Universidad Europea

TRABAJO DE FIN DE GRADO

Grado en Odontología

APPROACHING SCIENCE TO THE DENTAL CLINIC: IS IT POSSIBLE TO REGROW A TOOTH?

Madrid, curso 2020/2021

Número identificativo 250

ABSTRACT

Introduction: The definitive tooth loss usually results from the failure of an uncontrolled tooth pathology. Nowadays, replacement therapy options are restrained to prosthesis (placement of implant through surgery, partial removable or fixed prosthesis). The drawbacks resulting from such treatment introduced the need to develop more advantageous alternatives. The ambitious idea of regenerating dental tissues, either naturally or through bioengineering involves the association of stem cells, growth factors, bioactive molecules.

Objectives: This study aims to analyze the possibilities to regrow a tooth and the potential procedures associated. The possibilities of using stem cells to induce the generation of every tissue of a dental tooth will be analyzed along with the concerns and differences that exist for the regrowth of each of these tissue categories. The implication of other tools like scaffold, growth factors and bioactive molecules will also be studied. The practical aspects such as the source, type and storage of the stem cells will be included in the review.

Materials & Methods: Journal articles have been searched on the following websites: Science direct, Elsevier, Pubmed and Mendeley. Keywords: Tooth regrow, Regeneration, Stem cells, iPSC, MSC, ESC, DPSC, Odontogenesis, Growth factors, Tissue engineering, Dental scaffold. 46 articles from the last 20 years have been carefully selected.

Results: Various methods have been studied to permit the regrowth of the tooth, mainly through the tooth bioengineering which permitted the whole regrowth of a tooth in vivo, followed by its implantation into a mice alveolus, or by the generation of tooth germ, which was also implanted and leaded to the tooth regrowth *in situ*.

Conclusion: Further research regarding tooth development in addition to technological advances permits to potentially consider a positive outcome for the years to come and a promising alternative therapy for tooth loss in the future.

RESUMEN

Introducción: La pérdida definitiva de dientes suele ser el resultado del fracaso de una patología dental no controlada. Las opciones terapéuticas de sustitución se limitan a las prótesis (colocación de implantes mediante cirugía, prótesis parcial removible o fija). Los inconvenientes derivados de estos tratamientos introdujeron la necesidad de desarrollar alternativas más ventajosas.

Objetivos: Este estudio pretende analizar las posibilidades de hacer crecer de nuevo un diente y los posibles procedimientos asociados. Se analizarán las posibilidades de utilizar células madre para inducir la generación de cada uno de los tejidos de un diente, así como las preocupaciones y diferencias que existen para el recrecimiento de cada una de estas categorías de tejidos. Se estudiará la implicación de otras herramientas como andamios, factores de crecimiento y moléculas bioactivas. Los aspectos prácticos como la fuente, el tipo y el almacenamiento de las células madre se incluirán en la revisión.

Materiales y métodos: Se han buscado artículos de revistas en los siguientes sitios web: Science direct, Elsevier, Pubmed y Mendeley. Palabras clave: Regeneración de dientes, Regeneración, Células madre, iPSC, MSC, ESC, DPSC, Odontogénesis, Factores de crecimiento, Ingeniería de tejidos, Andamio dental.

Resultados: Se han estudiado varios métodos para permitir el recrecimiento del diente, principalmente a través de la bioingeniería dental que permitía el recrecimiento completo de un diente *in vivo*, seguido de su implantación en el alvéolo de ratones, o mediante la generación de germen dental, que también se implanta dando lugar al recrecimiento del diente *in situ*.

Conclusión: La continuación de la investigación sobre el desarrollo de los dientes, junto con los avances tecnológicos, permite considerar potencialmente un resultado positivo para los próximos años y una prometedora terapia alternativa para la pérdida de dientes en el futuro.

INDEX

I. INTRODUCTION

A. Causes and consequences of tooth loss

Tooth loss is a frequent consequence of aging along with factors such as disease or poor dental hygiene. According to the FDI, in people aged from 65 to 74 years, 30% have lost the totality of their teeth and 15 to 20% of middle-aged adults present severe periodontal disease which may lead to tooth loss. In the United States, the percentage of adults over 65 years old with untreated caries was 19% in 2011-2012. (1) Periodontal disease and tooth decay are the two most prevalent diseases; both have great risk of leading to tooth loss if left untreated, which explains why a great percentage of the population suffer from the lack of one or several teeth. A patient that has lost the integrality of his natural teeth will be described as edentulous. In 2010, the percentage of edentulous individuals was 2.3%, which corresponds to 158 million people worldwide. (2) The loss of a tooth could also occur due to a trauma, a failed endodontic treatment, or could be multifactorial. In the case of an agenesia, the patient suffers from a genetical anomaly resulting in the absence of one or several teeth, usually the lateral incisors. Nowadays, this condition can be solved through the use of prosthetics or orthodontics. In a study, it has been found that among these patients missing a tooth, the consequence that has been noticed the most was the difficulty of mastication. (3) Other consequences include the collapse of the face (especially in complete edentulous patients), drifting of the remaining teeth and supra-eruption. The implication of a missing tooth in the quality of life of the patient is significant, especially in the case of an anterior tooth loss. (4) The psychological effect of tooth loss could have major importance in the patient's daily life. A relationship between dementia and tooth loss has been demonstrated in a meta-analysis and systematic review. The

cognitive function could be indirectly impacted by the masticatory dysfunction associated with the lack of a tooth. A poor nutrition could lead to the reduction of cerebral blood flow, therefore producing loss of memory and eventually a decline in cognition. (5)

Nowadays, a limited amount of treatment options can be offered to the patient.

B. Implants: the treatment of choice

A missing tooth due to a pathology or an agenesia affects many patients and the solution is not always evident. In the best of the cases, the patient has the financial resources to receive an implant. Nowadays, an implant is the most effective way to replace a tooth and presents a success rate of 90% with a great predictability. (6) However, it raises some conditions, apart from the expensiveness. The treatment planning includes bone measurement and choice of placement, taking into account the antagonist and adjacent teeth and the type of prosthesis that the implant will support. In addition to these conditions, we should also consider the limitations of the treatment. Once the implant is placed, a large number of factors must be taken into account to avoid its failure.

Although the implant is considered the most effective way to replace a tooth, it does present an important number of disadvantages which must considered. For instance, the expensiveness, the bone availability and the risk of failure. The duration must also be taken into consideration, starting from the diagnostic and treatment planning until the end, when the artificial tooth is finally in mouth. All of these steps will take several months to a year to be achieved.

According to a study, implant failure during the 1st year after its placement occurred in 2% of the cases, due to various factors like lack of primary stability or failed osseointegration.(6) This percentage is quite low, nevertheless it exists and therefore should be considered. The lowest failure rate must be sought. The negative outcome of this surgery is not only the patient

remaining without a tooth, but also that he/she will suffer from bone loss and possible infections such as suppuration, soft tissue hyperplasia and other more serious complications. (7) The most prevalent disorders following implant surgery include peri-implantitis, peri-implant mucositis and failure of esthetic restoration. (6) The acceptation by the patient's body and mind is of primary importance. The psychological impact is often forgotten, during treatment planning and after treatment. Therefore, the dentist must take special considerations when choosing the patient. Respecting the physiological aspect of the patient's implant tolerance, the lack of proprioception due to the missing periodontal ligament, in addition with the absence of pulpal response to thermal stimuli impede the protective role of the natural tooth. (8)

Some risk factors associated with implant failure could be considered as a contraindication for the placement of the implant. When treating a patient who is a smoker, suffering from diabetes mellitus or with a compromised immunity, a removable prosthesis could be more adequate. Absolute contraindications include patients with heart disorders, for instance, patients who underwent a heart valve surgery, a recent myocardial infarction and patients under intravenous bisphosphonates. (6)

C. Removable and fixed Prosthesis

In some cases, a removable prosthesis will be the rehabilitation advised to the patient. For instance, the choice will be made according to possible financial limitation, hygiene difficulties or patient's state of mind. This non-invasive option to replace one or multiple teeth while restoring function usually adapts well to the patient's requirements. In 2020, a review from the Journal of Dentistry has estimated that one in five adults in the United Kingdom was wearing a removable prosthesis, either partial or complete. (9) However, it raises some disadvantages that the patient must be aware of. The design of the denture must be carefully planned to reduce

the risk of failure. Recent studies have proven the association between poor removable partial denture (RPDs) design and root caries, gingivitis, periodontal diseases and plaque retention. Considering that the main reason of tooth loss in the population is related with bad oral hygiene, practicians must consider the patient's motivation when planning the RPD, as its risk of failure will significantly increase in the case of patients with poor oral hygiene. (10) Moreover, the prosthesis will need to be rebased after some time, as the oral cavity could suffer a lot of changes as time passes by.

The outcome of the prosthesis treatment will hardly be entirely satisfying for every patient. Some of the patient's expectations will be more easily achieved than others but it is very likely that one of them will not, for instance the esthetics. A dental health impairment "may have a detrimental effect on psychological and social measures, such as self-confidence, social avoidance, anxiety, and emotional distress".(11) Some patients have presented high psychological disturbance even while adapting well to the denture because of their lack of adjustment to changes. The wellbeing, psychological and emotional state should be included when planning on the restorative procedure. (9)

The satisfaction of the patient is an important factor in the success of the prosthesis. If the patient doesn't feel comfortable while wearing it, the risk of reducing the time of wearing the implant during the day increases. A retrospective study has found that after 5 years, 39% of the RPDs were no longer used by the patient. (10)

Although fixed prosthesis is much more convenient because of the minimal care needed, the possibility of intentionally damaging neighboring teeth represents a great disadvantage.

