PULP REGENERATION: CURRENT STAGES OF RESEARCH A LITERATURE REVIEW

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ABSTRACT

This research base is a literature review in order to know the aspects of the pulp regeneration, conducted in experimental research "in vitro". The presence of stem cells is necessary to pulp regeneration occurs, which can be found in the pulp third molars, an extracellular matrix and growth inducing substance. To perform this technique, it is still necessary further studies. There is no a protocol for such procedure.The formed tissue also still does not know.

KEYWORDS: tissue engineering, stem cells, dentistry, endodontics
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INTRODUCTION

In 1963, the Canadian scientist called James Edgar Till, in one of his researches, accidentally discovered that transplanted marrow cells on rats' spleen self-replicate. This researcher was the first to isolate and identify stem cells, opening up a way to new discovers.

Over the years, several studies have been developed in order to prove that is possible carry out medical and dental treatments using stem cells because they have the capacity to divide themselves originating two cells similar to the mother. These studies aim to improve current procedures, further create new ones. Recent researches show that the tissue engineering study has demonstrated promising results with stem cells associated to biocompatible matrix and sensitive molecules.

Pulp death occurs in result of pulp necrosis, which is a specific type of necrosis localized in dental pulp, where after a set of morphological and physiological changes cause failure of the vascular and nervous system. Pulp is a non-mineralized connective tissue that fills the central part of tooth. It is richly innervated and vascularized, and its main function is vascularization, nutrition and oxygenation of cells to keep the dentin vitality. When the pulp necrosis occurs, consequently the dentin loses the vitality and sensitivity.

Efficacy of pulp regeneration treatment proposes that tooth vitality can be maintained and recovered. Restore pulp vitality and devolve health to the tooth are until then an unachievable dream. However, studies on tissue engineering show there is similar behavior to the pulp and it is possible manipulate them in a laboratory. These studies show that stem cells are able to improve the tissue recover capacity, making the tooth become healthy and performing its functions with sensitivity: aesthetical, functional, phonetics and masticatory.

In this context, this work intends to review the literature on pulp tissue regeneration presenting studies that search the appropriate match stem cells with pulp origin, able to recover the tooth vitality from one previously without life, in other words, in pulp necrosis.

LITERATURE REVIEW

Definition of tissue regeneration and stem cells with emphasis on experimental pulp regeneration

Tissue engineering is a multidisciplinary field that uses principles of engineering, biology and clinical sciences to develop biological substitutes able to keep, restore or improve the function of tissues and organs. This new Science bases itself on three components: cells, biocompatible matrix and bioactive molecules, which are responsible by morphogenetic signalization. Stem cells are frequently used.[1]

To the tissue regeneration occurs, some factors should be considered, such the presence of stem cells, growth factors and a growth matrix. The creation of this environment favorable to the proliferation and differentiation of stem cells starts with the control of infection on the root canal[2].

Stem cells are classified into two groups: multipotent and pluripotent. Multipotent are cells with capacity to specialize themselves in any cell that was originated in embryonic tissue, and the pluripotent are cells with capacity to specialize themselves in any other cell, regardless its origin. These cells are found in embryonic period and can be from mesenchymal or ectomesenchymal originally[2,3].

Stem cells present low differentiation degree, capacity for multiplication, further the self-reproductioncapacity, generating differentiated or specialized cells. From this principle they believe that the use of stem cells can make possible create an alternative method for endodontic treatment.[4]

Stem cells are defined as undifferentiated cells with high capacity for self-renovation and to produce at least one specialized cell type. There are two categories of stem cells: pluripotent embryonic cells and the lineage of unipotent or multipotent, called adult stem cells, which dwell in different tissues[5].

Stem cells are undifferentiated with high capacity for self-renovation and production of at least one high-specialized cellular type. They are divided into two categories: embryonic and adult. The advantage of an adult stem cell is because it is autogenic, and the embryonic is its capacity for proliferation and differentiation in several cell types[6-8].

Regarding to the several authors perspective, we conclude that adult stem cells, when removed from dental pulp and stimulated by some growth factors and proteins, are able to differentiate themselves in odontoblasts, becoming able to regenerate tissues like dentin and pulp[9].

