ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 06, pp. 37338-37350, June, 2020
https://doi.org/10.37118/ijdr.19179.06.2020

RESEARCH ARTICLE OPEN ACCESS

THERAPEUTIC APPLICATIONS OF CLONING IN ODONTOLOGY

***Sidqui, M. and Rhattas, S.**

Faculty of Dental Medicine of Casablanca, Hassan II University

ARTICLE INFO

Article History:
Received 18th March, 2020
Received in revised form
19th April, 2020
Accepted 06th May, 2020
Published online 30th June, 2020

Key Words:

Cell cloning, Periodontitis,
Applications in dentistry,
Bone regeneration, Teeth

****Corresponding author: Sidqui, M.,***

ABSTRACT

Therapeutic cloning opens new perspectives for regenerative medicine, it would represent a major advance in the fight against diseases now considered incurable, and would save many lives. Therapeutic cloning involves transferring the nucleus of a cell, taken from an adult organism in an enucleated oocyte to obtain embryonic stem cell lines used for therapeutic purposes, they are then genetically identical to the individual from whom was removed the cell of the transferred nucleus, and will result in no signs of rejection, which is a huge health benefit, but the difficulty of obtaining human blastocysts hinders their use leaving room for adult stem cells. Endodontics, periodontics and dental prosthesis are all returned in a new use of stem cells to repair and even to regenerate natural teeth.

Citation: ***Sidqui, M. and Rhattas, S.*** "Therapeutic applications of cloning in odontology*", International Journal of Development Research*, 10, (06), 37338-37350.

INTRODUCTION

Scientific news prompts us to think again about cloning. (1) Every clinician dreams of one day obtaining, for his patients, ad integrum regeneration of injured organs. The study of the healing mechanisms in the animal world allows us to observe, in cold-blooded animals, phenomena of regeneration of complex structures. This regeneration exists in amphibians, reptiles and in certain fish. In fact, when a salamander loses one of its members, or even part of its head or heart, this amphibian from the urodele family has the capacity to completely regenerate the lost organ (Figure 1). Thanks to these observations, a very large number of research laboratories have been interested in the identification, isolation of these stem cells and their use in therapy, hoping that this type of regeneration may one day be possible in the man. Scientific news prompts us to think again about cloning (1). Every clinician dreams of one day obtaining, for his patients, ad integrum regeneration of the injured organs. The study of the healing mechanisms in the animal world allows us to observe, in cold-blooded animals, phenomena of regeneration of complex structures. This regeneration exists in amphibians, reptiles and in certain fish. Indeed, when a salamander loses one of its members, or even part of its head or its heart, this amphibian of the urodele family has the capacity to completely regenerate the lost organ. Thanks to these observations, a very large number of research laboratories have been interested in the identification, isolation of these stem cells and their use in therapy, hoping that this type of regeneration may one day be possible in Hom Given the difficulty of using embryonic stem cells and knowing that embryonic cells cannot be "self" cells without going through cloning, research has focused on adult stem cells. Currently, it has been shown that adult mesenchymal stem cells can be multiplied in vitro and regenerate not only mesenchymal type tissues, such as bone tissue, but also other tissues, such as nervous tissue. At the dental level, several teams have identified adult stem cells in the dental pulp and in the periodontal ligament, allowing one day to hope to practice biological treatments and, thus, to obtain regeneration rather than repair of the organ dental. In addition, if adult stem cells prove their real usefulness in regenerative therapy, it may be possible to use stem cells from temporary teeth or wisdom teeth to regenerate bone tissue, cartilage or even nervous tissue.Many researchers believe that this hope is really founded. Indeed, in the United States, banks of dental pulp stem cells already exist. (2) The purpose of this thesis is to define cloning, to study the different types of stem cells and to understand how they can be used in cell therapy,

as well as the therapeutic applications and perspectives of cloning in dentistry.

Le clonage

Definition of cloning: The word clone, originally from the Greek "κλων", which means twig or slip, defines a method of replication that occurs in some plants, and therefore represents a means of asexual reproduction. For many plants, and some invertebrates and even some vertebrates (some lower animals like the hydra, sea anemone, and others such as planarians and annelids), cloning is the natural way to reproduce. (3)

The different types of cloning

Reproductive cloning: Reproduction, the term is well suited as opposed to procreation. To reproduce is to produce the same thing. This is the will of those who work there. The nucleus is removed from the cell of an adult and transferred to a previously enucleated egg. Thus an embryo can develop until it is transferred to a uterus. The being born of these operations will be genetically the same as its parent whose genome will not have known mixing with that of a woman. In 1996 appeared the result of the first reproductive cloning of a mammal, the famous Dolly sheep, obtained by asexual reproduction. Since then, reproductive cloning has been applied to other animal species such as cows, sows and mice. The publication on these works has rightly had immense media coverage. Nevertheless, the main author of this cloning of reproduction, Professor Ian Wilmut of the Roslin Institute in Scotland, did not stop insisting on the high rate of failures of the technique, on the anomalies which appear in certain organs of the clones obtained and on the accelerated aging of the cloned animals. It is for these reasons that the World Scientific Community absolutely condemns any idea of reproductive cloning applied to humans and considers as a crime attempts proposed by individuals and sects with the most suspicious motivations. (4) The proclaimed intention of the investigators is to use a new palliative technique for sterility in couples. (1)

Therapeutic cloning: The term "therapeutic cloning" is an ambiguous and improper term which has been proposed for ease of designating the use of stem cells multipotent derived from Human blastocysts, obtained by nuclear transfer of somatic cells. This term is unfortunate because it has become confused in the minds of the public with reproductive cloning. They share a common word, which wrongly evokes the worst connotations of the simplistic image of cloning (4, 5). This type of cloning aims not to give birth but to maintain, to treat. The procedures are the same for the nucleus of a cell taken from the patient to be treated, who is introduced into an ovum deprived of its own nucleus. But the resemblance stops there because there is no transfer of the embryo thus created but use of its cells for a care purpose. (1) (Fig 1). The underlying idea is to use the multiple differentiation capacity of the embryonic stem cells thus generated to repair diseased adult tissues (Parkinson's disease, diabetes, cancers, infarction...). This path has been explored with some success in animal models of these diseases, but its application comes up against the difficulty of obtaining human blastocysts by following this technique. At the same time, research is increasingly exploring the possibility of using stem cells from sources other than obtaining blastocysts by nuclear transfer. The sources of adult stem cells are mainly blood from the umbilical cord, the blood itself, but especially bone marrow. (6)

Cloning techniques

Cloning by cell dissociation: The first animal cloning by cell dissociation was carried out in 1891 in a species of sea urchin. In this experiment, the first four cells of the embryo were dissociated and each gave birth to a small, normal larva. The goal was not to clone the organism, but to test the potential of the first cells of the embryo. Similar experiments were subsequently attempted in amphibians, then in mammals. Morula stage sheep embryos (8/16 cells) were cut in half and then cultured in vitro. Both halves have reached the blastocyst stage, and have developed normally after implantation into the womb of a surrogate mother. These first mammalian clones were obtained in 1979. Cloning by dissociation of the first cells of the embryo, or embryonic cloning, is only possible in sea urchins and vertebrates. In other animals, the first embryonic cells, once dissociated, only form incomplete embryos. (7)

Cloning by core transfer : The first cloning by transfer of nucleus was carried out in 1952 in the frog Rana pipiens. The aim of the experiment was to test the potential of nuclei from various cells, therefore to determine whether or not they had the same genome. At the time, nothing was known about the mechanisms involved in cell differentiation. The question was whether the establishment of various cell lines resulted in irreversible changes to the genome. In this eventuality, a somatic cell nucleus that has undergone such alterations would no longer have the genetic information required to ensure full development. To test this hypothesis, it was "enough" to place the kernel in the context of a new development. Whether it is an amphibian or a mammal, the principle of nucleus transfer cloning is the same: it is necessary to replace the nucleus of the first cell of a potential individual ("recipient" cell: the ovum or oocyte), by that of another cell ("donor" cell). (7)

Stem cells

Definition: Stem cells are immature non-specialized cells, which have the potential to develop into many different cell lines via differentiation. By classical definition, these cells can renew themselves indefinitely by "self-renewal", and they vary depending on their location in the body and the type of cells they can produce. Recent studies have revealed that oral tissues, which are readily available to dentists, are a rich source of stem cells. Given their unique capabilities, stem cells are particularly important for the development of innovative technologies for tissue engineering strategies to regenerate or replace diseased or damaged tissue, and even missing organs through in vitro cell manipulation and design of the extracellular environment. (8) There are several possible "classifications" of stem cells: a physiological classification, which follows the chronological succession of stem cells successive in life, from the embryo which creates the tissues, to the fetus and to the adult where the tissues only renew or repair themselves in the event of injury, more or less effectively; we can also classify stem cells according to their differentiation potential, or even distinguish those which work in vivo, and therefore exert a recognized "physiological" role, and those which acquire ex vivo - after culture - properties of CS (CSE lines and CSM). (9) Pluripotency designates the capacity of a cell to differentiate in the three primary lineages resulting from gastrulation (which occurs very early in the embryo, day 10): the mesoderm, the endoderm and the ectoderm; in the case of totipotency, the placental

trophoblastic lineage must be added. Multipotency, which is more restrictive, indicates that a cell can only produce the types of differentiated cells that make up a single tissue. Totipotency and pluripotency are two properties restricted to embryonic cells: intuitively, we can understand that at a very early stage of development, before the specification of tissues, certain cells have the information required for an almost unlimited capacity for differentiation, which is no longer necessary in the fetus and the adult, once the organs have been formed. A certain regeneration is possible in the embryo and the fetus, but the adult Man does not have the power that has the salamander to regenerate a whole member, and a fortiori a heart or a brain. Just our adult stem cells can repair a point lesion. (9)