D. Challenges

The imitation of the embryonic process of odontogenesis is the leading idea of several research projects, with the objective of regenerating dental tissues. Six main stages lead to the development of the tooth: Initial stage, Bud stage, Cap stage, Bell stage, Appositional stage and Root development. These different steps result from the interaction between the ectomesenchyme and the ectoderm, guided by growth factors and signal molecules. (12) As it is already used in tissue regeneration, the implication of stem cells is for now essential in the process of regrowing a tooth. Dental tissue engineering consists of the association of endothelial cells, growth factors, bioactive molecules and biomaterials in order to achieve the most natural dental tissue regeneration possible. (13) Recent successful research in the field of dental bioengineering have studied pulp regeneration as endodontic treatment. (14)

The growth of a soft tissue like the pulp and a hard tissue like enamel contains different characteristics and presents the greatest challenge in the attempt of regrowing a tooth.

Several scientific research have considered different ways to regrow a tooth, either by creating a bioengineered tooth from a 3D biomimetic tooth bud model through the use of photopolymerizable gelatin methacrylamide (GelMA) hydrogel formulas (15), or by cell compartmentalization *in vitro* for further *in vivo* transplantation (16) or even by recovering the tooth replacement capability lost during mammalian evolution. The ultimate goal of tooth bioengineering would be to regrow a tooth in its socket, achieving adequate function and esthetic, outperforming the usual 2 or 3 years it takes for a tooth to grow. (17) Some experiments have been made using an alginate scaffold from a mold of a human tooth. Dental pulp cells were isolated from exfoliated temporal teeth and seeded into the scaffold. (18) This kind of cell is considered multipotent and therefore can generate pulp-like tissue *in vivo*. (19)

E. Stem cells

The next promising approach to face the challenges mentioned above are focused on the use of stem cells. Due to its great ability to differentiate and proliferate into many different cell types, stem cells have been widely studied in the field of regenerative dentistry.

The stem cell has the ability of self-renewal and differentiation into different cell types. They can be described according to a hierarchy established with respect to their origin (embryonic or adult) and their range of potential to give rise to different cell types (totipotent, pluripotent, multipotent, unipotent). (Fig. 1)

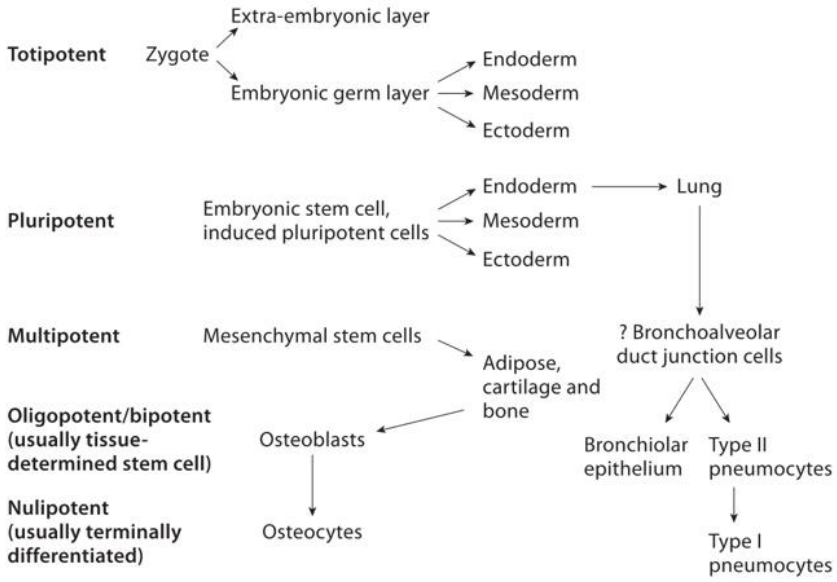


Figure 1. Stem cells hierarchy (20)

The very first undifferentiated cell involved in embryonic development is the totipotent or omnipotent cell. The endoderm, mesoderm and ectoderm originate from pluripotent stem cells that are found in the blastocyst stage. According to their origin, we will distinguish two types of pluripotent cells. The embryonic stem cell (ESC) presents a great ability for differentiation and self-renewal, as they arise from the inner cell mass of the blastocyst. In the presence of

transcription factors (Nanog, Oct4) they can be maintained in their undifferentiated state, frozen, and used for further experimentation by removing an inhibitory factor. (20)

Another type of pluripotent cell is the induced pluripotent stem cell (iPSC), which is derived from adult somatic cells. The pluripotency of these cells is regained through the expression of transcription factors (Oct3/4, Sox2, c-Myc and Klf4; or, Oct3/4, Sox2, Nanog, and Lin28) that are founds in ESCs. (21) Therefore, these cells have been able to differentiate into the three germ layers just like the ESCs do. (20) The use of the iPSC reduces the risks of immune rejection in comparison with allogenic cells. (22)

Mesenchymal stem cells (MSCs) are adult stem cells presenting a large differentiation capacity, although not as extensive as the pluripotent stem cells, they differentiate into cells from a single germ layer. However, their use still presents a great advantage, considering the ability of mesenchymal stem cells to differentiate into different tissues like bone, cartilage, muscle and adipose tissue. (23)

The oligopotent and unipotent stem cells are able to self-renew but present limited differentiation capacity compared to the cells that were previously described, as they have the ability to differentiate into only one or two lineages.

Dental pulp stem cells (DSPCs) belong to the MSC type. It has been shown that the pulp could regenerate in necrotic teeth of children with open apex, thanks to the presence of stem cells through the opened root apices. (13) After an injury, the DPSCs will be able to repair dentin and bone by differentiating into osteoblast and chondrocytes, but it has been demonstrated that these cells could potentially develop also into odontoblasts, cardiomyocytes, corneal epithelial cells, adipocytes, neuron cells, insulin secreting Beta cells and melanoma cells. Some research have been made regarding their capacity to regenerate *in vivo* dentin and pulp-like tissues. (24) (25)

Chondrogenic, neurogenic, adipogenic and odontoblast-like differentiation capabilities represent a great proof of the multipotency of the DPSCs. (26) The great interest for DPSCs depends on its location i.e., where it is derived from. Its accessibility represents a great advantage contrary to the MSC found in the bone marrow, placenta, umbilical cord and adipose tissue that are harder to obtain.(27) The easy accessibility of the MSCs isolated from dental tissue represents a significant advantage when compared to BMSCs, although both are great candidates for initiating the dentinogenesis with the help of growth factors. (28) Other sources of dental stem cells (DSC), apart from the DPSCs, have been found in human postnatal dental tissues: dental follicle stem cells (DFSCs), stem cells from human exfoliated dental tissues (SHEDs), stem cells from apical papilla (SCAPs), and periodontal ligament stem cells (PDLSCs). Although they share the capacity of self-renewal and differentiation into at least 3 lineages, some differences can be found regarding their proliferation rate, gene expression and plasticity. (26) (Figure 2)

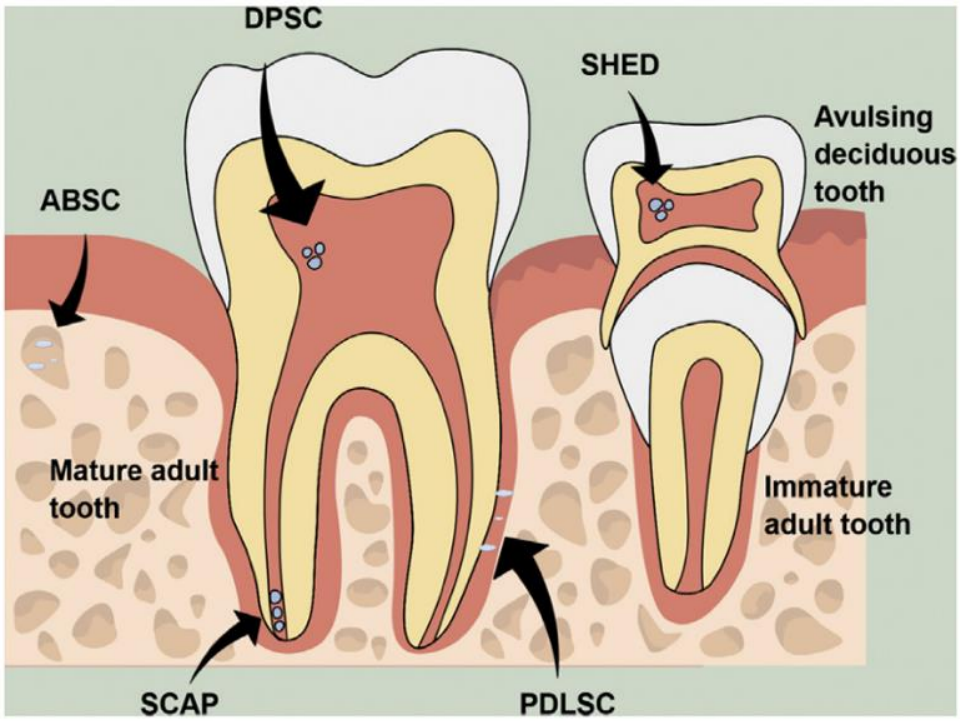


Figure 2. Most relevant dental stem cells sources in the tissues of the tooth (26)

The isolation of DSCs is achieved through the disaggregation of the tissue, either mechanically, enzymatically or by explant. The cells can then be cultured and maintained in a plastic dish under specific laboratory incubation conditions.