Adult stem cells are undifferentiated and found in tissues responsible by regeneration during their existence. However, they have as disadvantages they are not pluripotent, it is difficult to obtain, purify and cultivate them in vitro, further their lower quantity in the tissues. These cells have been identified in skin, kidney, adipose tissue and dental pulp, among others; in other words, they are able to self-renovate, differentiation of multiple lineage and high proliferation potential[6,10].

Among the main sources of stem cells in dentistry, dental pulp, periodontal ligament, deciduous teeth, dental follicle and apical papilla are detached. Adult cells identified on dental pulp are multipotent and present capacity to differentiate themselves into fibroblasts, connective tissue components and into odontoblasts involved in dentin formation[6].

Growth factors are proteins secreted extracellular, which rule the morphogenesis during these interactions and comprehend five proteins family: bone morphogenetic protein (BMP); growth factor (GF); Hedgehog proteins (Hh), wingless and int-related proteins (Wnt); and tumor necrosis factor (TNF)[11].

To develop techniques able to perform manipulation of high proliferative degree stem cells, what become bioengineering effective, three factors are necessary: the stem cells found in isolated pulp cells and in deciduous teeth; an extracellular matrix composed by synthetic or natural materials and growth factors. In several studies where the stem cells were isolated, the capacity for self-renovation were observed in them, further differentiation in various cell types[11].

Tissue engineering is the Science field that studies functional and physiological restoration of deteriorated or lost tissue structures because trauma or disease. It is based on three principles of tissue biology: mother stem cells; extracellular matrix (or scaffold) that maintain the tissue structure, substances which induce cellular growth, and differentiation[6,7,12].

Human genome decoding and scientific advances to comprehend the molecular regulation of tooth

morphogenesis, stem cells biology and biotechnology offer opportunities unprecedented to enable teeth regeneration in the future. The results obtained show that adult stem cells present in the pulp of deciduous and permanent teeth, as well as periodontal ligament can originate dental tissues. It has been demonstrated that stem cells are localized in dental germ, both in epithelium and in ectomesenchymal underlying[13].

However, we have to emphasize an organ regeneration is not simple because its development is determined by complex interactions and several growth factors, and the cellular differentiation is still linked to morphological changes during the tooth germ formation[6,10].

Tissue engineering has been proposed in several dentistry areas to promote dental regeneration, specifically in Endodontics, Periodontics and craniofacial surgery.[14]

Cells indicated for experimental evaluation and why they are required

Oral cavity has presented itself as a huge reservoir of multipotent cells, called *mesenchymal stem cells*. These cells are found in different sites and they are classified according to the Table 1. The exactly original site where stem cells are find in regeneration process in not known. They believe they are originally from apical papilla, because it is lacerated during the obtainment of intra root bleeding. How these cells survive inside so hostile environment is unknown, as it is observed in periapical inflammation. A possible explanation is the low density of blood vessels in that region[2].

Growth factors are proteins that link themselves to receptors on cells, and act as signaling to induce cell proliferation or differentiation[2,15].

Factors that appear more frequently on regeneration processes are the transformer factor growth (TFG) and bone morphogenprotein (BMP). Recent studies have demonstrated that dentin acts like a reservoir for these factors[2,16].

To the regeneration of new tissues occur inside the root canal, a growth matrix to provide favorable environment for cells organization, proliferation, differentiation and regeneration is necessary[2,3].

They identify a population of supposed postnatal stem cells in human third molar pulps, which presented regenerative capacity. After transplantation, a connective tissue was obtained, similar to the complex dentin-pulp, composed by mineralized matrix with tubules and odontoblasts aligned and fiber issue containing blood vessels in a set similar to that found in a normal tooth. Electron microscopy demonstrated that mineralized matrix formed presented a calcospherites globular pattern, similar to the primary dentin and different from that seen on ectopic lamellar bone observed in stem cell transplantation of bone marrow. These cells are also able to respond specific signals from the middle and generate new stem cells or select a particular differentiation program[17].

Generally, studies are concentrated in adult stem cells, whose sources are bone marrow, blood, cornea and retina, liver, skin, gastrointestinal tract, pancreas and dental pulp. Other sources are the periodontal ligament, dental follicle, apical papilla and deciduous teeth pulp[6,8].