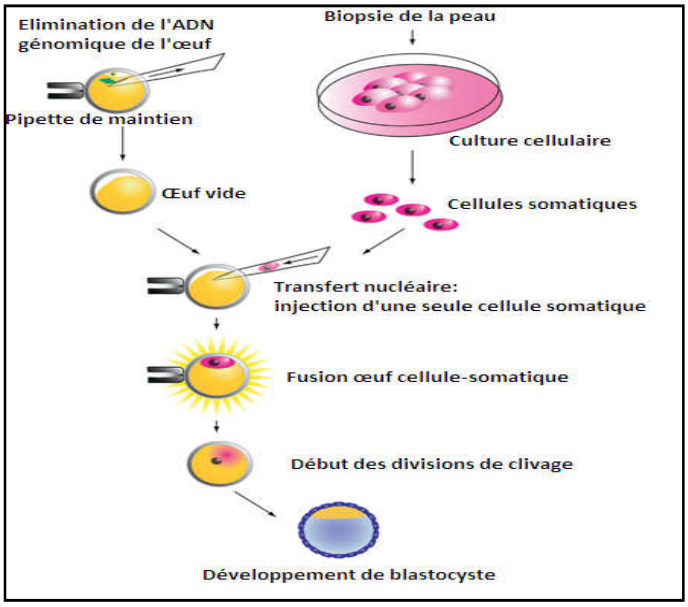

Figure 1. Cloning by nuclear transfer of somatic cells. (10)

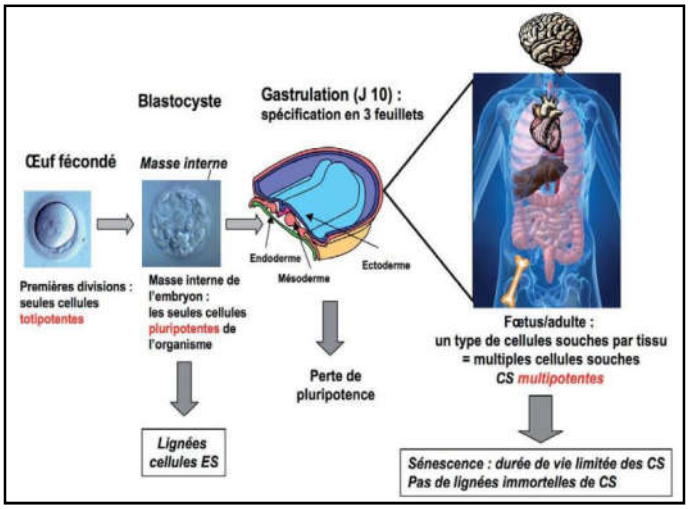

Figure 2. Embryonic and fetal / adult stem cells: very different entities succeeding each other during life. (9)

The different types of stem cells (Fig 2)

Embryonic stem cells: Embryonic stem cells are cells isolated in vitro from the internal cell mass (MCI) of the blastocyst between the 5th and 6th day of embryogenesis. MCI are cells which have the property in vivo to give birth to the three embryonic layers-endoderm, mesoderm and ectoderm - which are at the origin of all the tissues of an adult human being. It was demonstrated in 1981 in mice the possibility of isolating and cultivating these embryonic cells in vitro and of making them proliferate indefinitely while maintaining their capacity for pluripotency, that is to say the capacity to generate all differentiated tissues. These works were crowned by the Nobel Prize in Medicine in 2007. These results were reproduced in humans in 1998 by the teams of James Thomson, Joseph Istkovitz-Eldor and Benjamin Reubinoff by the derivation of the first hESC lines. (11) Embryonic stem cells have the capacity to proliferate indefinitely without entering senescence or having specialized functions. Consequently, they are in an undifferentiated state and, after a process of differentiation, they can produce the different tissues of the three germ layers, even the extraembryonic tissues in the case of human embryonic stem cells. (12) Up to the 8 cell stage (3 days), even dissociated cells from each other can form an entire organism, they are totipotent. (13) On the 5th or 6th day of embryogenesis, the internal mass of the blastocyst, counts 100 to 200 pluripotent cells, capable of evolving towards one of the 200 types of differentiated cells of an individual. (14)

Sources of human embryonic stem cells: Embryonic stem cells are taken from Human embryos from in vitro fertilization (IVF) or by cloning. (2) The derivation and maintenance of Human embryonic stem cells were carried out, for the first time, in 1998 by James Thomson, when his team cultivated the internal cell mass of blastocysts from embryos received from in vitro fertilization (IVF). (15) The great advantage of nuclear somatic cell transfer is that embryonic stem cells from blastocysts will be genetically similar to the cells of the individual who donated the nucleus. It is less likely, therefore, that the expressed proteins will be recognized as foreign and elicit an immune response in the host. (16)

Characteristics of embryonic stem cells: Their almost unlimited proliferation capacity in the laboratory (without being able to reform a viable embryo). This allows the accumulation of a very high number of cells, and the isolation of permanent lines having the same characteristics as the primary cells at their origin. This authorizes the practice of research work. Embryonic stem cells retain a normal genotype, despite their high proliferation rate, they do not accumulate mutations. The lines formed by these cells are pure, therefore their proliferation and differentiation potentials are identical. They lend themselves to a great facility of analysis and modification of their genome: one can insert or delete genes of which one supposes the importance for the realization of a given tissue program, which makes it possible to analyze the functional consequences of these disturbances. The triggering of their differentiation can take place, on demand, in this or that tissue, thanks to the addition of regulatory molecules. Embryonic cells certainly constitute a privileged source of inducing signals since it is at the embryonic stage that organogenesis is decided, that is to say the establishment of the various organs or tissues which will constitute the adult organism. These signals could be identified and purified from these ESCs or their daughter cells, and then used for the specification of adult tissue stem cells. (17). In mice, embryonic stem cells are particularly easy to grow. (18). However, even if the functioning of certain tissues is generally very similar in humans and in mice, it is however difficult to extrapolate to humans the conclusions drawn from the study of mouse embryonic stem cells. Indeed, the regulatory molecules diverge, and other criteria, such as the size of the animal, its

lifespan, often making the mouse model unusable from a therapeutic perspective. (17)

Fetal stem cells

The different types of fetal stem: Fetal stem cells come from fetal tissues between the 5th and 9th week, we will distinguish two classes: somatic cells which represent the cells of the body except the gametes which are called germ cells. (13)

Fetal somatic stem cells: Fetal tissues contain stem cells: two of these tissues are particularly important from a therapeutic perspective, in particular by their capacity for regeneration and repair of tissue damage (13/38):

- Stem cells of the central nervous system, in the treatment of certain neurodegenerative pathologies (Parkinson's or Huntington's diseases) (13).
- Fetal hepatocytes which are the subject of active research for transplantation (19).

Stem cells of the germ line: They come from the outline of the germinal tissue of the fetus. They are pluripotent. Their genome is less stable than that of embryonic stem cells, which makes them, for the moment, unusable from a therapeutic perspective, while they open important perspectives in fundamental research. (20)

Characteristics of fetal stem cells: Fetal SCs are much closer to adult stem cells than to embryonic stem cells in their molecular properties and characteristics. They have already acquired a tissue identity, and like adult SCs, are no longer pluripotent, but only multipotent. Present in all fetal tissues, they are distinguished from their adult counterparts by their intense proliferation activity and a much greater "reserve" of cell divisions, but their potential is not fundamentally different. Despite this robust proliferation, it is not possible to "spontaneously" establish immortal lines of multipotent fetal SC, they always end up entering senescence. On the other hand, access to fetuses in humans is very limited, and the number of fetal stem cells that can be isolated is very low, which compromises any realistic therapeutic use. Only cord blood is a reproducible and important source of fetal CS, but those found there are almost only hematopoietic (21). Mesenchymal stem cells are detected in small numbers there, and rare studies have reported that certain MSCs could express, in vitro after several weeks of culture, certain attributes of pluripotent CSs (22), but convincing evidence is lacking. Despite a very large proliferation and therefore a considerable number of cells, and despite recent controversies, often launched to fuel another debate questioning the usefulness of ESCs, cord blood is not a source of pluripotent CS, and remains above all a source of CSH. (9)

Adult stem cells: It appears that the best way to define an adult stem cell is its function: a somatic stem cell (to distinguish it from germ cells) ensures homeostasis, that is to say the physiological maintenance of an organ or a tissue, replacing dead cells, either naturally or after an injury, thus ensuring the sustainability and function of the organ during the life of the individual. It fulfills this function on the one hand by multiplying identically (which avoids the drying up of the stem cell reservoir), on the other hand by differentiating, thus acquiring the characteristics of the tissue to be repaired. (20)

Locations of adult stem cells

Extraoral locations: Stem cells have been identified in almost all adult tissues, including the skin, intestines, liver, brain and bone marrow. (5) These cells self-replicate, allowing the conservation of the cell pool, and differentiate themselves to regenerate tissues damaged or destroyed by trauma, disease, or by aging. (2) The tissue whose regeneration potential is best known is probably the bone marrow.