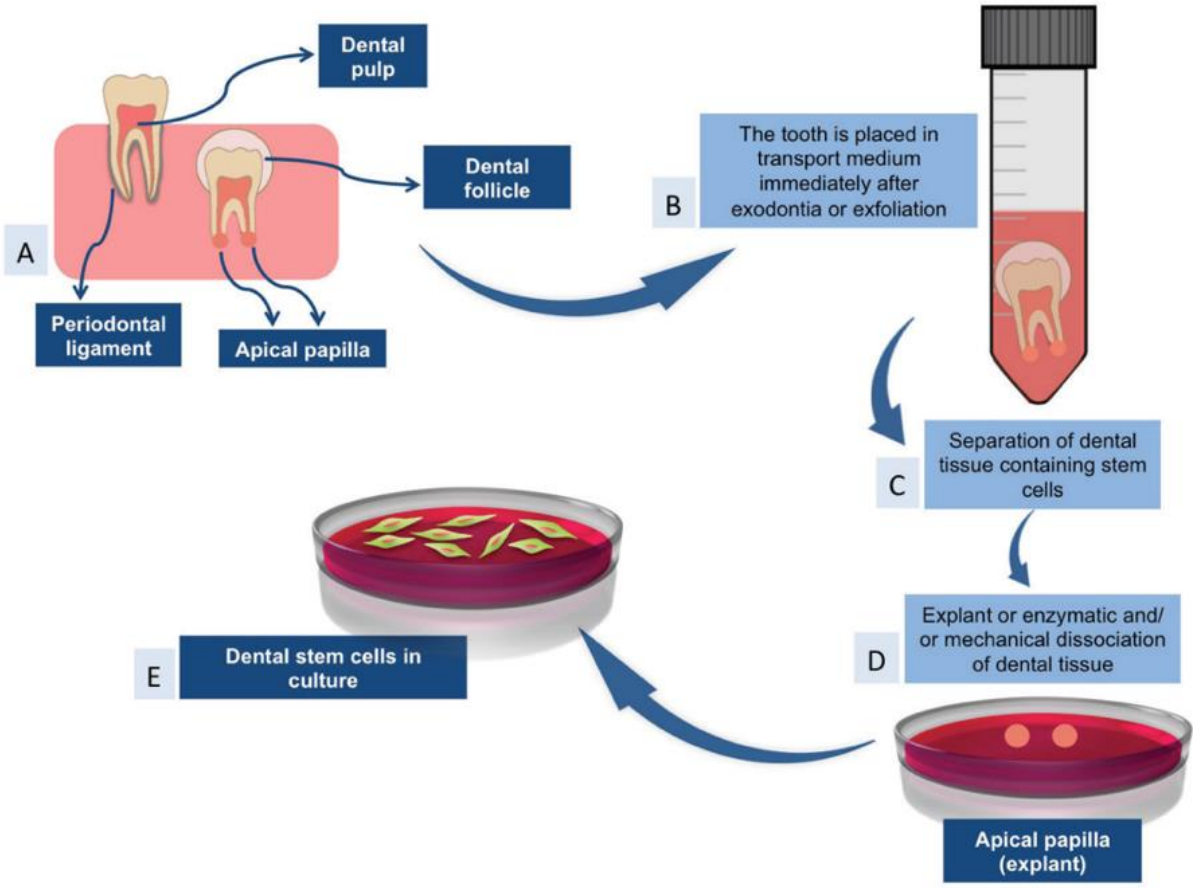


Figure 3. Isolation of dental stem cells (27)

A: DSCs sources

B: The tooth is brought to the lab right after the extraction/exfoliation

C: The dental tissue must be separated as one tooth possess different sources of DSCs

D: Enzymatic/mechanical dissociation or explant

E: Culture of DSCs

The absence of a tooth represents a major problem in the population and a recurrent demand in the dental clinic, therefore the need of finding a solution to this problem keeps on increasing. Nowadays, the implant is considered as the gold standard in tooth replacement therapy. Although it presents great advantages and a reasonably low percentage of failure, it could be less appropriate for some patients. The same applies for the removable prosthesis, which presents as main drawback the foreign object sensation. Therefore, the study of the possibility of regrowing a tooth by alternative means has become more and more relevant. The most interesting tools that remain to be explored further in detail are stem cells in order to regrow dental tissues, with the help of a scaffold and growth factors.

II. OBJECTIVES

PRIMARY OBJECTIVES

- Study the possibilities of using stem cells as a tool in tooth tissue bioengineering, permitting the regrowth of a tooth.

SECONDARY OBJECTIVES

1. Review the source, type and storage of stem cells
2. Analyze the implication of growth factors and bioactive molecules
3. Study the use of scaffolds to overcome the morphological challenges
4. Find the concerns and differences existing for the regrowth of each tissue constituting the tooth structure
5. Establish the differences between regenerating dental tissue from stem cells *in vitro* and *in vivo*: is tooth implantation after regrowth a good solution?

III. MATERIAL & METHODS

A literature review has been realized through the systematic research of electronic databases of different study designs. We have investigated the following websites: Science Direct, Elsevier, PubMed and Mendeley.

KEYWORDS

Teeth regrow, Regeneration, Stem cells, DPSCs, MSCs, ESCs, iPSCs, Odontogenesis, Growth factors, Tissue engineering, Dental scaffold, Tooth loss, Mesenchymal cells, Ectoderm, Implant failure, Removable prosthesis outcome.

INCLUSION CRITERIA

- Research articles, systematic reviews, meta-analysis, books, clinical trials
- Selection among the last 20 years
- Publications in English with complete text and free access

EXCLUSION CRITERIA

- Studies published more than 20 years ago
- Articles describing the use of stem cells for other medical purposes (regenerative therapy of organs etc.)

PubMed	Science direct	Elsevier	Mendeley
• Review: 15 • Articles: 28	• Review: 6 • Articles: 15	• Review: 17 • Articles: 29	• Review: 11 • Articles: 17

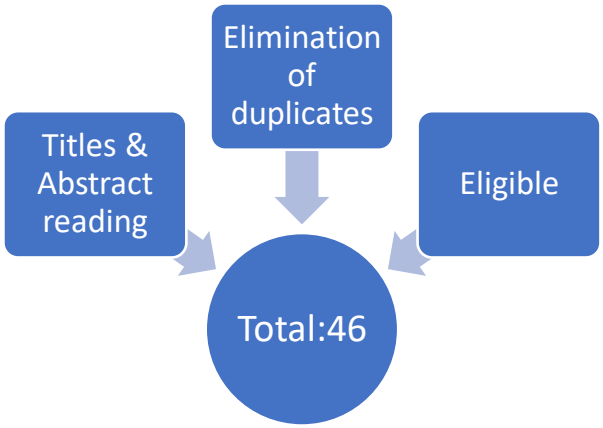


Figure 4. Process of articles selection

IV. RESULTS

A. Natural tooth regeneration

Most mammalians, including humans have the capacity of regrowing their teeth only once, whereas the majority of vertebrates are able to regrow their teeth many times throughout their life making the difference between a polyphyodont and a diphyodont. The reason why humans are not able to regenerate their teeth continuously throughout their life seems to be related with evolution, during which unknown factors have inhibited the capacity of the successional dental lamina to regrow teeth. The dental lamina plays an essential role in the tooth morphogenesis as it contains the stem cells necessary for the development of the tooth. (17) Therefore, finding a way to prevent this inhibition could lead to recover the capacity of generating further dentitions, subsequently inducing natural tooth regeneration *in situ*. The study of the mechanisms behind the regeneration of teeth in polyphyodonts could represent a lead in the tooth replacement researches.

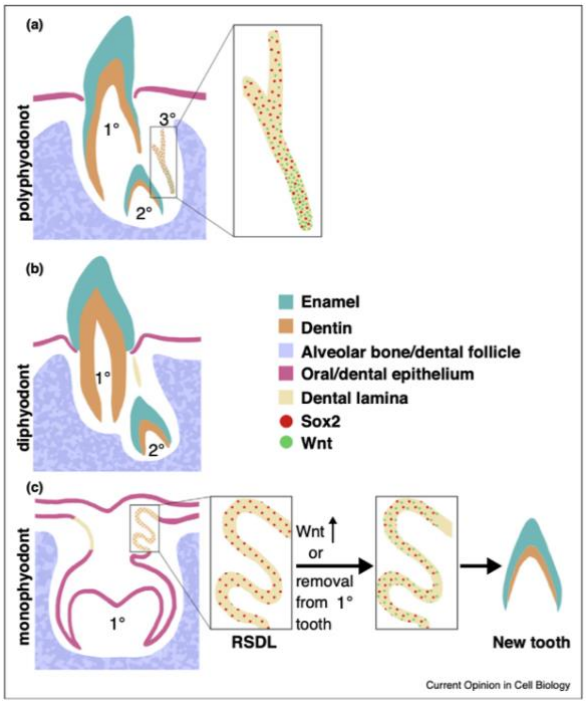


Figure 5. Diagram of tooth generation in a polyphyodont, diphyodont and monophyodont and its relationship with dental lamina (29)

While polyphyodonts possess a permanent continuous dental lamina, monophyodont do not replace their dental lamina once it has been involved in the tooth generation. Regarding diphyodonts, a sequence of mechanisms leading to the regression of the dental lamina only permits a second tooth development. The loss of the basement membrane by cell migration and apoptosis leads to the separation of the epithelial cells from the lamina. (29) In humans, if the degradation of the dental lamina is not achieved properly, the risk of developing epithelial pearls and, later, ameloblasts or tumors increases. This risk must be considered in the research for tooth regeneration from the dental lamina. (30) Since mouses are monophyodont, pigs have become the main model for studies about dental lamina, as they are diphyodonts and their dentition is similar to the own human's dentition. The dental lamina regression could be initiated by signals from the tooth or the surrounding mesenchyme. Therefore, by controlling these signals sent in order to regress the dental lamina, the production of additional tooth generations could be considered as replacement of lost teeth. (29) By the study of polyphyodonts like snakes or alligators, it has been proved the importance of the Wnt pathway for the expression of Sox2 by stem cells in the successional dental lamina, which is de-activated in diphyodonts or monophyodonts. Therefore, finding a way to re-activate the Wnt pathway could be studied in order to permit the tooth regrowth in humans. (22)

The inability of enamel regeneration once the tooth is erupted is explained by the apoptosis of the ameloblast and the loss of the epithelial stem cells. Regarding the periodontal ligament, its structure and cellular origin makes it the most challenging tissue to regenerate, in contrast to dentin whose repair capacity relies on the recruitment of mesenchymal stem cells through the vasculature. (31)

B. Tooth engineering

Tissue engineering is the generation of bioartificial tissues from the isolation and culture of stem cells in an appropriate environment with a three-dimensional structure. This will permit the correct differentiation and proliferation of stem cells, mediated by growth factors in order to generate the tissue required.