Dental pulp stem cells (DPSC) are potentially superior to other types of stem cells from adults. As teeth are easily to access and extracted routinely along life, the capacity to protect DPSC in a young person and store it for future use is extremely important. A personalized stem cell can be created from a DPSC, with no procedures that could cause ethical concerns, as the use of isolated stem cells in a supernumerary tooth. Then, this is a more convenient source of terminal cells because these teeth are easily removed along life, as previously mentioned. In addition, we believe the success in isolation and characterization of DPSC from pulp tissue of a mesiodent or in supernumerary teeth, which are normally discharged after extraction; then, they can represent a valorous source of human DPSC[8].

In this study was possible identify a postnatal stem cells in human third molar pulps that presented regeneration capacity. After transplanted, a connective tissue similar to the pulp-dentin complex was obtained, composed by mineralized matrix with tubules and odontoblasts aligned, and fiber tissue containing blood vessels in a set similar that find in a normal tooth. These findings provide evidences that pulp stem cells transplanted can origin odontoblast lineage, and dwell in the pulp connective tissue as cells similar to fibroblasts[6,17].

Description of pulp regeneration techniques in vitro

Application of pulp regeneration with stem cells for clinic will require the use of injectable scaffolds. The aim was evaluate the behavior of stem cells from exfoliated deciduous teeth (SHED) injected in root canals of human pre molar with open apex using scaffold of human collagen type I and based on self-arranged nanofibers (SA). To determine

the feasibility and differential potential of stem cells in vitro, non-instrumented roots were positioned with apex in culture medium of stem cells applied in self-arranged collagen nanofibers were applied in all the canals. Analytical tests showed high proliferative activity and few apoptotic cells. Injection of tetracycline evidenced neo-formation of dentin. Microvascular density and the number of odontoblasts delineating the dentin were similar in experimental groups and positive control. The association between stem cells and injectable scaffolds was able to origin a pulp tissue. It is capable to produce dentin and constitutes a step ahead to the aim to achieve the pulp regeneration in human patients[18].

The aim of the study was try to isolate pulp cells of healthy deciduous human teeth with different levels of root resorption and evaluate them after expansion in vitro for parameters presented for stem cells. Thereunto, pulp tissue was obtained from 30 children teeth from 6 to 12 years. From the total, 21 teeth were in advanced process of root resorption (group I), 17 presented the root complete resorption; and 4 presented residual root. The 9 teeth remaining did not present visible resorption (group II). The cells were evaluated by flow cytometry according to the specific phenotype and to determine presence or absence of gene expression. In the same passages, the cells were submitted to their growth evaluation in vitro, and they were induced to adipogenic, osteogenic and chondrogenic differentiation. Cells isolation was considered successfully in 25 samples, in which the cells were adhered. It was not possible establish culture to the group II (n=9). Establishment of cultures and proliferation capacity were independent on the amount of pulp tissue remaining collected. Cells on fifth and tenth passages were positives for specific phenotype and for specific gene expression. Stem cells localized in pericytes were described as possible niche of pulp stem cells. All the cultures were able for differentiation in the 3 cell lineages previously mentioned. However, isolated cells were stem ones, because they were positive for characteristic markers of mesenchymal and pluripotent stem cells. We can suggest that the perivascular niche is not the only one to provide pulp stem cells. Obtaining these cells is easy, and can be linked to the root resorption, because pulp tissue of group I present capacity for proliferation in vitro, while in the group II it was not enough to allow establishment of cultures[19].

When isolating dental pulp stem cells from impacted human third molars, we could observe the capacity to form a structure similar to the pulp dentin complex[6,17].

There is four techniques used or in speculation by scientists to confection of bio tooth: a) using biocompatible molds, b) tissue recombination, c) tooth construction “again”, and d) induction to a third dentition[6].

Biocompatible molds technique: The first step is the confection of molds that shape the teeth. These molds are made on biodegradable polymers, like polyglycolate / polylactide (PGA/PLLA) and poly-L-lactic-co-glycolic (PLGA)[6,12,20].

The second step is plating, on these molds, cells from dental germs dissociated enzymatically, which were cultivate by six days. The set molder/ odontogenic cell was placed in the ment of immunocompromised mice in order to provide an appropriate place for the cells grow up. After 20-30 weeks, histological analysis revealed the formation of small dental crowns with evident formation of enamel, dentin and pulp[6,21].

However, this technique presents some problems, like teeth formed did not assume the molder shape, and the molds provide a static growth model[6,20].