Bone marrow stem cells are made up of two cell types: (2)

- Hematopoietic cells, which will give all the cells of the blood line (polymorphonuclear cells, lymphocytes, macrophages, dendritic cells, red blood cells and platelets) (Fig 3);
- Mesenchymal (stromal) cells, which are located in the supporting tissue of hematopoietic cells.

These stem cells have the ability to differentiate and form different tissues in the body, including bone tissue, cartilage tissue and fatty tissue. (2)

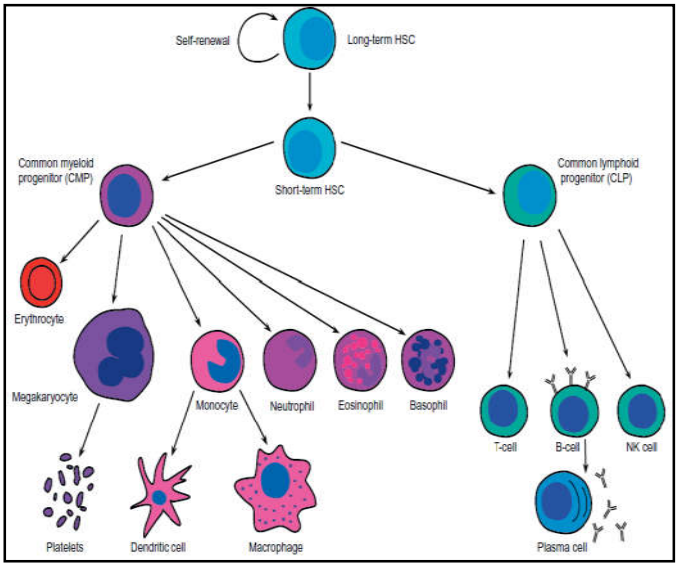

Figure 3. Simplified diagram of the differentiation of hematopoietic stem cells displaying the classic view of their development with two main branches, the myeloid and the lymphoid, and their descendants. (23)

Intra-oral locations: Stem cells of dental origin were first identified in the pulp tissue, then in the exfoliated baby teeth, the periodontal ligament, the dental follicle, and the developing dental papilla. These stem cells are derived from human dental tissues, which share the common characteristics of self-renewal, multipotency, and clonogenic potential. Although universal stem cell markers are not available for these cells at this time, these cells have been considered postnatal stem cells, which play an important role in healing the tissue that has been destroyed. Another type of dental stem cell is derived from the remains of Malassez epithelial cells, a type of cell of epithelial origin.(Figure 6) (24).

Stem cells from the dental pulp: Although the regenerative capacity of the dentino-pulp complex is not well understood, it is known that, during injuries, restorative dentin is formed as a protective barrier for the pulp. As a result, one could predict that the dental pulp contains dentinogenic progenitors which

are responsible for the formation of restorative dentin. (25) (Fig 4). Odontoblasts are the highly differentiated cells responsible for the formation of dentin. Odontoblasts are post-mitotic cells, and this implies that they can never renew themselves by cell division. During a dentino-pulpal aggression causing the degeneration of odontoblasts, Höhl cells (located in the sub-odontoblastic layer) can differentiate into odontoblasts, to form reactive dentin. However, if the aggression is too great, during a pulp break-in associated for example with pulp styling, the Höhl cells disappear. In this case, a first phase of neoangiogenesis induced by growth factors secreted by the pulp fibroblasts seems to be an essential step before the secretion of restorative dentin. This restorative dentin is secreted by odontoblast-like products derived from the differentiation of pulp stem cells. (2) Recently, stem cells from the dental pulp have been isolated from extracted third molars. These cells were exploited for their capacity to regenerate, they were explored by Gronthos et al. (17/27), which quantitatively determined the presence of stem cells in the dental pulp with conclusive evidence by demonstrating the expression of various perivascular markers, such as STRO-1, VCAM-1 (vascular cell adhesion molecule), MUC-18 and smooth muscle actin. (25)

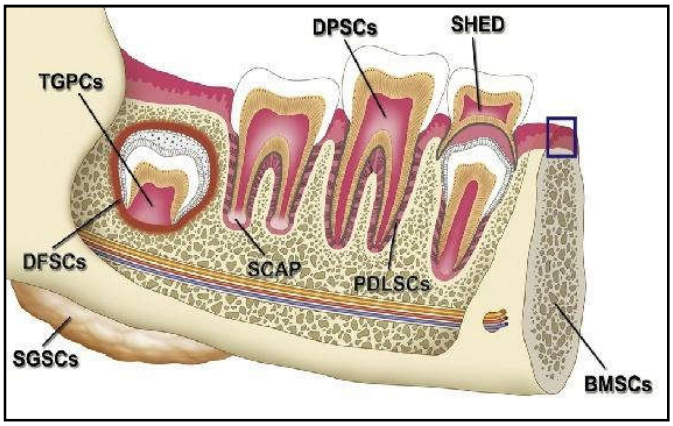

Figure 4. Sources of adult stem cells from the buccal and maxillofacial region. (8)

BMSCs: CSM of bone marrow from the orofacial marrow; DPSC: stem cells from dental pulp; SHED: stem cells from exfoliated baby teeth; PDLSCs: stem cells of the periodontal ligament; DFSCs: stem cells of the dental follicle; TGPCs: Stem cells of dental germ; SCAP: stem cells of the apical papilla; SGSCs: stem cells derived from the salivary gland.

In order to understand the mechanisms involved in the formation of dentine repair and to identify the precursors / stem cells capable of differentiating into odontoblast-like, the culture of the cells of the dental pulp has been widely developed. In culture, the cells of dental pulp, obtained by the explant technique or by enzymatic digestion, acquire a morphology which is similar to that of odontoblasts. In addition, the secreted proteins are the same as those which constitute the dentin matrix: dentin sialoprotein (DSP), dentin phosphoprotein (DPP) and the dentin matrix protein (DMP1). Finally, these cells express an enzyme involved in the mineralization process, alkaline phosphatase, and are capable of inducing mineralization similar to that observed in dentin. When dental pulp cells cultivated for 15 days are transplanted subcutaneously in immunocompromised animals, a dentino-pulp complex develops, confirming the presence of odontoblastic precursors in cell cultures of dentalpulp. These studies have shown the ability of cells in the dental pulp to differentiate and form new odontoblasts, which will secrete a dentin matrix in vitro and in vivo. (2)

Stem cells from exfoliated baby teeth: Stem cells isolated from the pulp of lacteal teeth (Stem Cells Human Exfoliated Deciduous) are capable of inducing bone formation, generating dentin, and differentiating into a variety of cell types, such as nerve cells , adipocytes, and odontoblasts. (26) Cordeiro et al. (10) have shown that SHEDs, seeded in prepared human teeth serving as biodegradable matrices, then implanted subcutaneously in immunodeficient mice, have differentiated into functional odontoblasts capable of generating tubular dentin and angiogenic endothelial cells. DPSC and SHED have the same origin as the ectomesenchymal cells of the neural crest, so their development and functioning seem to be identical. Compared to DPSCs, SHEDs display higher proliferation rates, higher expression of genes involved in the formation of the extracellular matrix, and several growth factors such as fibroblast growth factor (FGF) and TGF- β. TGF-β is important because it is released after dentinal lesions, and could act to mobilize pulp stem cells to differentiate into odontoblasts (27). Thus, the discovery of temporary tooth stem cells offers an interesting possibility of using them for tissue engineering. (28) The advantages of SHED are obvious: high yield, great plasticity, and easy access since children naturally lose 20 baby teeth. Commercial banks of these cells have spread, with the aim of using these cells when the child becomes an adult. However, some studies have shown that SHEDs do not keep their properties beyond two years of cryopreservation **(29).** Long-term storage (> 10 years) has not yet been evaluated. In comparison, DPSCs retain their stem cell characteristics after prolonged cultivation, allowing their use as a generic allogeneic source of mesenchymal stem cells. (30)

Stem cells of the periodontal ligament: The periodontal ligament (PDL), from which stem cells have been isolated and characterized, is a very thin sheet-like structure that supports a tooth and connects it to the surrounding alveolar bone. Although the existence of precursor cells in the periodontal ligament was discovered more than three decades ago in the mouse molar, they appear to have characteristics similar to current adult stem cells of the periodontal ligament, which harbors populations of multipotent cells. . This conceptual evolution in the dental field allows the development of therapy based on stem cells in dentistry. (24) The first step in characterizing PDLSCs is to isolate them from other cells in periodontal tissue. The method used is the same as that of the isolation of mesenchymal stem cells from the bone marrow and dental pulp. Adult stem cells originating from the periodontal ligament can generate cement and PDL-like tissue when transplanted into immunocompromised mice (Fig 5). (24).

