplantation; (C) - three years later (positive electrical pulp testing, low root development); (D) - twelve years after autotransplantation [55]

In another case of a 39-year-old patient, the left mandibular molar (Figure 4) was replaced by a third mandibular molar due to its unfavorable prognosis. Because the replaced tooth had complete root formation, root canal treatment began two weeks after autotransplantation and was completed after four weeks. The transplanted tooth was followed for 30 years and did not show any associated complications, its normal function was maintained [18].

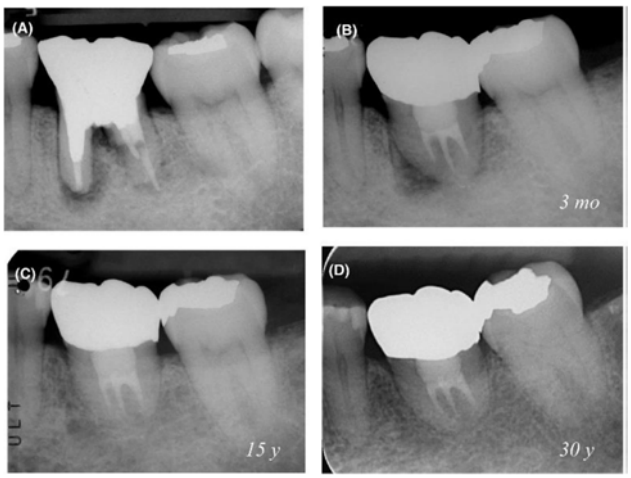

Figure 4 Patient 39 years old. (A) - X-ray before treatment; (B) - 3 months after autotransplantation and crown production; (C) - 15 years after follow-up; (D) - 30 years after surgery [18]

3.4 Gene therapy

The very term regenerative endodontics was adopted in 2007 by the American Endodontist Association based on an understanding of the concept of tissue engineering. Endodontic regeneration applies stem cells, scaffolds, tissue engineering, bioactive growth factors at the canal site to regenerate pulp tissue that has been damaged by trauma, infections, or congenital anomalies. Permanent

immature teeth with necrotic pulp are usually treated by apexifixation, where calcium hydroxide is used to induce the formation of an apical hard tissue barrier before filling the root canal. However, this procedure does not have the potential to repair damaged tissue at the canal site and promote thickening and maturation of the root canal walls. That is why a new therapy and treatment option has been introduced under the term revascularization to manage an immature permanent tooth with apical periodontitis [19].

The advantage of regenerative endodontics is the revitalization of the tooth, but also the subsequent development of the roots and increased resistance to fracture. It consists of three steps: disinfection of the root canals, induction of bleeding to form a scaffold, ie the carrier for the cell stem, and coronal sealing of the blood clot with biocompatible material [20].

In experimental studies by Nygaard-Ostby (1961) and Nygarrd-Ostby & Hjortdal (1971), they attempted to induce bleeding from periapical tissues into the canal space of the teeth, which was partially filled with root filling. Histological examination of the extracted teeth revealed that connective tissue and cellular cement subsequently formed in the apical canal, which contained the vital marrow. However, no regenerated tissue was formed in the apical canal space in the necrotic marrow teeth. Iwaya et al. consulted the first group to apply revascularization treatment to immature permanent teeth with apical periodontitis. The concept was based on experiments in the revascularization of reimplanted and autotransplanted dog teeth, as well as on the disinfection of root canals. In 2004, Banchs & Trope added the antibiotic minocycline to the antibiotic used by Iwaya, and the treatment became known as triple antibiotic paste. This procedure led to the elimination of clinical symptoms of apical periodontitis, as well as to the promotion of thickening of the canal walls. Subsequently, this regenerative therapy was recommended as a suitable alternative to the traditional apexification for immature permanent teeth with necrotic marrow [19].

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Murray et al. defined regenerative endodontics as "biologically based procedures to replace damaged dental structures, including dentin and root structures, as well as pulpentin complex cells" [21].