The National Institute of Health defines tissue engineering as: "the reconstruction of living tissues to be used for the replacement of damage or lost tissue/organs of living organisms, founded on principles of cell biology, developmental biology and biomaterial sciences"(32). Principles of developmental biology will therefore be copied in order to regenerate tissues. (33)

A cascade of events leading to *de novo* formation of the lost tissue to be regenerated will be based on the teamwork of the stem cells specific to the tissue, the adapted scaffold and bioactive molecules.(33) While tissue engineering consists of the implantation of cells that have been seeded *in vitro,* guided tissue regeneration is the implantation of a matrix in the host once the implantation is completed. (32)

The first experiment of tooth tissue regeneration *in vivo* was achieved in 1934 by Huggins through the autogenous transplantation of cells from the epithelial and mesenchymal layer of the developing tooth of a young dog in its abdominal wall. It resulted in the formation of enamel only when ameloblasts were in contact with odontoblasts, proving the necessity of the mesenchymal layer with respect to the production of enamel by the epithelial layer. (34)

The experiments conducted *in vivo* aiming to regenerate a tooth often involve the autologous transplant of stem cells from the post-natal tooth buds of animals.

The reforming of the embryogenesis steps by using epithelial and mesenchymal cells and the interaction between them leads to tissue reconstitution. The dental epithelium derives from the ectoderm and the dental mesenchyme from cranial neural crest; both of them interact through

signaling molecules. (35) Many studies about tooth bioengineering have been executed in mice, as its tooth development is very similar to the human's and the differences in the gene expression are minor. (17) (36) The presence of epithelia-derived signals (bone morphogenetic protein and fibroblast growth factor) permits the proliferation of mesenchymal cells and therefore the condensation around the thickened epithelium. This will permit a bud-like structure to be created. In 2018, research has successfully managed to recreate a tooth bud exhibiting the natural characteristics of a tooth, by recreating an odontogenic microenvironment using endothelial cells from human umbilical veins, cultured with post-natal dental cells. (37)

To reach the cap-like enamel organ, the dental mesenchyme's role will include the delivery of signaling molecules to the dental epithelium. The elongation of epithelial cells leads to its differentiation into pre-ameloblasts, while the odontoblasts will arise from the differentiation of the adjacent layer of the dental mesenchyme. The dentin matrix will be deposited by the odontoblasts which will be followed by the transition and maturation of ameloblasts.

Once the eruption of the tooth is completed, the loss of enamel-forming ameloblasts complicates the process of tooth bioengineering, as the regenerative capacity of enamel is lost. It is however possible to regenerate the dentin-pulp complex through mesenchymal stem cells found in the dental pulp. This dental pulp can be removed from exfoliated deciduous teeth, extracted molars and also from root apical dental papilla. (35) The inductive ability of the mesenchyme to form enamel on epithelium has been proved to be maintained even on epithelia with non-oral sources. The epithelium alone does not possess the ability of generating a whole tooth, since a tooth arises from two different tissues and the cooperation of epithelial cells with mesenchymal cells is required.(Fig. 6) (38) The use of clonal cell lines to be incorporated into the bioengineered tooth prepared with dental mesenchymal tissues has been studied and can therefore regenerate teeth. In the study of Takahashi et al. in 2010, the clonal cell lines were

obtained from the oral epithelium of embryonic mice fetuses. With these cell lines and fetal dental mesenchyme, a tooth germ cell has been reconstructed and implanted under a kidney capsule for 2 to 3 weeks. This experience has led to the formation of developed teeth with calcified structures similar to the ones encountered in a natural tooth. Therefore, the ability of tooth regeneration from dental epithelia has been proven. (39) In 2009, Ikeda et al. reconstructed a tooth germ *in vitro* from separated epithelium and mesenchyme acquired from the tooth germ in cap stage of mouse embryo molars. This tooth germ was then reimplanted in the murine alveolar bone. Adequate results were reached as the presence around the tooth of odontoblasts, periodontal ligament, cementum, pulp, blood vessels, and alveolar bone has been demonstrated. However, the morphology and orientation of the tooth obtained can't be controlled due to the lack of scaffold.

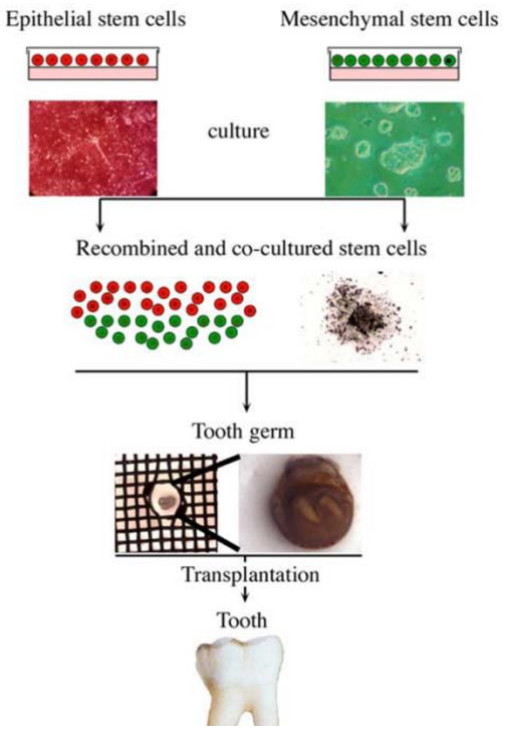


Figure 6. Tooth formation achieved through the association of dissociated epithelial and mesenchymal cells. The tooth germ is generated in vitro for further implantation into the alveolar bone to complete the development of a fully functional tooth. (39)

So far, two conventional methods have been developed with respect to the regeneration of a tooth. While both use the complete dissociation of epithelial and mesenchymal cells *in vitro*, one is directed towards the method of biodegradable scaffold and the other towards cell aggregation.

1. Scaffold

To permit the differentiation and proliferation of the implanted cells, and therefore the formation of the dental tissues, a biomimetic scaffold combined with bioactive molecules will create the ideal environment. Regarding the material, the use of macro biomolecules such as collagen, alginate and hyaluronic acid derivatives has been studied. The function of the scaffold in the tooth bioengineering will be to provide support for the cells and growth factors to be properly delivered at the site where the tissue regeneration will take place. (13) Therefore the type of scaffold must be wisely chosen, as it will have to present specific physical and mechanical properties due to its exposure to a challenging environment: the oral cavity. The scaffold will be subjected to strong mechanical forces through the mastication, and other conditions related with the presence of microorganisms, changes in temperature and pH. (22) An important physical property will be, for instance, the porosity and the factors influencing the cell adherence to the material. The matrix must be highly hydrated, allowing the transport of nutrients. This property will be encountered in polymeric materials, which counterbalance their potential lack of mechanical and biological properties. Inorganic materials will be more likely to possess great biological properties but could suffer from brittleness. Therefore, the material of choice to use as scaffold for tooth engineering will be composite materials, including organic and inorganic components. (22) Regarding the mechanical aspect, the scaffold material must present viscoelasticity. The biocompatibility of the material is imperative, and its

degradation process should follow the rate of tissue regeneration. The main objective of the scaffold will be to reproduce the processes executed by the extracellular matrix (ECM), involving the adhesion of the cell followed by its migration, differentiation and proliferation. (36)

Regarding the scaffold-base technique, the stem cells will be seeded into a biodegradable scaffold which will then be transplanted in the renal capsule or omentum of the animal. Although this method has been proved to be able to mimic the odontogenesis regeneration pattern, the timing, shape and size of the tooth created can hardly be controlled, resulting in several small tooth-like structures. To avoid this phenomenon, Honda et al. in 2007 have developed a technique, seeding epithelial and mesenchymal cells sequentially from the tooth bud of a pig third molar, resulting in the formation of a single tooth structure. (8)

Several scaffold-free techniques have been studied in order to overcome the disadvantages of the scaffold-base tooth regeneration. In 2007, Nakao et al. achieved the maturation of a tooth germ in a renal capsule through the co-culture of mesenchymal and epithelial cells in a collagen gel. This was later transplanted into the jaw, completing the formation of the tooth with adequate structure and growth of nerve fibers and blood vessels.

2. Signaling molecules

Signaling molecules participating in the process of tooth development must be included in the research in order to reach a whole-tooth regeneration similar to the natural growth of a tooth. In the dental mesenchyme, these molecules permit to regulate the macro-patterning of the size of the tooth. (40) By regulating the morphogenesis and development of the tooth, cytokines and other signaling molecules must be considered in the tooth regeneration. (40) Most of these molecules are tumor growth factor beta (TGF-β), fibroblast growth factor (FGF), wingless

integrated (Wnt), platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP) and hedgehog. (36) These growth factors can be included in the biomimetic scaffold with the objective of releasing them following the degradation rate of the scaffold, which must be similar to the cell´s growth rate as to not interfere with the growth of the tissue. The size of the pores is also involved in the capacity of the scaffold to release the bioactive molecules. (36)

C. Dental tissue regeneration

1. Enamel regeneration

The understanding of the enamel formation during the tooth generation plays an important role in the research of the enamel regrowth through the tooth bioengineering process. The interaction between the epithelium and the mesenchyme initiates the formation of enamel. While the epithelium is responsible for the enamel production, the mesenchyme will be the origin of the dentin-pulp complex and periodontal apparatus. (22) The amelogenesis depends on the signaling centers and growth factors such as Wingless/Int1 (Wnt) and Bone Morphogenetic Proteins (BMPs) which, for instance, will play a role in the differentiation phases post-natal. (22) Proteins such as amelogenin and ameloblastin serve as scaffold in order to guide the deposition of calcium and phosphorus ions. This will monitor the generation of enamel rods through the aggregation of hydroxyapatite crystals. Eventually, enamel will become an acellular tissue through the apoptosis of ameloblasts and degradation of the scaffold by matrix proteases, making a great challenge out of the enamel formation through bioengineering. (22) Moreover the attempt of ameloblast culture *in vitro* is, for now, vain. To overcome this issue, successful research was accomplished with the chemical synthesis of enamel, more precisely of hydroxyapatite nanorods. Fluorapatite acid paste was used in order to develop the growth of enamel-like structures on a human tooth. (36) The extreme conditions needed to produce

enamel (high temperature, high pressure, high pH) in addition to the use of some surfactants such as sodium hypochlorite make the direct synthesis in the oral cavity difficult. (33) With the use of an enamel protein matrix, the growth of elongated parallel apatite crystal has been achieved as a result of the interaction and combination of the elements involved in enamel engineering (enamel protein and calcium phosphate growth solutions). The protein amelogenin plays a big role in the production of enamel as it contributes to almost 90% of the development of the enamel layer. It has been possible to achieve the growth of enamel-like apatite *in vitro* (33).