Stem cells of the dental follicle: The dental follicle is an ectomesenchymal tissue that surrounds the organ of the enamel and the papilla of the developing dental germ. This tissue contains cell precursors that will form the periodontium, that is, the cementum, the periodontal ligament and the alveolar bone. Precursors have been isolated from dental follicles from the third molar germ. These stem cells are called Dental Follicle Precursor Cells. By analogy with other dental stem cells, these cells form a small number of colonies of adherent clones following their extraction by enzymatic digestion. (31, 32)

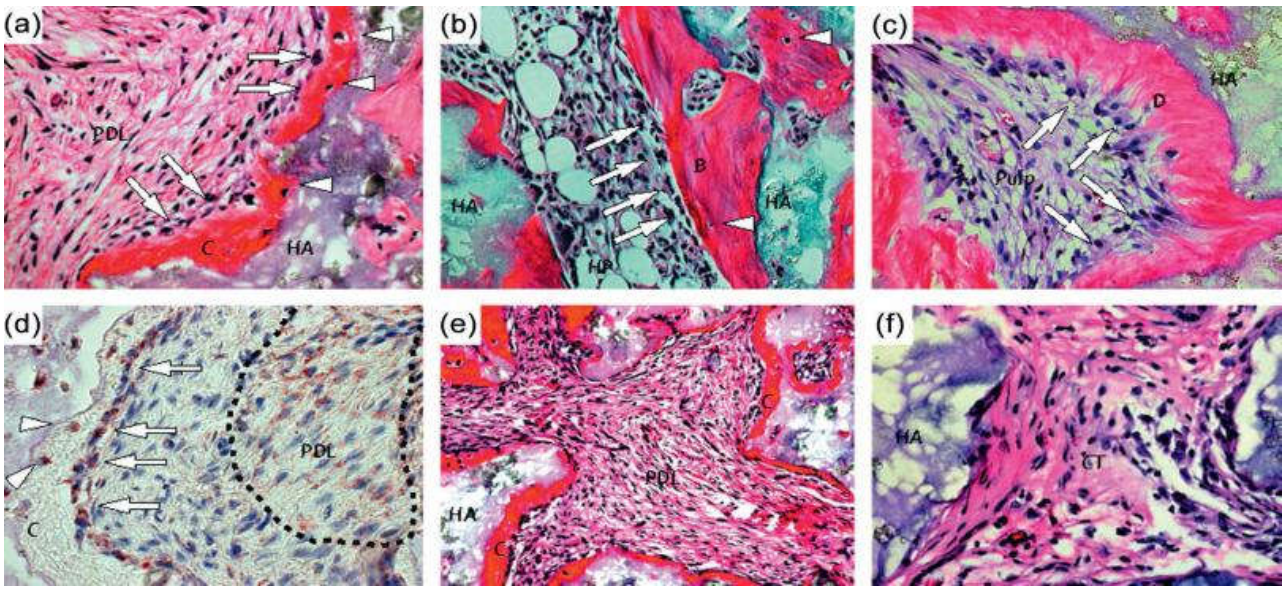

Figure 5. Generation of cement-like structures and to PDL in vivo by PDLSCs. (24)

DFPCs are capable of differentiating into osteoblasts, cementoblasts, chondrocytes and adipocytes according to the culture medium used (adipogenic, osteogenic, etc.). (32) The results of in vitro research seem to show that DPSCs have a better potential for hard tissue formation than DFPCs. This could be explained by the stages of development of the dental germ: the DPSC isolated from the dental pulp, at the stage of formation of the crown, present a greater accumulation of calcium than the DFPC extracted from the dental follicle. The DPSC express odontoblastic markers while the DFPC express cementoblastic markers. (33) DFPCs, whose division capacity is `` infinite '', that is to say that they do not age as they divide, are capable, in mice, of recreating a new periodontal ligament after in vivo transplantation (79). However, hard tissue such as dentin, cementum or alveolar bone has not been identified after transplantation of these cells into immunodeficient mice. (33)

Stem cells from the dental apical papilla: A single stem cell population isolated from human teeth is found at the apex of the root being formed. These stem cells from the dental apical papilla (called Stem Cell from the root of Apical Papilla), are able to differentiate into odontoblasts, adipocytes, osteoblasts and chondrocytes… (34). SCAPs have higher rates of proliferation in vitro than DPSCs. This cell population seems more suitable for the regeneration of dental root cells. The apical papilla is loosely fixed tissue at the apex of the developing root that can be easily elevated. It is only present during the development of the root, before the eruption of the tooth. Therefore, wisdom teeth are a clinically and easily exploitable source for collecting SCAP. (35)

Properties: The very numerous experimental works carried out in vitro or after transplantation in animals, make it possible to attribute to adult stem cells the following characteristics which distinguish them from embryonic stem cells: (20)

- They are not considered to be pluripotent and are generally programmed by a given tissue. They are said to be multipotent.
- They do not multiply endlessly in an undifferentiated state.
- They are very heterogeneous given the diversity of the tissues of the organism to which they belong.
- They are multipotent, they can produce cells of very different morphology and function, generally grouped within the same organ or tissue.

This is the case of hematopoietic stem cells, which produce all blood cells: red and white blood cells, lymphocytes and certain vascular structures. This is also the case for nerve stem cells, which produce neurons, but also accessory cells of the nervous system (astrocytes, olygodendrocytes). (20) Similarly, in human skeletal muscle tissue, there are stem cells (MDSCs: muscle derived stem cells) which can be activated to ensure tissue renewal in the event of injury, illness or destruction. These cells, which were described as monopotent, have been shown in culture to be able to differentiate and be at the origin of the mesodermal, adipocyte and osteogenic lines. (36)

Therapeutic applications of cloning in dentistry: Thanks to stem cells, important medical advances have taken place in several fields: neurodegenerative diseases, those linked to a cell deficit, certain aspects of senescence caused by cell depletion, the treatment of cancers, when these damage the healthy tissue in which the malignant cells have grown. (37) Stem cells are the natural source of differentiated cells that generate new tissue / organs, or that regenerate existing tissue. In recent years, stem cell research has grown exponentially due to the recognition that stem cell therapies have the potential to improve the lives of Alzheimer's patients with ischemia heart, or bone loss. (13) Endodontics, periodontics, and dentures have all entered a new era of using stem cells to repair and even regenerate bio-teeth or natural teeth. (38) Many approaches for repairing or replacing organs or tissues have recently been published, but the methodology for generating an entire tooth from human cells has not yet been established. (39)

Therapeutic cloning in endodontics: The very large number of articles published in 2006 and 2007 testify to the interest shown in stem cells from teeth and their potential use in dentistry. Various tracks of clinical applications have so far been discussed, including pulp regeneration. More and more

research teams are interested in the use of pulp stem cells to regenerate the pulpo-dentin complex. (2)

Regeneration of the dental pulp is part of regenerative endodontics, which includes the isolation, propagation, and transplantation of stem cells into the prepared root canal. The formation of new blood vessels by angiogenesis is mandatory to increase the survival rate of the transplanted tissues. Angiogenesis is defined as the formation of blood vessels from preexisting capillaries, which is of great importance in pulp regeneration and homeostasis. (40)

strategies are shown schematically, and each has advantages and limitations, as follows. (Fig 6) (41)

(a) Induction of the migration of stem cells from periapical tissues into the root canal by the generation of a blood clot, the injection of a scaffold containing chemotactic signals, or the mobilization of chemotactic signals derived from dentin. The translational potential of such a strategy is relatively high, since it does not involve cell transplantation. On the other hand, it is difficult to know if this type of therapy will allow the regeneration of the dental pulp over the entire extent of the root canal.

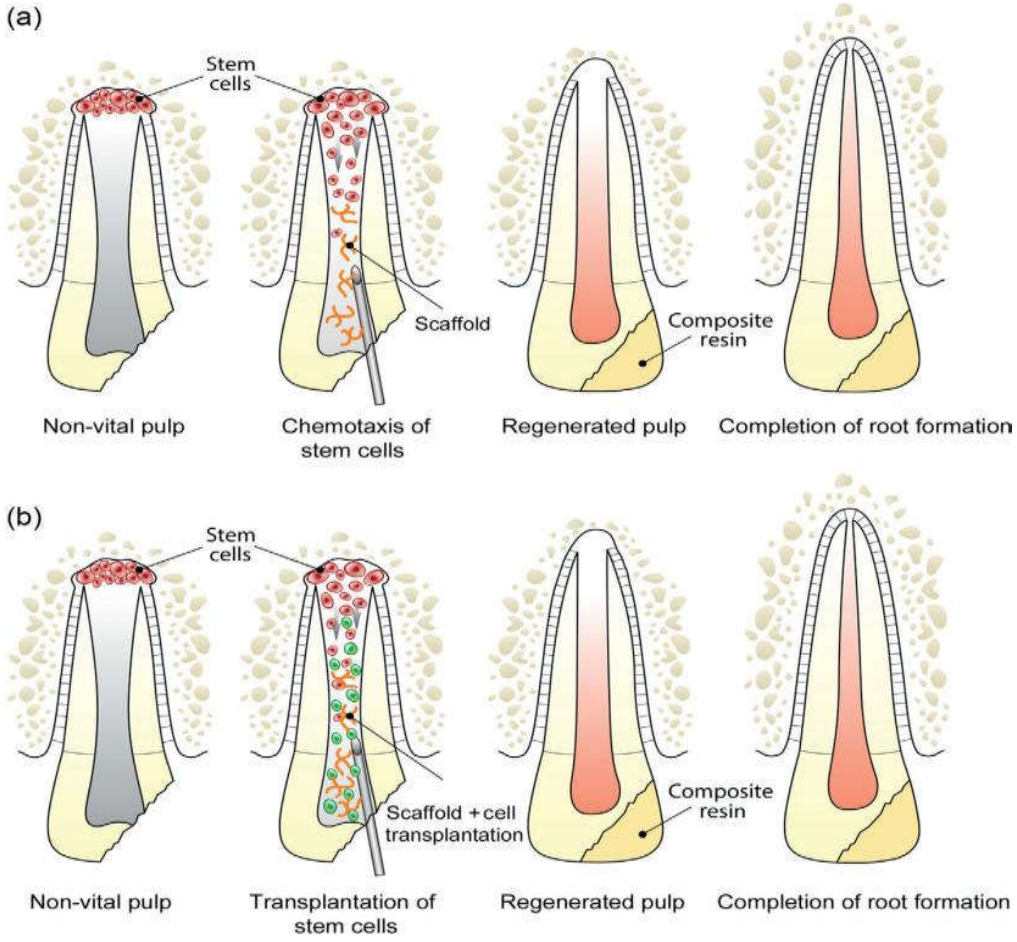

Figure 6. Diagram showing two strategies for the regeneration of dental pulp. (41)