3.4.1 Revascularization and regeneration

Revascularization has been mentioned in studies in the healing of pulpal wounds after replantation of immature permanent teeth. As mentioned above, the term was first used by Iwaya and colleagues. Treatments included sodium chloride lavage along with an intracanal antibiotic paste. The result was the elimination of clinical symptoms and remission of apical periodontitis, thickening of the canal walls and development of roots. Induction of periapical hemorrhage into the canal space is considered a necessary step in regenerative endodontic procedures, and it has been suggested that blood clots in the canal space could serve as a scaffold to promote healing of soft pulp tissue. In a study carried by Lovelace et al., they showed that provoked periapical hemorrhage brought mesenchymal stem cells into the canal space. Blood contains many growth factors that are derived from platelets, so periapical hemorrhage brings the fibrin skeleton, bioactive growth factors, and mesenchymal stem cells to the canal site. Growth factors embedded in the dentin matrix after demineralization of the dentin by rinsing ethylenediaminetetraacetic acid in endodontic procedures are also released [20,22].

Mesenchymal stem cells in the apical papilla of immature permanent teeth with necrotic pulp, which are introduced into the canal site during endodontic procedures, could be able to differentiate into odontoblasts and dentin production. Hertwig's epithelial root canal is able to signal these cells in the dental follicle to differentiate into cementoblasts and thus regulate root development. Histological studies of teeth with necrotic pulp and apical periodontitis after regenerative therapy revealed that the tissues formed were similar to cement, bone, or periodontal tissue. In many animals, it was not a matter of requiring marrow tissue. Wall thickening or root maturation was caused by the deposition of cement-like tissue or bone on the canal walls. In another study, the formation of nerve fibers in newly formed tissue in the duct space of a revascularized tooth was demonstrated [22].

Clinical examinations in a specific study by Nosrat et al. were targeted at two patients aged 9 years (female) and 10 years (male) and showed asymptomatic results after regenerative endodontics. X-rays after four months showed root maturation in both patients. The roots showed a well-developed layer of dentin surrounded by periodontal ligament at the outer edges of the root. The MTA material was recorded in the coronal parts of the tooth and a layer of hard tissue was observed beneath it. No inflammatory cells were found in the canal spaces. In a 10-year-old patient (Figur 5), parts of the roots were surrounded by dentin at the outer edges of the root and the primary dentin layer in the canal spaces was preserved. A much larger layer of dentin was observed in this tooth than in another tooth. The walls of the root canal were covered with a layer of repair cement mixed with osteoid tissue.

The patients developed roots (Figure 6), which is considered a sign of success after regenerative endodontic treatment. However, histological reports indicate that the tissue formed in the root canal is not true marrow tissue. It resembles periodontal tissue and the hard tissues on the walls of the dentin are similar to cement or bone tissue [23].

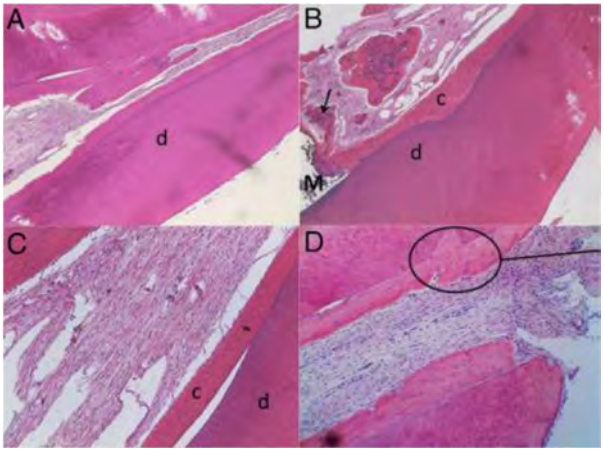

Figure 5 Histological images of a 10-year-old patient. (A) the presence of primary dentin (d) surrounding the canal space. (B) A layer of mineralized tissue is formed under the MTA (M). (C) the presence of repair cement (c) on the walls of the dentin. (D) cement ingrowth on dentin walls [60]