Tissue recombination technique: the aim is reproducing within an adult mouth cavity the tooth development, like occurs during the embryogenesis. Thus, by recombination, epithelial cells are used as sources to origin enamel, and mesenchymal cells to form dentin, dental pulp, periodontal ligament and other support tissues[6,22].

Most used epithelial sources comes from the odontogenic epithelium of the dental lamina in embryos of rats and mice, from mesenchymal tissue of the own ectomesenchyme which is subjacent to odontogenic epithelium or other non-odontogenicmesenchymal sources, like those from marrow bone[6,23,24].

Although, this technique present some embarrassment, like find viable cell substitutes, mainly from human, as alternative sources to epithelium and dental germ mesenchyma in initial stages of development; and control the dental shape[6,22].

New tooth construction: different population of dental and non-dental stem cells (e. g.: marrow bone stem cells) have distinct proprieties. The idea is enjoy the better what each cell can offer and form the different tissuesthat compose a tooth[6,20].

Nevertheless, the great technique impasse is to put the different cell sources together in order to interact harmonically among them and reproduce the dental tissue in appropriate shape and function.

Induction to third dentition: presents the aim to discover biomolecular mechanisms able to induce the odontogenesis in adult tissues, and then could apply them on the place where the new tooth formation is necessary[6,20].

When isolated and cultivated pulp cells from 30 deciduous human health teeth in different degrees of root resorption and evaluate them after expansion in vitro for stem cells parameters, 25 samples were considered successful, in which were verified cells adhesion after 24 hours. Only 17 achieved 90% confluence. Establishment of cultures and proliferation capacity were independent on the pulp tissue amount remaining. All the cultures were able for differentiation in the 3 cited lineages. However, isolated cells were stem cells, because they were positive for characteristic markers of mesenchymal and pluripotent stem cells. Easy of obtaining these cells seems to be linked to the root resorption process[19].

Teeth treatment with incomplete root resorption and pulp necrosis represents the great challenge for endodontic therapy. Current panorama is the concept of pulp tissue regeneration. The article describes and discusses the three main protocols, their variables, specifying the shadiest spots of this therapy, which is the newest field of Endodontic, and whose advances will provide inestimable benefits to population.Regeneration success must achieve three main aims: first eliminate symptoms and evince periapical tissue reparation; second promote canal walls thickening and/or continuing root formation (just a wish, not essential); and third, obtaining positive response to vitality tests; if achieved, indicates more organized pulp tissue[2].

Physical chemical properties and biological applicability of materials for Tissue Engineering (TE) usage have great interest and growing importance to develop innovation on biotechnology area.In Dentistry, researches advance every day and show the possibility to apply regeneration therapy of dental pulp in clinical practice in the future. This transition will demand capacity to build a pulp tissue that fill the root canal completely, produces dentin and has enough vascularization to perform tissue metabolically changes. Thereunto, advances still need to occur; standardization of techniques and materials that produce safe results are imperative for therapies based on TE usage in human clinical trials. Then, the authors performed a study to evaluate the influence of scaffold pores size on the proliferation and differentiation of stem cells.To obtain two different size pores, cell proliferation was evaluated after specific periods (3, 7, 14 and 21 days) using the indicated method, and cell proliferation rates were similar in experimental groups. However, after 14 days cultivation, cells cultivated on scaffolds with less porosity presented a proliferation significantly higher ($p<0.05$). After 21 days cultivation on dentin fragments, stem cells were able to sustain proliferation and differentiation[25].

Cell events occurred in dental development stage are determined by genetic information, which can start from a series of interactions between epithelial and mesenchymal cells. Epithelial cells form the enamel, and the cells that surround them are mesenchymal ones.These interactions involve signaling proteins and specific receptors; once they are activated, dental development process starts[6,8].

Different irrigating substances have been proposed on regeneration protocols; among them there are Chlorhexidine 2%21, EDTA22 andSodium hypochlorite on several concentrations 1,25%23, 2,5%24 e 5,25%25. In vitro studies have demonstrated the inefficiency of Chlorhexidine 2% to keep the cell vitality, and it is potentially indispensable in regenerative processes. EDTA has shown itself as an irrigating liquid totally tolerate by those cells, besides to be able to release growth factors present in dentin[2].