Several studies have shown that stem cells are mainly found in specialized niches, and that certain tissues contain more stem cells than others. Among these tissues, dental pulp is considered a rich source of mesenchymal stem cells. The dental pulp contains progenitor cells committed to a specific destiny, as well as pluripotent stem cells. Human stem cells from dental pulp were discovered based on their ability to form isolated colonies in culture, their ability to self-renew in vivo, and their pluripotency in vitro. It is known that stem cells from dental pulp have the potential to differentiate into several cell types including odontoblasts, neural progenitor cells, osteoblasts and endothelial cells. Stem cells from dental pulp can be very proliferative. This characteristic facilitates ex vivo expansion, and increases the translation potential of these cells. In particular, dental pulp is without doubt one of the most accessible sources of postnatal stem cells. Regarding the source of cells for pulp tissue engineering, there are two main strategies: approaches based on transplanting stem cells into the root canal; and cell-free approaches that depend on the chemoattraction of host cells in the root canal. The two

(b) the use of injectable scaffolds containing stem cells. Preclinical data suggest that this approach can lead to functional regeneration.

Therapeutic cloning in periodontology

Periodontitis is an inflammatory disease induced by bacteria. It involves the progressive loss of supporting tissues of the teeth, such as the gum, the periodontal ligament (PDL), the cementum and the alveolar bone. The loss of alveolar bone is a particularly typical phenomenon of periodontitis and is not easily reversible. (42) If left untreated, it can lead to tooth loss. It is also closely associated with several systemic diseases, such as cardiovascular disease, diabetes and lung disease. As a result, researching more effective and predictable clinical methods to cure periodontitis is necessary for clinicians and dental researchers. (43) Over the past few decades, a number of procedures have been studied in an attempt to restore tissue after periodontitis. Non-surgical anti-infective therapy to

control biofilm and other risk factors is the cornerstone of periodontal therapy. Conventional treatments such as scaling, root planing, and the open flap reduce the depth of the pockets and allow clinical attachment gain. However, healing of periodontal tissue is usually dominated by the formation of a long, unstable junctional epithelium instead of periodontal regeneration. The basic concept behind conventional periodontal regenerative therapy is first to eliminate the source of the infection and provide a space in which neighboring cells can grow. (44) Bone grafting and biologically active regeneration materials, as well as guided tissue regeneration (RTG) are the two techniques with histological documentation of periodontal regeneration. RTG, performed by inserting a barrier membrane, is one of the most widely used treatments for periodontal regeneration. Periodontists also use autografts, taking the bone from another site to cure periodontal defects. However, the results of these existing therapeutic methods remain generally unpredictable. The desired regenerative results have not been achieved, particularly for advanced periodontal lesions.

Recently, strategies for periodontal tissue regeneration have shifted from RTG and bone grafting to cell-based tissue regeneration approaches. The healthy periodontal ligament contains, in adulthood, niches of stem cells which play a key role in maintaining the capacity for periodontal regeneration. However, due to the constant existence of inflammatory factors in periodontitis, tissue repair does not occur due to the absence of robust stem cells. As a result, the expanded / manipulated stem cells ex vivo will be a source of reconstruction of the host cell niche and will facilitate tissue regeneration. In pre-clinical studies, it has been shown that cell-based periodontal regeneration is likely to produce more reliable and effective results in the management of periodontal defects, and several clinical trials involving the use of expanded stem cells ex vivo to patients have already started or are in preparation. In such therapeutic approaches, stem cells manipulated in vitro are administered to patients as dynamic biological agents. Transplanted cells can participate in the repair of damaged or diseased tissue, serve to produce building blocks, or regulate regeneration through the secretion of trophic factors, instead of or in addition, directly participating in the tissue regeneration. (43)

Therapeutic cloning in implantology: The alveolar ridge plays an important role in the performance of oral functions, including chewing, speaking, and bolus formation. After tooth loss, especially in edentulous and semi-edentulous patients, the alveolar ridge plays an essential role in supporting the dental prosthesis. The height and width of the alveolar ridge are crucial for the stability of removable prostheses and the installation of dental implants. In the case of implant restoration, the alveolar ridge directly supports the implant via functional connections, called osseointegration. The vertical and horizontal volume of the alveolar ridge limits the size and angle of the dental implant; thus, maintaining a sufficient volume of the alveolar ridge is vital for successful implant-supported prostheses (45) The clinical effectiveness of stem cell therapies has been evaluated primarily in increasing the alveolar crest for insertion of dental implants (Fig 7). (46). Currently, stem cell-based clinical approaches to bone augmentation are divided into two broad categories: a tissue engineering approach and an armchair cell transplant approach. In both approaches, the bone marrow-derived mesenchymal stem cells (BMSC) from the iliac crest are the most commonly used cells because they are the most well characterized among the clinically available stem cells, and it has been shown that they have a higher osteogenic capacity. (46) A 20 year old male patient who presented with a lost 21 following an accident. (A) X-ray showing the significant defect in the alveolar bone resulting from the loss of the tooth. (B) X-ray showing increase in alveolar bone (arrow) by stem cell therapy. Mesenchymal stem cells derived from the iliac crest, expanded ex vivo, were applied at the defect level in a hydroxyapatite scaffold. (C) The radiograph shows the insertion of the dental implant four months after the stem cell treatment. (D) Vestibular view of the implant restoration (arrow). Replacement of teeth with a biocompatible or bioactive alloplastic material was often accompanied by additional problems such as mechanical and biological failure. The biomechanics of force distribution on supra-implant prostheses is qualitatively different from that of natural teeth. The essential difference is the absence of the dynamic role of the periodontal ligament which allows micro-movements and acts as a shock absorber. In the case of osseointegrated implants, there is no fibrous capsule. High resolution microscopy revealed an afibrillary area at the bone-implant interface. This area is rich in non-collagenous proteins (proteoglycans and glycoproteins), and in certain plasma proteins. Thus, osteointegrated dental implants have a rigid bone-implant interface which does not have the plasticity and biological remodeling that the natural tooth has, which is responsible for the decrease in mobility under functional load, and the transfer of excessive destructive stresses on the surrounding bone which cause marginal bone resorption. (47)

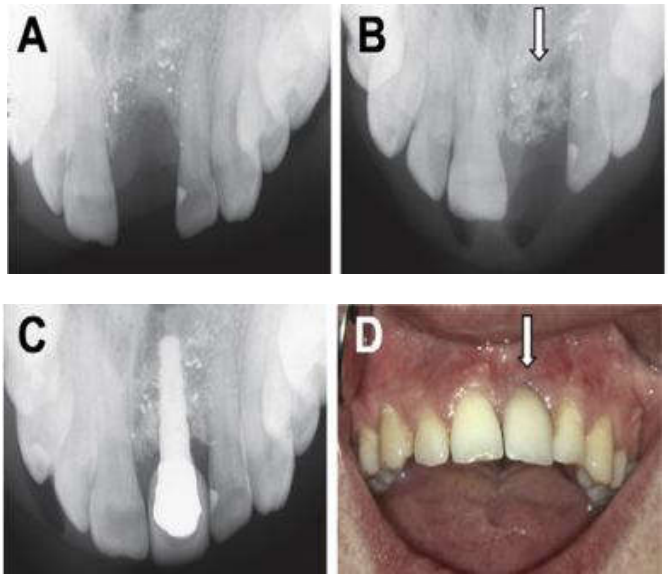

Figure 7. A clinical case of alveolar bone regeneration based on stem cells for the insertion of a dental implant. (46)

A three-dimensional hollow scaffold, which has the shape of a root, formed from biodegradable DL-lactide-co-glycolide (PLG), with 80% porosity, was seeded with bone marrow stem cells which were cultured and inserted around of a titanium implant fixation in a goat model. The scaffold acted as a delivery vehicle for the cells, and the geometric shape of the scaffold enabled the distribution of the masticatory load throughout the viscoelastic wall of the scaffold and interconnected pores. After ten days, the remains of the scaffold could not be identified and were replaced with periodontal tissue around the implants. (47) A more ideal solution would be to completely restore the periodontal

ligament on the implanted surface to completely replace the extracted tooth. This is the promise of tissue engineering which aims to integrate specific cell types into an appropriate scaffold, as well as appropriate signaling factors which, when implanted, gradually regenerate ligament tissue, on the implant surface, which closely resembles normal periodontal tissue and restores the integrity of functionality. The ligament integration of dental implants, accompanied by the formation of a PDL-like, requires the construction of a new cementum on the surface of the implant as well as the complete development of the periodontal attachment. The latter should include functionally oriented periodontal fibers and Sharpey fibers inserted into the newly formed cementum layer as well as the alveolar bone. This ligament tissue is equivalent to the PDL found in gomphosis which acts as a buffer for occlusal loads, allowing bone remodeling and curative orthodontic movements for poorly positioned dental implants. (47)

Therapeutic cloning in dento-facial orthopedics: Tissue engineering in orthodontics (OTE) operates in two major theaters: **(36)** repair, regeneration, or replacement of existing phenotype; selective alveolar decortication (SAD) or corticotomy; and **(19)** the restructuring of a new phenotype which better accommodates facial aesthetics and the shape of a wider dental arch (periodontal accelerated osteogenic orthodontics (PAOO) / accelerated osteogenic orthodontics (AOO). An integral part of tissue engineering in orthodontics is the new biology of stem cell therapy. This option is powerful because it lends faster healing to injuries and stability to the clinical outcome. In a nutshell, stem cell therapy is faster, safer and better. Stem cells are unique in their "asymmetric" replication. In other words, stem cells divide into multiple generations of daughter stem cells that self-replicate, in addition to specific cells of fully differentiated function, and therefore, provide exponential growth and a young and healthier environment for l 'TAKES AWAY. Stem cells used in biological research can include either adult stem cells or embryonic stem cells. The latter, limited to the United States by law, have become largely irrelevant for orthodontic purposes. Tissue engineering in orthodontics is based on the concept that creating larger alveolar bone is better than extracting healthy teeth.