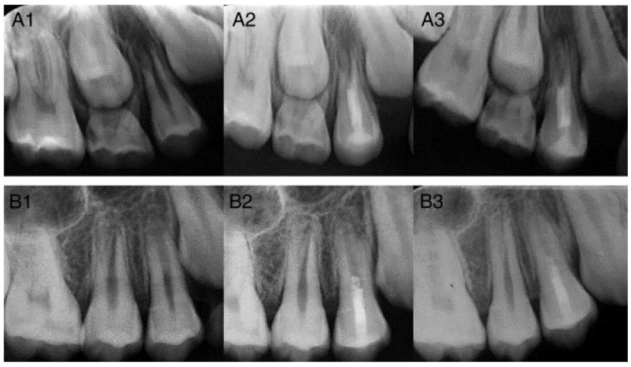

Figure 6 Root development in both patients [23]

Cohort et al. investigated 20 cases of regenerative endodontic treatment with necrotic teeth, where 100% success rate or survival rate of treated teeth in terms of remission and disappearance of apical periodontitis or tooth retention was demonstrated. Coagulation of the canal walls or root development in these necrotic pulp teeth is unpredictable. Thus, it has been shown that this therapy could be a suitable alternative to non-surgical root canal therapy in terms of elimination of clinical symptoms and disappearance of periodontitis. Regenerative endodontic therapy is not suitable for all teeth with necrotic pulp. If the primary goal is to achieve the disappearance of periodontitis, then this therapy can be used, but it is not a suitable alternative for teeth that require a coronary restoration pin [22].

3.5 Regenerative periodontal therapy

Improving the survival of teeth and reducing the progression of periodontitis are provided by reconstructive periodontal interventions, which also provide stability of the result in the long term. Natural teeth provide better long-term survival and marginal bone changes compared to dental implants. Periodontal reconstruction is a complex biological process that involves the formation of lost dental structures, including alveolar bone, cement, and periodontal ligament on a previously diseased root surface. These reconstructive procedures have advantages over conventional surgical procedures in terms of long-term stability, improved tooth survival, less progression of periodontitis, and less need for long-term interventions. Periodontal regeneration can be achieved by applying barrier membranes, grafts, wound healing modifiers and their combination [24].

3.5.1 Guided tissue regeneration and enamel matrix derivative

Guided tissue regeneration (GTR) mechanically isolates the defect and is based on the application of a separation barrier membrane. This approach based on biological and mechanical concepts has been shown to be successful in both clinical and preclinical studies, but several shortcomings have been reported. These are complications caused by membrane exposure and recession of adjacent teeth, shortcomings in the treatment of several proximal defects and incomplete adaptation of the tooth by the membrane around asymmetric roots. Enamel matrix derivates (EMD) are the most evaluated in clinical and preclinical models. It is a biologically active compound which, when applied to the exposed root surface, triggers a process of biological processes. This process increases the migration of mesenchymal cells and their attachment to the root surface, as well as their differentiation into cementoblasts, osteoblasts and fibroblasts.

EMDs increase gene expression, which stimulates protein and mineralized tissue synthesis in PDL cells. The process itself later leads to the reconstitution and regeneration of the periodontal apparatus. The use of EMD during therapy improves bone formation [24].

No significant differences were found when comparing EMD to GTR, but GTR is associated with a greater recession and postoperative complications than swelling. EMD is a good treatment alternative for multiple proximal defects without reducing blood valve nutrition and this leads to extensive membrane exposure. While GTR is known to be controversial in bone loss defects, EMD improves outcomes in these defects. EMD also improves oral wound healing by promoting blood vessel and collagen fiber formation in connective tissue, increasing gingival fibroblast proliferation, and influencing inflammatory and healing responses by various cellular mechanisms with a positive effect [24,25].

Since the separate use of EMD as a therapy can be used mainly for narrow defects, combination therapy is also used. It focuses on various alternatives to periodontal reconstruction and includes regenerative principles such as conductivity and inductance, matrix development and cell differentiation, as well as wound space and stability. The combination of biomaterial grafts, biological agents together with EMD reduces postoperative recession.

Combination therapy includes the application of autogenous connective tissue grafts using EMD.