Using the cell culture method, the effect of different concentrations of poly-antibiotics pastes and calcium hydroxide on the papilla cells noticed out all antibiotics tested reduced significantly these cells viability. On the other hand, calcium hydroxide did not present damage effect over the cells, what corroborates several studies that prove its potential for repairing and contradict studies that did not indicate it on dental regeneration[2,26].

Viability in current clinical applying

It is important highlight that a healthy person loses20 deciduous teeth, which are naturally exfoliated.Permanent tooth pulp can also be easily obtained in any moment of life; periodontal tissue can be accessed in a simple way, and third molars with embryonic tissues (apical papilla and dental follicle) can be extracted in any moment in adult life with no morbidity to the person. Then, posteriorly these cells can be used to treat caries, periodontics and endodontic treatments, alveolar repairing, dental implant, increase alveolar bone height, repairing temporomandibular cartilage joint, as well as other human body areas[6].

This technique is still far from a clinic application, due to its complexity, quantity of bimolecular signaling involved in the cytological and morph

differentiation processes during the dental genesis[6,27].

There is great advance in adult stem cell experiments from mouth tissues. Their easy access and because they are not vital organs constitute an attractive for test practicality and viability of bioengineering techniques. In a near future there is the possibility for bioengineering usage in endodontic and periodontics therapies, despite nowadays this Science is far from develop complete teeth from stem cells due to their complex mechanisms for dental formation[2].

With advance in stem cells research and development of tissue engineering techniques, we assume the replace a lost tooth by a biological organ in a near future, able to substitute it in all biological, aesthetical and functional aspects[6,8,22].

Studies analyzed showed there is the possibility to obtain dental germs from stem cells appropriately signalized, which develop themselves when transplanted in appropriate niches. Despite genes and regulatory signals involved in dental genesis are known, and the advances obtained in dental structures development from stem cells, still there are obstacles to overcome to replace a human tooth lost through tissue engineering.Before the fast evolution in researches in this field, perspectives show themselves as promising regarding to the use BMPs and stem cells for teeth regeneration, specifically in endodontics, periodontics and craniofacial surgery. In the future, we hope achieve complete dental regeneration to use for replace lost teeth[13].

Learn more on stem cells isolation is necessary, also on their niches, as well as molecular growth and differentiation mechanisms to enable the use of this cell therapy in Dentistry[6,7,28,29].

Despite the increase on knowledge about molecular pathways, that regulates tooth morphogenesis and regeneration, their application on dental engineering remains in an initial stage. Notwithstanding a more detailed frame on complexes and redundant signalizing pathways on initiation, formation pattern, morphogenesis and cellular differentiation, it is not completely clear on these pathways specifies the distinct tooth morphology: incisors, canines, premolars and molars.We conclude there is the possibility for use in short term for stem cells in dental regeneration, specifically in endodontics, periodontics and craniofacial surgery.In the future, we hope achieve the complete dental regeneration for use in lost teeth replacement[13].

We believe that, in a near future, the use of stem cells will represent a common procedure, meaning a great advance for Dentistry field[6,30].

Based on the previous exposition, it seems to be permissible conclude that a definitive protocol still is not established for pulp regeneration procedures.The studies still do not clarify the nature of the new tissue, when formed[2].

DISCUSSION

According to Casagrande (2009), tissue engineering is based on three principle, engineering, biology and clinical sciences, in order to develop biological substitutes able to improve or restore the function of an organ or tissue. Chen (2012) defines tissue engineering as the Science that studies functional and physiological restoration of deteriorateor lost tissue structures.

Segundo (2012) suggests that stem cells represent low differentiation degree, capacity for multiplication and self-reproduction in specialized and differentiated cells.Odorico (2001) defines stem cells as undifferentiated; with high capacity for self-renovation and produce, at least, one type of specialized cell, like Borges (2014) alleges that stem cells are able to generate another specific one. Gomes (2008) states that stem cells are undifferentiated adult cells found in tissues responsible by regeneration.

Souza (2014), Chen (2014), and Odorico (2001) classify the cells into two groups, which are multipotent or adult cells, and the pluripotent or embryonic cells.

Borges (2014) advocates that an adult stem cell, when removed from the dental pulp and stimulated by growth factors and proteins, it is able to differentiate itself in odontoblasts, and then capable to regenerate tissues like dentin and pulp.