If teeth are extracted due to the insufficient alveolar size for the dental mass, then making the bone larger is one of the great merits of stem cell therapies. This is because therapy without extractions is often an imperative for some patients, and enlargement of the alveolar bone regardless of its underlying skeletal corpus may avoid the need for major orthognathic surgery. Before the prodigious collaboration of Wilcko-Ferguson research groups in 2003 (48) in leading American universities, the consensus of some orthodontists was that the shape of the dental socket was immutable. But the evidence presented in the past decade raises arguments against the misleading perception of alveolar immutability. (48) There will likely never be a complete replacement for extraction of premolars or its justification in clinical art. The need for camouflage biomechanics in case of skeletal dysplasia of class II and the correction of disfiguring bimaxillary protrusion certainly define a perpetual need to occasionally sacrifice certain dental units. Even stem cell therapies, while offering a formidable alternative for extractions, do not entirely dispense with the need to remove premolars. Indeed, bone tissue engineering is not conceptually incompatible with an extraction treatment. Stem cell supplements aid in retention and healing of the extraction site, and surgical manipulation of the vacant socket can accelerate mass removal of the anterior teeth without loss of anchorage, while safely ensuring adequate bone support. (48)

Therapeutic cloning and complete dental regeneration: Teeth are composed of three types of hard, highly mineralized tissue: enamel, dentin, and cement; supported by the periodontium which includes the cementum, the alveolar bone, the periodontal ligament, and the gum. Dental development results from a set of sequential and reciprocal interactions between the oral epithelium and the underlying mesenchyme. (38). Tooth loss caused by cavities, periodontitis, or mechanical trauma is a major public health problem worldwide. People with tooth loss have a poor quality of life involving eating, smiling, and communicating problems. Current treatments for tooth loss rely on artificial dentures, such as fixed bridges, removable dentures, and dental implants. However, compared to natural teeth, dentures are not biological and have some drawbacks, including a feeling of a foreign body, which displeases patients. Thus, biological teeth are considered to be necessary (49). Teeth regeneration is considered an optimistic approach to replace current treatments for tooth loss. (49) Recently, many approaches have included simulating the natural process of dental training to recreate tooth-like structures, such as dental regeneration based on scaffolds, induction of a third dentition, the assembly of different transgenic components, and the tooth regeneration by gene manipulation. However, among the approaches mentioned above, synthetic scaffolding is currently the most recognized technique for the regeneration of teeth using a preconceived and optimized scaffold (Fig 8). (50)

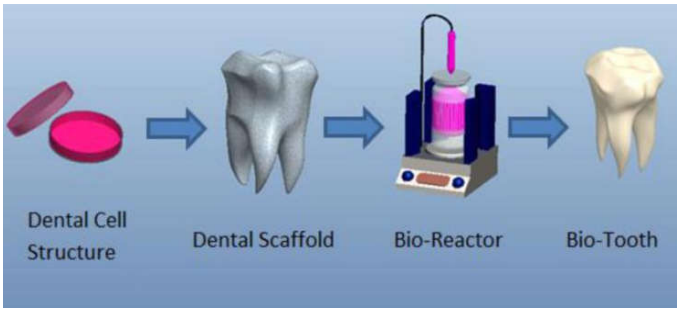

Figure 8. Schematic illustration of tooth tissue engineering. (50)

Initial approaches to tooth tissue engineering using conventional tissue engineering techniques have successfully reproduced complex dental structures from porcine postnatal dental cells (Fig 9). At the stage of coronary formation, the dental bud contains the three tissues required (the enamel organ, the dental papilla and the dental follicle) for the regeneration of the enamel, the pulpo-dentin complex, as well as the periodontium. To access these required cell populations, hard tissue is removed, then the remaining complete tissue is dissociated into cell-only suspensions by enzymatic digestion. These mixed cell populations are placed in a PGA mesh scaffold, and the scaffolds are wrapped in epiploons of immunocompromised rats and allowed to grow. This simple method has shown that the generation of dental structures, including enamel and the dentin-pulp complex, is possible. Following these experiments, a similar process was used to regenerate a tooth with all of its structures, including the root (Fig. 10a). Examination of the regenerated tooth at high magnification showed that a reduced adamantine epithelium

remained above the enamel (Fig. 10b), and that the cementum (Fig. 10c) and the Hertwig epithelial sheath were developed (Fig. 10d). These results suggested that progenitor / epithelial and mesenchymal stem cells were present in the early stages of dental crown formation. However, most of the regenerated teeth showed an irregular dental morphology (Fig. 10e). (39) The third molar is harvested from the mandible of a 6-month-old pig. The dental buds are dissociated into single-cell populations which are sown on the PGA scaffolds and after transplanted into naked rats.

distraction osteogenesis has been successfully used to reconstruct condylar osteochondral defects. (51) In addition, a human-shaped mandibular condyle has been successfully designed from osteogenically and chondrogenically induced rat bone marrow stem cells, encapsulated in a biocompatible polymer. These results may provide initial evidence of the ultimate stem cell-based tissue engineering for degenerate joint condyles in the context of conditions such as rheumatic arthritis. (44)

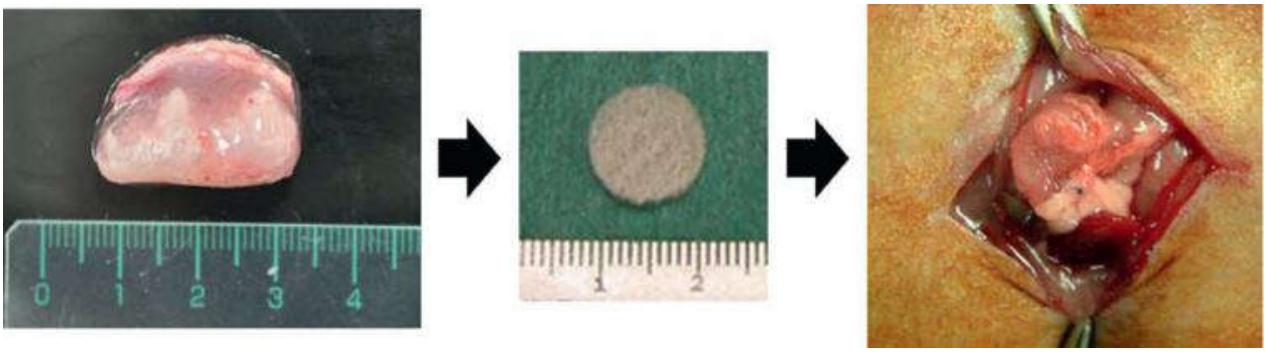

Figure 9. The strategy for the production of a tooth by tissue engineering. (39)

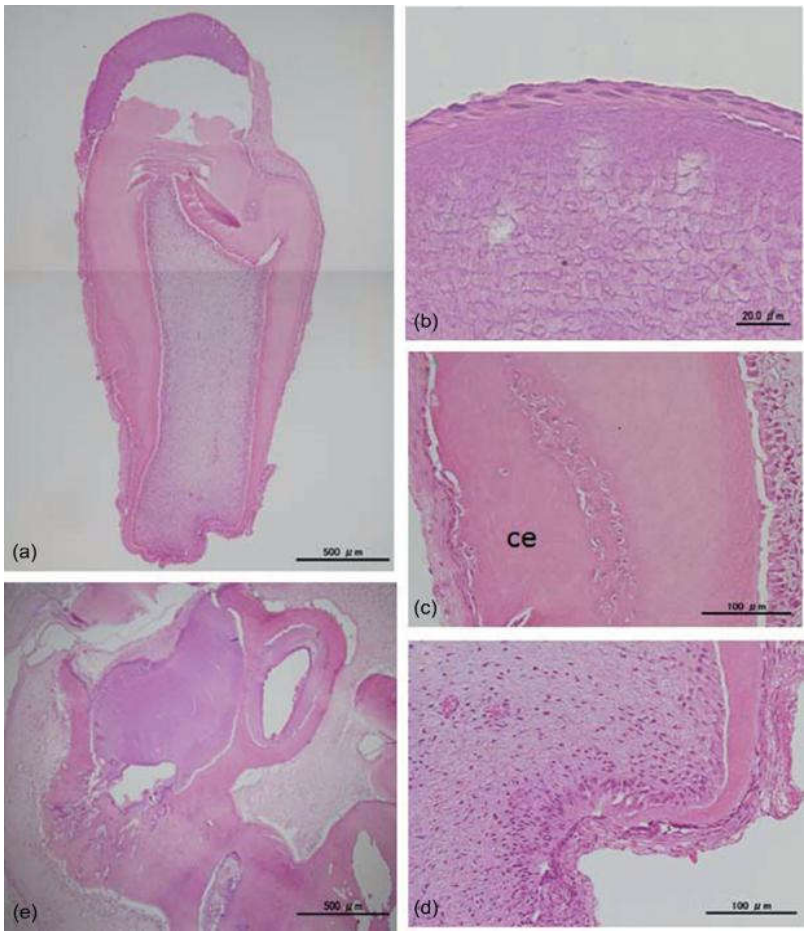

Figure 10. Dental structures from tissue engineering observed at 25 weeks after transplantation. (39)

Therapeutic cloning and regeneration of the mandibular condyle: Damage to the disc or condyle at the temporomandibular joint resulting from trauma or arthritis can cause lifelong pain and disrupt patients' chewing function. Tissue regeneration strategies for these defects may hold promise for affecting the quality of life of these patients. In a goat model, the combination of cartilage tissue engineering using cartilage-derived stem cells led into a hydrogel, and

(a) The tooth of tissue engineering has a shape similar to natural teeth. The enamel covering the dentin and the pulp surrounded by dentin can be seen in the tooth produced by tissue engineering. The cementum is recognized on the surface of the dentin.