Histological reports in humans have shown the formation of new cement, bone and adhesion. EMD has a positive effect on inflammatory reactions and enhances the effect on fibroblasts, thereby increasing proliferation. The study by Nemcovsky and Beitlitum includes a series of cases where a modified approach to the treatment of lower periodontal incisors was performed. In all cases, a combination therapy consisting of a single access valve, a conditioned root, an EMD application to the root surfaces, and an autogenous connective tissue graft was used for treatment. The results were satisfactory as there was an increase in the width and keratinization of the gums (Fig. 9), without pulling the bridle and muscles along with the radiographic bone filling of the defects. The results showed that the combination therapy for reconstructive peridontal treatment was able to successfully treat severely affected periodontal lower incisors with gingival recession [24].

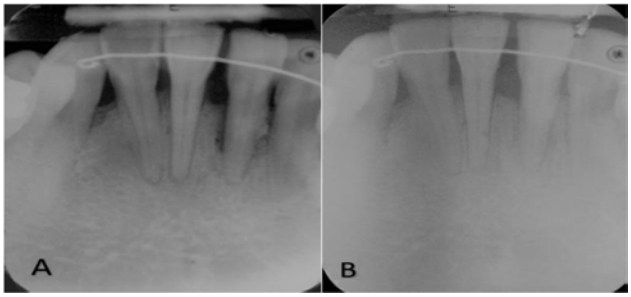

Figure 7 A - before combination therapy (gum recession, bone loss); B - three years after therapy stable results [24]

3.6 Spheroids and organoids

Organoids and spheroids are considered to be new treatments and therapies in dentistry. It is the development and study of stem cells from *in vitro* three-dimensional culture in order to mimic the physiological properties and tissue architecture of embryonic cells. In 2011, Berahim and colleagues grown spheroids from human periodontal ligaments and subsequently transplanted them into collagen- and polyglycolic acid-enriched membranes [26].

They have demonstrated the ability to grow, migrate and proliferate these cells. In 2017, she and the team created a bioengineering tooth (Figure 8) on a dog model that was physiologically similar to a normal human tooth. Through these studies, the authors have shown that the combination of epithelial tissue with mesenchymal tissue, or mesenchymal and epithelial cells provides better tooth formation. By culturing these tissues and cells, they formed a germ organoid, which they transplanted into the dog's jaw, which then developed into a bioengineered tooth with dentin, enamel, and marrow tissue several weeks later [27].

In another study by Jeong et al. developed organoids by culturing DPSCs with matrigel, which were similar to dentin pulp. These characteristics have also revealed the differentiation of odontoblast-like cells, which represents a promising prospect for the use of these structures in dentistry [27].

Figure 8 Autologous transplantation of a dental germ into the lower jaw of a dog [64]

Other representatives who received a bioengineering tooth were Wang and the team. They combined isolated epithelial and mesenchymal cells to form a dental organoid, and after transplantation into the mouse jaw bone, the organoid later developed into a full-size tooth [28].

Dental organoids and spheroids represent a promising future and an opportunity for advancement in dental procedures. These 3D cultures make available a system for modeling human organogenesis, disease modeling and regenerative medicine. They make complex interactions between cells, the flow of molecules and nutrients available for self-organization to help scientists understand the physiology of the tooth and dental tissues. 3D cultures support the interaction between cells and the macroenvironment, which play an important role in cell migration, proliferation and differentiation [11].

4 Conclusions

As regenerative medicine is a broad area of research that consists of various disciplines and uses knowledge from materials, cell transplantation and biomedical engineering to develop biological replacements, dental therapy research can also be considered a leader in this field. Whereas in dental medicine they focused mainly on the restoration of dental function with the use of implants and prosthetic replacements, nowadays the focus is on the restoration and regeneration of the entire craniofacial area, including the surrounding structures and tissues. The central focus of RM is stem cells, which are able to regenerate and replace damaged organs, soft and hard tissues. Revealing the real cause of defects and diseases in the dental area helps to treat them. The output of this work was to present at the same time therapies based on the principles of RM, whether using SC, scaffolds, biological mediators, or a combination thereof. Although much research and studies are still needed into the potential benefits of regenerative medicine, its benefits and risks, there are now many clinical studies demonstrating the potential of regenerative therapies based on stem cells, biomaterials, scaffolds and growth factors. In the future, RM represents a wide range of uses in treatments and therapies, both in humans and animals.

Acknowledgement
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