2. Dentin-pulp complex regeneration

The odontoblasts are cells of mesenchymal origin which execute the dentin formation when migrating towards the pulp. The deposition of collagen, proteoglycans and other matrix proteins contributes to the formation of acellular calcified tissue constituting the dentin (70% hydroxyapatite, 20% organic materials, 10% water). Once the dentinogenesis is completed, the odontoblasts remain beneath the dentin, playing the main role in the dentin-pulp complex after an injury; the generation of tertiary or reparative dentin can be produced due to the activation and differentiation of DPSCs into odontoblasts. The dentin regeneration is triggered by changes in the environment, creating a stimuli (signaling molecules, pH, cytokines..). (22)(36) The odontoblasts possess the ability to secrete growth factors, especially TGF-β, which will induce the regenerative events in the dentin-pulp complex either *in vitro* or *in vivo*. Regarding the scaffold and the inductive microenvironment, treated dentin matrix (TDM) could play an important role. (36) However, this repair system occurring after an injury is not beneficial for research in tooth tissue engineering as the process of mobilizing DPSCs to the site of injury and differentiating into odontoblast-like cells is still unknown. (31) Moreover, the limited capacity

for regeneration of the dentin creates the need of choosing an appropriate source for the stem cell and differentiation strategy. The dentinogenic potential of DPSCs, SHEDs, iPSCs and BMSCs seem to be for now the most interesting information regarding dentinogenesis in the tooth bioengineering field. (22)

3. Cementum

Cementum is a calcified tissue produced by cementoblasts which is located between the periodontal ligament and the dentin. The cells involved in the cementogenesis could be DFSCs, PDLSCs, iPSCs. The cementum volume enlarges over time and its regeneration can be initiated by cementoblast precursors present in the periodontal ligament. In the case of a resorption due to a disease, cementum will not present any possibility of remodeling and regrowing, creating an issue regarding the whole-tooth regeneration objectives. (22)

D. Organ germ method

Also called the three-dimensional cell manipulation method, it aims to replicate the interactions between the epithelial and mesenchymal cells during organogenesis, by compartmentalizing these cells at high cell density. (40) In 2011, research by Oshama et al. obtained successful results through the generation of a tooth with correct structure *in vitro*, subsequently transplanted into the oral cavity. Between the bioengineered whole tooth and the alveolar bone, a bioengineered tooth unit constituted of periodontal ligament, mature tooth and alveolar bone have been grafted. In addition, to replicate the developmental mechanism of the bioengineered

tooth accurately, the imitation of epithelial-mesenchymal interactions through transcription factor pathways have been supplemented. (Figure 7) (41)

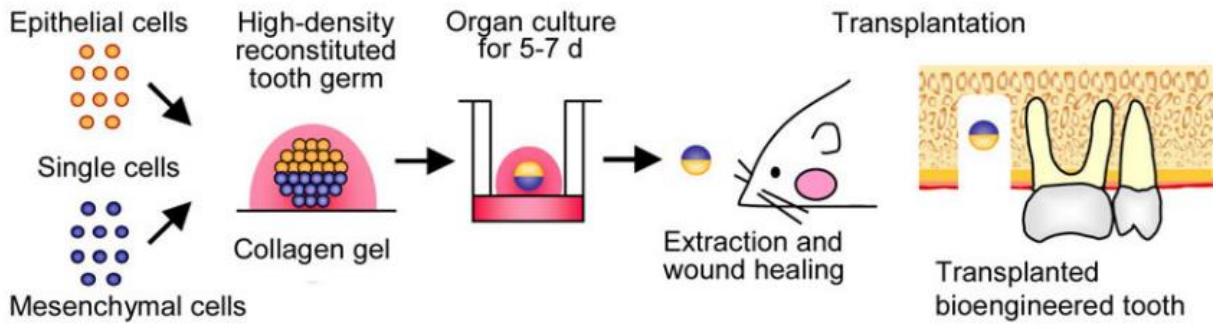


Figure 7. Schema of the method for generation and transplantation of reconstituted tooth germ (41)

Year	Author	Source of cell	Method	Outcome
1934	Huggins	Young dogs developing tooth germ: epithelial & mesenchymal layer	Recombined + transplanted into the abdominal wall	Where there was no mesenchymal layer, the epithelial layer did not generate enamel.
1970	Koller et al.	Mouse embryo: dental epithelium + papilla	Tissue recombination + transplant in the eye chamber (anterior part)	Proof of the odontogenic capacity of mesenchymal cells
1987	Mina et al.	Non-odontogenic ectomesenchyme of second arch	Combination with mandibular arch epithelium before embryonic day 12	The epithelium already possesses the odontogenic capability before Bud stage of tooth morphogenesis.
2004	Ohazama et al.	Oral epithelium from mouse embryo	Recombination technique at embryonic day 10 with non-dental cell aggregates (embryonic stem cell…)	Tooth primordia development.
2007	Nakao et al.	Co-culture of epithelial and mesenchymal cells seeded into collagen gel drop	3-dimensional organ-germ culture. Regeneration into renal capsule, implantation of tooth germ in jaw.	Correct tooth structure, growth of blood vessels and nerve fibers.
2009	Ikeda et al.	Epithelial and mesenchymal cells of the tooth germ of the embryonic molar of a mice at embryonic day 14	3-dimensional organ-germ culture (5-7 days) reaching the early bell stage, transplant in upper alveolar bone (molar level)	Correct tooth structure, size too small compared to the natural molar of mice..
2011	Oshima et al.	Cells of the tooth germ of a mice molar at embryonic day 14	3-dimensional organ-germ culture, size control method, mature tooth developed in sub-renal capsule, transplantation in the jaw	Development of single correctly structured tooth with periodontal tissue, alveolar bone regeneration, response to mechanical stimuli

Table 1. Summary of the most relevant studies on tooth bioengineering

V. DISCUSSION

Research regarding whole tooth bioengineering have evolved starting from the simple production of dental tissues towards the full growth of a complete and functional tooth structure. The tooth generated by Ikeda et al. does not only contain a good structure but also achieves the connection with nerve fibers allowing the bioengineered tooth to respond to stimulus. (41) The recovery of the nervous system through the reintegration of nerve fibers allows the peripheral nervous system to complete its role in the regulation of organ functions and permits the perception of external stimuli. This sensory feature of the tooth is crucial for its own protection and function. (41)

The lack of a functional periodontal ligament (PDL) represents a significant drawback of the implant as its essential roles in repair, tooth support and homeostasis cannot be imitated. In order to assess the reintegration of the PDL with the regenerated tooth, orthodontic tooth movements can be intended to verify the pathogenic and physiological response of the bone through extreme forces. (41) One disadvantage experienced in the tooth regeneration of Ikeda et al. is the small tooth size obtained. Oshima et al. developed the idea of transplanting the whole tooth unit in the alveoli instead of only the tooth germ. The tooth was grown inside the sub renal capsule of the mouth and was then implanted after reaching the adequate size and shape. (40) A functional occlusion must be reached through the development of cusps, fissures and grooves in order to fulfill the mastication purpose of the tooth. The low level of mineralization of enamel achieved through tissue bioengineering is, for now, not sufficient to succeed an adequate quality for the biomechanical loading of dental hard tissues. (22)

The therapeutic capability of mesenchymal stem cells in many medical fields raises the need to extract them and store them. However, the ethical drawbacks of collecting them from bone marrow or umbilical cords resulted in the need for studies to find another source. Regarding

embryonic stem cell therapy, the ethical disadvantages remain and create strong societal limitations. (42)

The availability of DPSCs and the non-invasiveness of their collection represent significant benefits in comparison with the other sources of MSCs. The use of autologous dental stem cells represents a meaningful advance in tooth tissue bioengineering and presents a great quantity of advantages in comparison with allogenic stem cells transplant. The main benefit relies in the guarantee of compatibility since the donor receives stem cells from its own body. However, in the case of DPSCs, the need for a tooth extraction could represent an ethical limitation. Therefore, the banking of stem cells throughout one's life by cryopreservation of young and proliferative stem cells could result in a major advance for the future of bioengineering. This technique of banking stem cells for later use could solve some of the ethical problems arising from the source of mesenchymal stem cells. The exfoliated deciduous teeth of children around 6 to 12 years old are of great interest since the tooth extraction is no longer needed and the population of SHEDs becomes easily available. (42) The stem cells from human exfoliated deciduous tooth appears to be more proliferative and have a higher survival rate while having the same functional ability as stem cells from the pulp of a permanent tooth. Their capacity of differentiating into specialized tissues remains comparable as both are able to create dentin-pulplike tissues.(26) Considering the small quantity of stem cells contained in the pulp, the possibility of expanding their quantity in order to reach a therapeutical amount of stem cells reflects the great benefit of SHEDs. (42) Regarding the state of the tooth selected for stem cell banking, it is unclear whether it must be healthy or if the presence of carious lesion prevents the tooth from being manipulated. (42) This lack of information causes some uncertainty regarding stem cell banking from extracted or exfoliated teeth.