(b) Enamel stems and a reduced adamantine epithelium can be seen above the enamel in a high magnification view (a).

(c) A cell cementum can be seen on the surface of the dentin. Line odontoblasts are observed in the pulp.
(d) A Hertwig epithelial sheath is present at the top of the root formation.
(e) An irregularly shaped dental structure can be seen on the collagen scaffold.

Therapeutic cloning and regeneration of the tongue: The loss of the tongue after surgical resection can deeply affect the quality of life, because the tongue plays an essential role in speech, swallowing and protection of the respiratory tract. Therefore, rebuilding tongue defects has always been a challenge in dentistry. Cell-based tongue reconstruction has been reported in a rat model where myoblasts / progenitor cells transported in collagen gel have been implanted in a hemi-resected tongue to successfully provide muscle regeneration with reduced scar contracture. (44) The tongue is a complex structure that includes fibers of skeletal muscle, mucous membrane with papillae, and nervous tissue; therefore, functional regeneration is difficult. Egusa et al. (52) have shown that the application of the cyclic strain of bone marrow stem cells has greatly accelerated skeletal myogenesis in vitro by producing aligned myotube structures, suggesting the importance of cell alignment for the creation of environments. physiologically relevant to skeletal muscle engineering. Advances in stem cell biology and tissue engineering may allow reconstruction of the resected or damaged tongue with normal physiological function. (44)

Therapeutic cloning and regeneration of the salivary gland: The regeneration of the salivary glands by stem cell transplantation is an important subject of study in oncology and head and neck surgery, because radiotherapy inevitably affects their function thus causing xerostomia (dry mouth syndrome) as a side effect.

Two main regenerative approaches have been applied to functionally restore damaged salivary glands:

- One approach is to develop an artificial salivary gland using tissue engineering technologies.
- A second is to apply stem cells to damaged saliva tissue. (44)

In a mouse model, mesenchymal stem cells from adipose tissue transplanted into irradiated submandibular glands restored the function of the salivary gland. (53) Recently, salivary gland stem cells have been isolated from mice, and intraglandular transplantation of these cells has been able to successfully repair the function of the irradiated salivary glands. (54,55). These reports suggest that stem cell transplantation can be used to functionally repair damaged salivary glands. The detailed regeneration mechanism should be clarified if the stem cells repair damaged host cells by replacing them or activating their turnover. (46)

Prospects for therapeutic cloning in dentistry

Regeneration of the alveolar bone: The scaffolding-based approach to bone augmentation has been combined with cell-based regeneration therapy. (56)

Pittenger et al. (57) reported that adherent cells isolated from human bone marrow maintain their undifferentiated state in long-term culture and differentiate to an osteogenic lineage when the appropriate treatment is carried out. Based on this finding, many studies have sought to regenerate bone tissue using mesenchymal stem cells derived from bone marrow. However, if the cells are transplanted without scaffolding, the transplanted cells do not remain at the transplant site, and are washed by extracellular fluids and / or blood circulation. Therefore, cells must be transplanted with an appropriate scaffold, such as a porous scaffold, a cell sheet with secreted extracellular matrix, or a hydrogel of encapsulated cells. In addition, the scaffold can provide bioactive clues to the proliferation or differentiation of transplanted cells. In order to successfully achieve bone-based bone augmentation, a sufficient number of osteogenic cells is necessary; Thus, autologous osteoprogenitor cells must be isolated, expanded in vitro, and transplanted to a specific anatomical site where bone is needed. (56)

Regeneration of the periodontal ligament: Osteointegrated dental implants have been used successfully as a prosthetic therapy for missing teeth for many years. However, osteointegration, representing a direct connection between the implant and the bone tissue, causes some inevitable problems, such as the concentration of the chewing force and the immobility of the dental implant. Thus, an ideal dental implant should have its own peri-implant periodontium, like natural teeth. (21) Over the years, many strategies have been developed to improve the integration of implants through surface modifications to promote mechanical, physical, and chemical characteristics. Tissue engineering has ushered in a new era in periodontal regeneration, and more so in implant therapy. New technologies using guided proliferation of purified periodontal ligament stem cells appear to promote periodontal regeneration around dental implants, which is a major goal for optimizing dental implant therapy by providing the patient with a dynamic biological implant rather than an ankylosed and inert dental substitute. (47) The creation or regeneration of functional tissue is achieved through the use of an appropriate combination of three basic tools, namely cells, engineering scaffolds, and signaling molecules, which together are known as the triad tissue engineering (58). In addition to this triad, the texture of the titanium implant surface influences the final morphology and cell proliferation:

Souches Stem cells in tissue engineering of the periodontal ligament: Human mesenchymal stem cells were originally isolated from adult bone marrow punctures for their ability to form clonogenic clusters of adherent fibroblasts. In addition, they display the potential for widespread proliferation and can differentiate into cells of the mesodermal line, such as adipocytes, chondrocytes, and osteoblasts, both in vitro and ex vivo. Human mesenchymal stem cells are also reported to have endodermal and neuroectodermal differentiation potentials. (47)

Scaffolding in tissue engineering of the periodontal ligament: The delivery of periodontal ligament cells via nonwoven glycolic acid (PGA) is a viable approach in promoting the regeneration of periodontal tissue, and offers the possibility of ligament regeneration on dental implants. Human cells of the periodontal ligament were seeded and cultured on three-dimensional PGA scaffolds, where they adhered well and exhibited excellent matrix secretion capacity under optical and scanning electron microscopy; type I collagen was positively expressed in the cell-scaffold complex by immune histochemical staining. (47)

Growth factors: Growth factors are natural biological molecules that bind to surface receptors and regulate key cellular activities during tissue repair, proliferation, chemotaxis, differentiation and matrix synthesis. (59) Basic fibroblast growth factor (bFGF) has been shown to increase proliferation, production of hyaluronic acid, heparin sulfate and osteopontin, expression of MMP-1 (Matrix Metalloproteinase-1), MMP-3, MMP-9 and mRNA while the expression and activity of ALP (Alkaline Phosphatase) are inhibited, the formation of mineralized nodules, type I collagen, MMP-2, and tropoelastin mRNA. (47) Transforming growth factor β (TGF-β) has been reported to increase the cell surface proteoglycan genes of PDL such as syndecan-2 and β-glycan, and to promote DNA synthesis, fibronectin, the protein acid, osteonectin, and connective tissue growth factor (CTGF). In addition, it has an essential role in the fibroblastic differentiation of PDL from stem / progenitor cells, and in the maintenance of the PDL apparatus under physiological conditions. (47)

Titanium surface and regeneration of the periodontal ligament: The implant surface character is a factor in the design of the implant which affects the rate of its development and success. The modification of surface characteristics compared to topography or surface chemistry modify the behavior of cells and their morphology (47). Human cells of the periodontal ligament (hPDLCs) have shown improvement in cell adhesion, proliferation, and differentiation when assessed by cell morphology, cell count, and immunofluorescent staining of osteocalcin (OC) on a rough titanium surface (44). Interestingly, on rougher surfaces, cells showed reduced proliferation based on the reduced cell amount by fluorescent staining, and the expression of alkaline phosphatase mRNA was decreased. (21)

Conclusion: The innovative biotechnical advances of recent years are represented by stem cells and therapeutic cloning. Therapeutic cloning is a technique that has caused a lot of ink to flow and has fantasized more than one. This biothechnology offers a multitude of potential applications that could help treat diseases previously considered incurable. However, we are far from being able to apply cloning because it poses technical and ethical problems. Presumably, over time, these problems will diminish. Indeed, the technical limits are always pushed back, as for ethical problems we could compare them to those posed by human dissection or even more recently in vitro fertilization about thirty years ago: which seemed unacceptable has now passed into our customs.

So would therapeutic cloning be like this for tomorrow?

REFERENCES

Andrea C, Sinanan M, Nigel P et coll. Human adult craniofacial muscle-derived cells: neural-cell-adhesion-molecule (NCAM; CD56)-expressing cells appear to contain multipotential stem cells. Biotechnol Appl Biochem 2004; 40/25-34.

Andreoletti M, Pages JC, Mahieu D et coll. Preclinical studies for cell transplantation: isolation of primate fetal hepatocytes, their cryopreservation, and efficient retroviral transduction. Human gene Ther (1997) 8/267-274.