Souza (2014) reports that to the tissue regeneration occurs, stem cells, growth factors and a growth matrix are necessary.Chen (2012), Huang (2013) and Neel (2014) suggest the tissue engineering is based three principles: stem cells, extracellular matrix that maintain the tissue structure and substances that induces the growth. On the other hand, Soares (2007) suggest stem cells, an extracellular matrix and growth factors for an effective tissue bioengineering.

According to Feques (2014), among the stem cells main sources in Dentistry, we detach dental pulp, periodontalligament, deciduous teeth, dental follicle, apical papilla, and these are multipotent cells. For Hau (2009), the stem cells can be found in pulp of deciduous teeth and permanent ones, in the periodontal ligament and the dental germ.Huang (2008)states the best stem cells source is Supernumerary and mesial

teeth, easily extracted. Gronthos (2000), however, suggests the third molars would be a good indication to obtain stem cells.

Murray (2014) defined growth factors as proteins that link themselves to the cells receptors, acting like signalizing for cells proliferation and/or differentiation. Bansal (2014) states the growth factors appear more frequently on regeneration processes, and they are Transformer factors Growth (TFG) and Bone Morphogenetic Protein (BMP).

Gronthos (2000), after perform the third molars stem cell transplantation, obtained formation of connective tissue similar to the pulp-dentin complex found in a health human teeth.

Rosa (2010) evaluated the stem cells behavior with scaffolds obtained from exfoliated deciduous teeth, when injected in pre molars with opened apex and non-instrumented root canals was possible observe high proliferative activity, few apoptotic cells and dentin formation, in other words, obtained a pulp tissue able to produce dentin.

Bernadi (2009) isolated and cultivated stem cells from deciduous teeth, and established culture and capacity for proliferation, regardless the quantity of pulp tissue present, what evidenced the capacity to obtain stem cells from deciduous teeth, mainly in incomplete rhizogenesis cases.

Daltoé (2014) suggests that for tooth confection, the use of biocompatible molds is necessary. They would give the tooth shape and would be made with biodegradable polymers. However, this technique still presents failures, because the cells did not dispose themselves in the mold form. Ohazama (2008) and Keishi (2014) indicate the way as from tissue recombination, and the development occurs as in embryonic genesis, using epithelial and mesenchymal cell sources, but one of the problems in this technique is find alternatives for epithelial and mesenchymal formation, and also controlling dental shape. Daltoé (2014) also presents as option a "new" dental formation; thereunto, assemble different cell sources is necessary, in a way they interact in balance between them and reproduces dental tissue.The great problem, in this case, would assemble cells able to interact in balance.

Souza (2014) concludes that to use pulp regeneration successfully, there are three aims to achieve: eliminating symptoms by periapical tissues reparation, promoting thickening canal walls and/or continuing the root formation in incomplete rhizogenesis cases, further obtaining positive response for vitality tests.

Rai (2014) describes that cell events that occur in tooth development stage are determined by several interactions between epithelial and mesenchymal cells. Epithelial cells form the enamel and the mesenchymal cells surround it, where these interactions involve signalizing proteins and specific receptors that start the tooth development when activated.

Conde (2012) believes there is a growing development in technology area, what demonstrates future possibilities to apply pulp regeneration therapies in clinic. Soares (2007) states there is a great advance in experiments with adult stem cells from mouth tissue; then, in a near future is possible use bioengineering in endodontics and periodontics therapies. However, the Science is still far from develop a complete tooth, due to the complex mechanisms of dental formation. Ohazama, Ferreira and Mostajo-Radji (2014) believe the advances in stem cells researches and tissueengineering development, in a near future, can replace a lost tooth by a biological one. Hau (2009) advocates still there are obstacles to overcome to achieve a human tooth replacement through tissue engineering, because this Science is in a beginning stage. Rai (2014) highlights that tooth regeneration is not simple, because its development is determined by several complex interactions. Koussoulakou (2009) states this technique is far from a clinic application due to its complexity.

Applying this technique in the current clinic looks like distant, because more studies and researches are necessary to enable it in a near future to perform successfully in clinic.

CONCLUSIONS

After literature review on the pulp regeneration, we conclude: (1) the main source of stem cells used on the experimental evaluation were multipotent stem cells found in third molar dental pulp; (2) to perform the "in vitro" pulp regeneration technique, stem cells, an extra cellular matrix and substances to induce the growth are necessary; (3) there is no a definitive protocol for pulp regeneration procedures; more studies on the nature of the tissue formed are required.

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