Although some researchers managed to regenerate a tooth *in vivo*, in every cases it has been achieved in ectopic places rather than *in situ*, and some crucial elements where lacking, for instance the periodontal tissue which is essential to anchor the unit into the alveolar bone. (43) The research made on tooth morphogenesis are mainly carried out on mice as its dentition is very similar to the human. As mice are monophyodont, the comparison regarding the signaling molecules pathway can be made with polyphyodonts, for example during the analysis of the Wnt pathway or to study the modulation of stem cell homeostasis. (30)

Creating the perfect "bio-tooth" is for now utopian. Although researchers have managed to develop a functional bioengineered tooth *in situ*, many questions remain unanswered regarding its viability. For now, it is still unclear the influence of the type of bone on the success of the regeneration, as well as the impact of potential systemic diseases, infections and aging of the patient.

Stem cells could potentially be considered as candidates for malignant transformation since many similarities can be found with cancer cells (e.g., relative apoptosis resistance, long life span, replication ability extended in time, growth regulators, independent growth). (44) The tumorigenic potential of stem cells also depends on other factors such as the site of administration, the culture *in vitro*, and the manipulation of these cells. The risk increases when culturing pluripotent stem cells such as ESC and iPSC.(45) When cultured in vitro, some mechanisms (cell cycle arrest or DNA repair) supposedly preventing the tumor formation could be defective. The tumorigenicity has been proved to increase proportionally with the length of the culture *in vitro* of stem cells. Therefore, the culture *in vivo* of these cells is favored but does not eliminate completely the tumorigenic potential of the procedure. The direct link between pluripotent stem cells and tumorigenesis has been confirmed in many research. (44)

Nevertheless, somatic stem cells (SSC) can and have been safely used for decades. Still, a lack of long-term follow-up impedes to exclude with safety the harmfulness of SSCs.

The different methods used to generate iPSCs could induce the growth of tumors, for instance through genetic modification by means of retroviruses and lentiviruses or by transgene reactivation. However, almost non-harmful methods have been found to reduce the risk of tumor formation, such as the replacement of viral integration by adenoviral vectors to encode reprogramming factors. (44) By introducing the transcription factor Nanog-iPSC which permits to silence four factors (Klf4, Oct3/4, cMyc, Sox2) after de-differentiation, the retroviral-based expression system becomes cancer-free through switching towards an adenovirus-based system. (46)

Apart from the risk of tumorigenesis, the response of the host immune system could present a complication in the stem cell therapy. Initially, ESCs present a low immunogenic potential and therefore almost doesn't require the immunosuppression of the host. Nevertheless, when these cells are used for a different function or in a different physiology, their differentiation increases the immunogenic potential, thus potentially creating an immune response from the host. The rejection of the graft could lead to losing the functions of the stem cells and should therefore be prevented by the intake of immune suppressants, which should be nonetheless taken carefully considering the side effects associated with the medication. As mentioned previously, an interesting option to avoid the immunological risk could be the banking of autologous stem cells from the dental pulp. (44)

VI. CONCLUSIONS

The increasing life expectancy plays a role in the society's growing need to find an alternative to existing tooth replacement therapy. Furthermore, several limitations associated with the wear of prosthesis favors the investigation for an option more natural, eliminating the potential drawbacks: risk of failure, sensation of wearing a foreign object, possible harm to surrounding tissues etc. The study of polyphyodonts has permitted to understand the operation by which these animals were able to generate teeth naturally. The re-activation of the Wnt pathway in the successional lamina, mimicking the mechanism of tooth regrowth in polyphyodonts could be the key to natural whole tooth regeneration as tooth replacement therapy. Further research will be necessary in order to reproduce this operation in humans.

The bioengineering of a tooth represents the ultimate goal of regenerative dentistry, attempting the recovery of a whole tooth complying with all masticatory and sensory functions, associated with an adapted size and morphology. According to MacArthur and Oreffo in 2005, tissue engineering aims at "understanding the principles of tissue growth, and applying this to produce the functional replacement tissue for clinical use."(43) For now, the regrowth of a tooth is still studied as possible therapy for tooth replacement but has not been fully achieved. While the clinical results are promising, the achievement of whole tooth regeneration in the everyday dental clinic *for now* is not conceivable. It consists most frequently in the culture of stem cells *in vitro*, which will then be seeded in the scaffold and then implanted *in vivo*, into the tooth socket. (36) The increasing knowledge in matter of tooth morphogenesis, stem cells and biomaterials plays an essential role in the whole tooth regeneration project. Further research regarding tooth development in addition to technological advances permit to potentially consider a positive outcome for the years to come and a promising alternative therapy for tooth loss in the future.

VII. BIBLIOGRAPHY

1. Dye BA, Thornton-evans G, Li X, Iafolla TJ. Dental Caries and Tooth Loss in Adults in the. 2015;(197):2011–2.
2. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJL, Marcenes W. Global Burden of Severe Tooth Loss: A Systematic Review and Meta-analysis. J Dent Res. 2014;93(July):20S-28S.
3. Dosumu OO, Ogunrinde JT, Bamigboye SA. Knowledge of consequences of missing teeth in patients attending prosthetic clinic in u.C.h. Ibadan. Ann Ibadan Postgrad Med [Internet]. 2014;12(1):42–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25332700%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4201933
4. Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM, Creugers NHJ. Tooth loss and oral health-related quality of life: A systematic review and meta-analysis. Health Qual Life Outcomes [Internet]. 2010;8(1):126. Available from: http://www.hqlo.com/content/8/1/126
5. Fang WL, Jiang MJ, Gu BB, Wei YM, Fan SN, Liao W, et al. Tooth loss as a risk factor for dementia: Systematic review and meta-analysis of 21 observational studies. BMC Psychiatry. 2018;18(1):1–11.
6. Castellanos-Cosano L, Rodriguez-Perez A, Spinato S, Wainwright M, Machuca-Portillo G, Serrera-Figallo MA, et al. Descriptive retrospective study analyzing relevant factors related to dental implant failure. Med Oral Patol Oral y Cir Bucal. 2019;24(6):e726–38.
7. Sakka S, Coulthard P. Implant failure: Etiology and complications. Med Oral Patol Oral Cir Bucal. 2011;16(1):42–4.

8. Bhanja A, D'Souza DSJ. Mapping the milestones in tooth regeneration: Current trends and future research. Med J Armed Forces India [Internet]. 2016;72:S24–30. Available from: http://dx.doi.org/10.1016/j.mjafi.2016.05.004

9. Kudsi Z, Fenlon MR, Hill K, Baysan A. Developing a questionnaire to measure psychological disturbance associated with tooth loss. J Dent [Internet]. 2020;98(January):103353. Available from: https://doi.org/10.1016/j.jdent.2020.103353

10. Campbell SD, Cooper L, Craddock H, Hyde TP, Nattress B, Pavitt SH, et al. Removable partial dentures: The clinical need for innovation. J Prosthet Dent [Internet]. 2017;118(3):273–80. Available from: http://dx.doi.org/10.1016/j.prosdent.2017.01.008

11. Mijiritsky E, Lerman Y, Mijiritsky O, Shely A, Meyerson J, Shacham M. Development and validation of a questionnaire evaluating the impact of prosthetic dental treatments on patients' oral health quality of life: A prospective pilot study. Int J Environ Res Public Health. 2020;17(14):1–19.

12. de Sousa-Romero L. Growth and Transcription Factors in Tooth Development. Int J Oral Craniofacial Sci. 2016;2:015–29.

13. Horst O V., Chavez MG, Jheon AH, Desai T, Klein OD. Stem Cell and Biomaterials Research in Dental Tissue Engineering and Regeneration. Dent Clin North Am [Internet]. 2012;56(3):495–520. Available from: http://dx.doi.org/10.1016/j.cden.2012.05.009

14. Schmalz G, Widbiller M, Galler KM. Clinical Perspectives of Pulp Regeneration. J Endod [Internet]. 2020;46(9):S161–74. Available from: https://doi.org/10.1016/j.joen.2020.06.037

15. N Monteiro, EE. Smith, S Angstadt, W Zhang, A Khademhosseini PCY. Dental Cell Sheet Biomimetic Tooth Bud Model. Physiol Behav [Internet]. 2017;176(10):139–48.

Available from: file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentar na qualificação/The impact of birth weight on cardiovascular disease risk in the.pdf

16. Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, et al. The development of a bioengineered organ germ method. Nat Methods. 2007 Mar;4(3):227–30.
17. Li L, Tang Q, Wang A, Chen YP. Regrowing a tooth: in vitro and in vivo approaches. Curr Opin Cell Biol [Internet]. 2019;61:126–31. Available from: https://doi.org/10.1016/j.ceb.2019.08.002
18. Smith AJ, Smith JG, Shelton RM, Cooper PR. Harnessing the Natural Regenerative Potential of the Dental Pulp. Dent Clin North Am [Internet]. 2012;56(3):589–601. Available from: http://dx.doi.org/10.1016/j.cden.2012.05.011
19. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental Pulp Tissue Engineering with Stem Cells from Exfoliated Deciduous Teeth. J Endod [Internet]. 2008;34(8):962–9. Available from: http://dx.doi.org/10.1016/j.joen.2008.04.009
20. Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. Respiration. 2012;85(1):3–10.
21. Zhang F, Citra F, Wang DA. Prospects of induced pluripotent stem cell technology in regenerative medicine. Tissue Eng - Part B Rev. 2011;17(2):115–24.
22. Baranova J, Büchner D, Götz W, Schulze M, Tobiasch E. Tooth formation: Are the hardest tissues of human body hard to regenerate? Vol. 21, International Journal of Molecular Sciences. MDPI AG; 2020.
23. Sobhani A, Khanlarkhani N, Baazm M, Mohammadzadeh F, Najafi A, Mehdinejadiani S, et al. Multipotent stem cell and current application. Acta Med Iran. 2017;55(1):6–23.
24. Potdar PD. Human dental pulp stem cells: Applications in future regenerative medicine.

World J Stem Cells. 2015;7(5):839.

25. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000;97(25):13625–30.

26. Sedgley CM, Botero TM. Dental Stem Cells and Their Sources. Dent Clin North Am. 2012;56(3):549–61.

27. Almeida PN, Cunha KS. Dental stem cells and their application in Dentistry: a literature review. Revistas. 2016;73(4):331.

28. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, et al. Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell. 2007;99(8):465–74.

29. Buchtová M, Štembírek J, Glocová K, Matalová E, Tucker AS. Early regression of the dental lamina underlies the development of diphyodont dentitions. J Dent Res. 2012;91(5):491–8.

30. Tsai S, Abdelhamid A, Khan MK, Elkarargy A, Widelitz RB, Chuong CM, et al. The Molecular Circuit Regulating Tooth Development in Crocodilians. J Dent Res. 2016;95(13):1501–10.

31. Balic A. Biology explaining tooth repair and regeneration: A mini-review. Gerontology. 2018;64(4):382–8.

32. Nigar G, Khawaja NG, Dempsey J, Lecturer S. Principles and applicaiton of cell delivery system in periodontal regeneration. Corp Gov [Internet]. 2007;15(4):585–608. Available from: http://www.sciencedirect.com/science/article/pii/S0883902608000475

33. Pandya M, Diekwisch TGH. Enamel biomimetics—fiction or future of dentistry. Int J Oral Sci. 2019;11(1):1–9.

34. Dahlberg AA. Transplantation of tooth germ elements and the e x p e r i m e n t a l heterotopic formation of d e n t i n and enamel*. 1934;(8).

35. Zheng L, Warotayanont R, Stahl J, Kunimatsu R, Klein O, DenBesten PK, et al. Inductive ability of human developing and differentiated dental mesenchyme. Cells Tissues Organs. 2013;198(2):99–110.

36. Zivkovic P, Petrovic V, Najman S, Stefanovic V. Stem cell-based dental tissue engineering. ScientificWorldJournal. 2010;10:901–16.

37. Smith EE, Angstadt S, Monteiro N, Zhang W, Khademhosseini A, Yelick PC. Bioengineered Tooth Buds Exhibit Features of Natural Tooth Buds. J Dent Res. 2018;97(10):1144–51.

38. Bluteau G, Luder HU, De Bari C, Mitsiadis TA. Stem cells for tooth engineering. Eur Cells Mater. 2008;16(July):1–9.

39. Takahashi C, Yoshida H, Komine A, Nakao K, Tsuji T, Tomooka Y. Newly established cell lines from mouse oral epithelium regenerate teeth when combined with dental mesenchyme. Vitr Cell Dev Biol - Anim. 2010;46(5):457–68.

40. Oshima M, Ogawa M, Tsuji T. Functional tooth regeneration. Methods Mol Biol. 2017;1597:97–116.

41. Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A. 2009;106(32):13475–80.

42. Zeitlin BD. Banking on teeth – Stem cells and the dental office. Biomed J [Internet]. 2020;43(2):124–33. Available from: https://doi.org/10.1016/j.bj.2020.02.003

43. Peng L, Ye L, Zhou X dong. Mesenchymal stem cells and tooth engineering. Int J Oral Sci. 2009;1(1):6–12.

44. Herberts CA, Kwa MSG, Hermsen HPH. Risk factors in the development of stem cell therapy. J Transl Med. 2011;9:1–14.

45. Wuputra K, Ku CC, Wu DC, Lin YC, Saito S, Yokoyama KK. Prevention of tumor risk associated with the reprogramming of human pluripotent stem cells. J Exp Clin Cancer Res. 2020;39(1):1–24.

46. Clément F, Grockowiak E, Zylbersztejn F, Fossard G, Gobert S, Maguer-Satta V. Stem cell manipulation, gene therapy and the risk of cancer stem cell emergence. Stem Cell Investig. 2017;4(7):1–15.

NCHS Data Brief ■ No. 197 ■ May 2015

Dental Caries and Tooth Loss in Adults in the United States, 2011–2012

Bruce A. Dye, D.D.S., M.P.H.; Gina Thornton-Evans, D.D.S, M.P.H.; Xianfen Li, M.S.; and Timothy J. Iafolla, D.M.D., M.P.H.

Key findings

Data from the National Health and Nutrition Examination Survey, 2011–2012

- Among adults aged 20–64, 91% had dental caries and 27% had untreated tooth decay.
- Untreated tooth decay was higher for Hispanic (36%) and non-Hispanic black (42%) adults compared with non-Hispanic white (22%) and non-Hispanic Asian (17%) adults aged 20–64.
- Adults aged 20–39 were twice as likely to have all their teeth (67%) compared with those aged 40–64 (34%).
- About one in five adults aged 65 and over had untreated tooth decay.
- Among adults aged 65 and over, complete tooth loss was lower for older Hispanic (15%) and non-Hispanic white (17%) adults compared with older non-Hispanic black adults (29%).

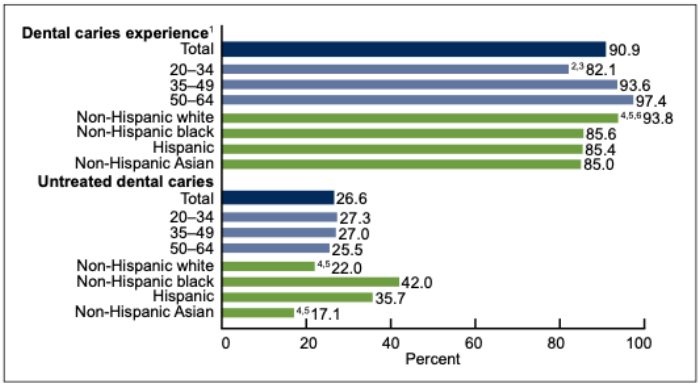
Dental caries and tooth loss are important oral health indicators for adults and are key measures for monitoring progress toward health promotion goals set by Healthy People 2020 (1,2). Although tooth decay and complete tooth loss have been declining in the United States since the 1960s, disparities have remained between some groups (3,4). As adults age, oral health-related quality of life is negatively affected by tooth loss and decay (5). This report describes U.S. adult dental caries and tooth loss by age and race and Hispanic origin for 2011–2012.

Keywords: tooth decay • edentulism • disparities • NHANES

What percentage of adults had dental caries in permanent teeth?

Approximately 91% of U.S. adults aged 20–64 had dental caries in permanent teeth in 2011–2012 (Figure 1). Dental caries among adults aged 35–64 was

Figure 1. Prevalence of dental caries in permanent teeth among adults aged 20–64, by age and race and Hispanic origin: United States, 2011–2012



[1]Includes untreated and treated (restored) dental caries.
[2]Significantly different from ages 35–49, $p < 0.05$.
[3]Significantly different from ages 50–64, $p < 0.05$.
[4]Significantly different from non-Hispanic black adults, $p < 0.05$.
[5]Significantly different from Hispanic adults, $p < 0.05$.
[6]Significantly different from non-Hispanic Asian adults, $p < 0.05$.
NOTE: Access data table for Figure 1 at: http://www.cdc.gov/nchs/data/databriefs/db197_table.pdf#1.
SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2011–2012.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Center for Health Statistics

CLINICAL REVIEW

Global Burden of Severe Tooth Loss: A Systematic Review and Meta-analysis

N.J. Kassebaum[1,2], E. Bernabé[3], M. Dahiya[4], B. Bhandari[4], C.J.L. Murray[2], and W. Marcenes[4]*

Abstract: *The goal of the Global Burden of Disease 2010 Study has been to systematically produce comparable estimates of the burden of 291 diseases and injuries and their associated 1,160 sequelae from 1990 to 2010. We aimed to report here internally consistent prevalence and incidence estimates of severe tooth loss for all countries, 20 age groups, and both sexes for 1990 and 2010. The systematic search of the literature yielded 5,618 unique citations. After titles and abstracts were screened, 5,285 citations were excluded as clearly not relevant to this systematic review, leaving 333 for full-text review; 265 publications were further excluded following the validity assessment. A total of 68 studies—including 285,746 individuals aged 12 yr or older in 26 countries—were included in the meta-analysis using modeling resources of the Global Burden of Disease 2010 Study. Between 1990 and 2010, the global age-standardized prevalence of edentate people decreased from 4.4% (95% uncertainty interval: 4.1%, 4.8%) to 2.4% (95% UI: 2.2%, 2.7%), and incidence rate decreased from 374 cases per 100,000 person-years (95% UI: 347, 406) to 205 cases (95% UI: 187, 226). No differences were found by sex in 2010. Prevalence increased gradually with age, showing a steep increase around the seventh decade of life that was associated with a peak in incidence at 65 years. Geographic differences in prevalence, incidence, and rate of improvement from 1990 to 2010 were stark. Our review of available quality literature on the epidemiology of tooth loss shows a significant decline in the prevalence and incidence of severe tooth loss between 1990 and 2010 at the global, regional, and country levels.*

Key Words: toothless, epidemiology, missing teeth, extracted teeth, global health, edentate.

Introduction

Tooth loss is a complex outcome that reflects an individual's history of dental disease and its treatment by dental services over the life course (Petersen *et al.*, 2005; Baelum *et al.*, 2007). Tooth loss reflects not only dental disease but also patients' and dentists' attitudes, the dentist-patient relationship, the availability and accessibility of dental services, and the prevailing philosophies of dental care (Baelum *et al.*, 2007; Fejerskov *et al.*, 2013). A good understanding of current trends in tooth loss is important for planning dental services and workforce as well as for updating the dental curriculum.

Tooth loss is considered an effective marker of population oral health and is therefore monitored in many countries. However, the epidemiology of tooth loss is not yet fully understood. Previous attempts to synthesize the epidemiology of tooth loss are limited to the developed world (Mojon *et al.*, 2004; Müller *et al.*, 2007) or the elderly (World Health Organization, 2003). Although it is accepted that the prevalence of edentate people has declined in all age groups in many developed countries (Hugoson *et al.*, 2005; Dye *et al.*, 2007; Steele *et al.*, 2012), data from developing and emerging countries are lacking. Moreover, data on the incidence of tooth loss are sparse (Müller *et al.*, 2007). This is further complicated by the quality of epidemiologic data, which vary considerably among surveys and make comparison among countries difficult (Mojon *et al.*, 2004). In addition, the rapidly changing dental health during the past 4 decades indicates that new data are required regularly.