Barrandon Y, Bonfils F, Brezin C et coll. Les cellules souches adultes et leurs potentialités d'utilisation en recherche et en thérapeutique. Comparaison avec les cellules souches embryonnaires. La Doc Fr (2000)/1-67.

Botero M.T, E.Nör J. Tissue Engineering Strategies for Endodontic Regeneration. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/419-430.

Chen L, Qing SD, Lei W, Jun R Z, Li AW, Bao LL. Dental implants with the periodontium: A new approach for the restorationof missing teeth. Med Hypo 72 (2009)/58-61.

Chen MF, Sun HH, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials (2012) 33(27)/6320-44.

Chen Y, Bei M, Woo I et coll. Msx1 controls inductive signaling in mammalian tooth morphogenesis. Development (1996) 122(10)/3035-3044.

Chen Y, Yu Y, Chen L, Ye L, Cui J, Sun Q, Li K, Li Z, Liu L. Human Umbilical Cord Mesenchymal Stem Cells: A New Therapeutic Option for Tooth Regeneration. Stem Cells Int. (2015)/549432.

Coulombel L. Cellules souches: un seul nom pour de multiples entités. Rev. Fr. des laboratoires -Décembre 2010- N°427.

De Vos J, Assou S, Tondeur S, Dijon M, Hamamah S. Les cellules souches embryonnaires humaines : de la transgression de l'embryon humain à la médecine régénératrice de demain. Gynécologie Obstétrique & Fertilité 37 (2009)/620-626.

Ding Y, Lian Q. Optimal cells for cardiac repair and regeneration. Pathology and Therapies (2014)/63-98.

Duailibi SE, Duailibi MT, Zhang W et coll. Bioengineered dental tissues grown in the rat jaw. J Dent Res (2008) 87(8)/745-750.

Egusa H, Kobayashi M, Matsumoto T, Sasaki JI, Uraguchi S, Yatani H. Application of cyclic strain for accelerated skeletal myogenic differentiation of mouse bone marrow-derived mesenchymal stromal cells with cell alignment.Tissue Eng Part A, (2012)/0164.

Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry – Part I: Stem cell sources. J. of Prosth. Res. 56 (2012)/151-165.

Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry – Part II: Clinical applications. J. of Prosth. Res. 56 (2012)/229-248.

Fischbach D.G, L.Fischbach R. Stem cells: science, policy, and ethics. J Clin Invest. (2004 Nov 15) 114(10)/1364-1370.

Gebhardt M, Murray PE, Namerow KN et coll. Cell survival within pulp and periodontal constructs. J Endod (2009) 35(1)/63-66.

Geenen V. Cellules souches embryonnaires, clonage reproductif, clonage thérapeutique: discussion et discernement bioéthiques. Science & Culture n°401 Mai-Juin 2006.

Germain D. Les cellules souches pluripotentes induites. Pathologie Biologie 57: (2009)/555-559.

González S, Ibáñez E, Santaló J. Establishment of mouse embryonic stem cells from isolated blastomeres and whole embryos using three derivation methods. J Assist Reprod Genet. (2010 Dec) 27(12)/671-682.

Gronthos S, Mankani M, Brahim J, Gehron Robey P, Shi S.Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Cell Biology. 2000; 97(25)/13625-13630.

Honda JM, Toriumi T, Oka K, Saito Y, Isokawa K. Dental Stem Cells for Tooth Tissue Engineering. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/347-358.

http://bite-it.helsinki.fi/

http://www.embryology.ch/francais/sdigestive/ gesicht05.html

Hung CN, Mar K, Chang HC, Chiang YL, Hu HY, Lai CC, Chu RM, Ma CM. A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. Biomaterials 32 (2011)/6995-7005.

Kaku M, Akiba Y, Akiyama K, Akita D, Nishimura M. Cell-based bone regeneration for alveolar ridge augmentation–Cell source, endogenous cell recruitment and immunomodulatory function. J of prosth res., Vol 59, Issue 2 (2015)/96-112.

Kalmani M.K, Treves S, Yafee M et coll. The Lamina Propria of Adult Human Oral Mucosa Harbors a Novel Stem Cell Population. Stem Cells 2010; 28(5)/984-995.

Kögler G, Sensken S, Airey JA, et coll. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med (2004) 200(2)/123-35.

Kojima T, Kanemaru S, Hirano S, Tateya I, Ohno S, Nakamura T, et coll.Regeneration of radiation damaged salivary glands with adipose-derived stromal cells. Laryngoscope (2011) 121/1864-9.

Krebsbach P.H, G.Robey P. Dental and skeletal stem cells: potential cellular therapeutics for craniofacial regeneration. J Dent Educ (2002) 66 (6)/766-773.

Langer R, Vacanti JP. Tissue engineering. Science (1993) 260(5110)/920-6.

Li B, Jin Y. Periodontal Tissue Engineering: Current Approaches and Future Therapies. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/471-482.

Lombaert IM, Brunsting JF, Wierenga PK, Faber H, Stokman MA, Kok T, et coll. Rescue of salivary gland function after stem cell transplantation in irradiated glands. PLos One 2008; 3/e2063.

Manipal S, John J, Saravanan S, Arumugham I.M. Stem cells in dentistry-A review. Ind. J. of Den. (2013)/1-5.

Maria OM, Khosravi R, Mezey E et coll. Cells from bone marrow that evolve into oral tissues and their clinical applications. Oral Dis 2007; 13(1)/11-16.

Masaru K, Yosuke A, Kentaro A, Daisuke A, Masahiro N. Cell-based bone regeneration for alveolar ridge augmentation–Cell source, endogenous cell recruitment and immunomodulatory function. J. of Prosth. Res. Vol 59, Issue 2, (2015)/96-112.

Mcculloch CA. Progenitor cell populations in the periodontal ligament of mice. Anat Rec 1985; 211(3)/258-262.

Michaud J. Les Clonages. Méd & Droit (2002) 54/1-1.

Mitsiadis TA, Smith MM. How do genes make teeth to order throught development? J Exp Zool B Mol Dev Evol (2006) 306(3)/177-182.

Moussa MR, H.Yassin H, M.Saad M, B.Nagy N, K.Marei M. Periodontal Tissue Engineering Around Dental Implants. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/765-774.

Murphy C.N, F.Bissada N, Davidovitch Z, Kucska S, Dashe J, H.Enlow D. Tissue Engineering and Stem Cell Therapy for Orthodontists. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/799-822.

Nanduri LS, Maimets M, Pringle SA, V.Zwaag M, V.Os RP, Coppes RP. Regeneration of irradiated salivary glands with stem cell marker expressing cells. Radiother Oncol 2011; 99/367-72.

Ogier J. Principes fondamentaux et approches en ingénierie des tissus dentaires. UFR Odontologie Strasbourg, 2010.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et coll. Multilineage potential of adult human mesenchymal stem cells. Science (New York) (1999) 284/143-7.

Prochazkova M, G.Chavez M, Prochazka J, Felfy H, Mushegyan V, D.Klein O. Embryonic Versus Adult Stem Cells Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/249-262.

Renard E, S.Lopez-Cazaux, Guicheux J, Weiss P, Laboux O, Alliot-Licht B. Les cellules souches de la pulpe dentaire. C. R. Biologies 330 (2007)/635-643.

Roelen B. Cloning: History and Current Applications. Stem Cells (2014)/131-161.

Saghiri M.A, Asatourian A, M.Sorenson C, Sheibani N. Role of Angiogenesis in Endodontics: Contributions of Stem Cells and Proangiogenic and Antiangiogenic Factors to Dental Pulp Regeneration. J Endod. (2015) 41(6)/797-803.

Scheller EL, Krebsbach PH, Kohn DH. Tissue engineering: state of the art in oral rehabilitation. J Oral Rehabil (2009) 36(5)/368-398.

Seo BM, Miura M, Gronthos S et coll. Investigation of multipotent postnatal stem cells from human peridontal ligament. Lancet (2004) 364(9429)/149-155.

Seo BM, Song IS, Um S, Lee JH. Periodontal Ligament Stem Cells. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015) 291-296.

Shin Y.S, F.Rios H, V.Giannobile W, Oh TJ Periodontal Regeneration: Current Therapies. Stem Cell Biology and Tissue Engineering in Dental Sciences (2015)/459-469.

Soggia A , Hoarau E , Bechetoille C, Simon MT , Heinis M , Duvillié B. Cell-based therapy of diabetes: What are the new sources of beta cells? Diabetes & Metabolism 37 (2011)/371-375.

Swelstad B.B, Kerr C.L Current protocols in the generation of pluripotent stem cells: theoretical, methodological and clinical considerations. Stem Cells Cloning. (2010) 3/13-27.

Takeuchi T, D.Palermo G. Implications of cloning technique for reproductive medicine. Reproductive BioMedicine, Vol 8. No 5. (2004)/509-515.

Tubiana M. Colloque cellules souches : conclusion C. R. Biologies 325 (2002)/1065-1071.

Wegnez M. Clonages. Paris: CNRS (2007)/94-103.

Yu H, Yang X, Cheng J, Wang X, Shen SG.Distraction osteogenesis combined with tissue-engineered cartilage in the reconstruction of condylar osteochondral defect. J Oral Maxillofac Surg (2011) 69/558-64.

Zhang L, Morsi Y, Wang Y, Li Y, Ramakrishna S. Review scaffold design and stem cells for tooth regeneration. Japanese Dental Science Review (2013) 49/14-26.

****