The goal of the Global Burden of Disease (GBD) 2010 Study has been to systematically produce comparable estimates of the burden of 291 diseases and injuries and their associated 1,160 sequelae from 1990 to 2010 (Murray *et al.*, 2012a; Murray *et al.*, 2012b). We aimed to report here internally consistent

DOI: 10.1177/0022034514537828. [1]Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, USA; [2]Department of Anesthesiology and Pain Medicine, University of Washington and Seattle Children's Hospital, Seattle, WA, USA; [3]Division of Population and Patient Health, King's College London, Dental Institute at Guy's, King's College, and St. Thomas' Hospitals, London, UK; and [4]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; *corresponding author, w.marcenes@qmul.ac.uk

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

Ann Ibd. Pg. Med 2014. Vol.12, No.1 42-48

KNOWLEDGE OF CONSEQUENCES OF MISSING TEETH IN PATIENTS ATTENDING PROSTHETIC CLINIC IN U.C.H. IBADAN

O.O. Dosumu, J.T. Ogunrinde and S.A. Bamigboye

Department of Restorative Dentistry, Faculty of Dentistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Correspondence:
Dr. S.A. Bamigboye
Department of Restorative Dentistry,
Faculty of Dentistry,
College of Medicine,
University of Ibadan, Ibadan.
E-mail: drbamigboye@gmail.com
Phone: 08038044905

SUMMARY

Background and Objective: **Various causes of tooth loss such as caries, trauma, periodontal diseases, and cancer have been documented in the literature. In addition, factors that can modify these causes such as level of education, age and sex have been studied. There is however paucity of information on whether patients or people with missing teeth are aware of the side effects of tooth loss on them or on the remaining teeth. This study investigated the knowledge of consequences of missing teeth among partially edentulous patients in a teaching hospital.**

Patients and Method: **Self-administered questionnaires were distributed to the patients to collect information relating to demography, cause and duration of tooth loss, awareness of the consequences of tooth loss and their sources of information. Four clinical conditions including supra-eruption, mastication, teeth drifting, and facial collapse were used to assess the level of awareness of consequences of missing teeth.**

Result: **Two hundred and three participants were included in the study. Their mean age was 45.5±1.8 years. There was no significant difference between the knowledge of the consequences of missing teeth and sex or on level of education ($p > 0.05$). Dentists constituted the largest source of information to these patients (25.6%) while the media constituted the least (0.5%).**

Conclusion: **The result of this study showed poor knowledge of the consequences of missing teeth among partially edentulous patients and the media that should be of assistance were equally unaware, signifying urgent need for public awareness on this subject.**

Key words: Tooth loss, Level of awareness, Consequence of missing teeth.

INTRODUCTION

Tooth loss could result from caries, periodontal disease, trauma, infection, malignancies, or failed endodontic treatments [1,2,3,4] and can present adverse consequences on the remaining dentition and on the patients' general wellbeing. [3, 4]

Petridis *et al*[5] reported drifting of adjacent teeth and supra-eruption of the opposing teeth to the edentulous space in their study which looked at positional changes of adjacent teeth to edentulous spaces. Also, Kini and Muliya[6] observed in a case report supra-eruption of a first mandibular premolar into the space left by an upper first premolar whose coronal tissue had broken down although the whole tooth was not lost.

In addition to the drifting and supra-eruption mentioned above is a possible facial/oral asymmetry or collapse that may result following loss of teeth. Martins-Junior and Marques[7] showed that premature loss of a lower right deciduous canine in an 8-year old patient resulted in deviation of the lower arch from the midline to the affected side just as Tallgren *et al*[8] reported facial collapse in patients with teeth and alveolar bone loss.

Mastication as a consequence of tooth loss are known by many patients[9] and this may be why people in this class of condition impose dietary restriction upon themselves and thereby incurring health risk.[10] In addition, loss of posterior teeth has been associated with impaired chewing and inadequate nutrition, the patients having the tendency to over-prepare food in an attempt to make it soft thereby loosing important nutrients.[11]

Gerritsen et al. Health and Quality of Life Outcomes 2010, 8:126
http://www.hqlo.com/content/8/1/126

HEALTH AND QUALITY OF LIFE OUTCOMES

REVIEW Open Access

Tooth loss and oral health-related quality of life: a systematic review and meta-analysis

Anneloes E Gerritsen[1*], P Finbarr Allen[2], Dick J Witter[1], Ewald M Bronkhorst[3], Nico HJ Creugers[1]

Abstract

Background: It is increasingly recognized that the impact of disease on quality of life should be taken into account when assessing health status. It is likely that tooth loss, in most cases being a consequence of oral diseases, affects Oral Health-Related Quality of Life (OHRQoL). The aim of the present study is to systematically review the literature and to analyse the relationship between the number and location of missing teeth and oral health-related quality of life (OHRQoL). It was hypothesized that tooth loss is associated with an impairment of OHRQoL. Secondly, it was hypothesized that location and distribution of remaining teeth play an important role in this.

Methods: Relevant databases were searched for papers in English, published from 1990 to July 2009 following a broad search strategy. Relevant papers were selected by two independent readers using predefined exclusion criteria, firstly on the basis of abstracts, secondly by assessing full-text papers. Selected studies were grouped on the basis of OHRQoL instruments used and assessed for feasibility for quantitative synthesis. Comparable outcomes were subjected to meta-analysis; remaining outcomes were subjected to a qualitative synthesis only.

Results: From a total of 924 references, 35 were eligible for synthesis (inter-reader agreement abstracts $\kappa = 0.84 \pm 0.03$; full-texts: $\kappa = 0.68 \pm 0.06$). Meta-analysis was feasible for 10 studies reporting on 13 different samples, resulting in 6 separate analyses. All studies showed that tooth loss is associated with unfavourable OHRQoL scores, independent of study location and OHRQoL instrument used. Qualitative synthesis showed that all 9 studies investigating a possible relationship between number of occluding pairs of teeth present and OHRQoL reported significant positive correlations. Five studies presented separate data regarding OHRQoL and location of tooth loss (anterior tooth loss *vs*. posterior tooth loss). Four of these reported highest impact for anterior tooth loss; one study indicated a similar impact for both locations of tooth loss.

Conclusions: This study provides fairly strong evidence that tooth loss is associated with impairment of OHRQoL and location and distribution of tooth loss affect the severity of the impairment. This association seems to be independent from the OHRQoL instrument used and context of the included samples.

Background

It is increasingly recognized that the impact on quality of life (QoL) of disease and treatment of disease and its consequences should be taken into account when assessing health status and evaluating treatment outcomes. Clinical indicators only are not sufficient to describe health status and it has been reported that people with chronic disabling disorders can perceive their quality of life as better than healthy individuals, i.e., poor health or presence of disease does not inevitably mean poor quality of life [1,2]. Adaptive capacity and personal characteristics appear to influence patient's response to chronic disease. This can result in reports which seem counterintuitive, for example, the finding in a large German survey that having fewer than 9 teeth had more impact on health-related QoL than having cancer, hypertension, or allergy [3]. Therefore, clinical indicators only are not sufficient to describe health status. This is also true for oral diseases and its consequences for oral health-related quality of life (OHRQoL). The two most prevalent oral diseases, caries and periodontal disease often do not cause symptoms in early stages.

* Correspondence: a.gerritsen@dent.umcn.nl
[1]Department of Oral Function and Prosthetic Dentistry, College of Dental Science, Radboud University Nijmegen Medical Centre, Philips van Leydenlaan 25, 6525 EX Nijmegen, The Netherlands
Full list of author information is available at the end of the article

Fang et al. BMC Psychiatry (2018) 18:345
https://doi.org/10.1186/s12888-018-1927-0

BMC Psychiatry

RESEARCH ARTICLE Open Access

Tooth loss as a risk factor for dementia: systematic review and meta-analysis of 21 observational studies

Wen-li Fang[1†], Mu-jun Jiang[2†], Bei-bei Gu[3], Ying-mei Wei[1], Sheng-nuo Fan[1], Wang Liao[1], Yu-qiu Zheng[4], Shao-wei Liao[1], Ying Xiong[1], Yi Li[1], Song-hua Xiao[1] and Jun Liu[1,5,6*]

Abstract

Background: Tooth loss is suggested to be associated with an increased risk of dementia in many studies. But the relationship between tooth loss and dementia is not yet fully understood. This systematic review and meta-analysis aimed to determine the relative effect of tooth loss on dementia risk.

Methods: An electronic search of PubMed, Scopus, Embase, and Web of Knowledge was conducted in March 2018 to identify relevant observational studies with the English language restriction. Studies were included if they assessed the relationship between tooth loss and risk of dementia. Study quality was detected by the modified Downs and Black scale. Odds risks (ORs) were pooled using a random-effects model in the crude model.

Results: The literature search initially yielded 1574 articles, and 21 observational studies published between 1994 and 2017 were finally included for the analyses. The crude results with random-effects model showed that patients with multiple tooth loss had higher incidence of dementia (OR 2.62, 95% CI 1.90–3.61, $P < 0.001$, $I^2 = 90.40\%$). The association remained noted when only adjusted results were pooled from 18 studies (OR 1.55, 95% CI 1.41–1.70, $P = 0.13$, $I^2 = 28.00\%$). Meta-regression analysis showed that study design explained about 16.52% of heterogeneity in the crude model. The overall quality rating scores of studies ranged from 11 to 16.

Conclusions: Findings from this review evidenced that tooth loss is positively associated with an increased risk of dementia in adults. Future well-designed longitudinal researches examining the direct and indirect relationship between tooth loss and dementia risk are encouraged.

Keywords: Dementia, Cognitive impairment, Tooth loss, Risk assessment, Meta-analysis

Background

Dementia is characterized by cognitive and functional decline and neuropsychiatric symptoms caused by irreversible neurodegenerative diseases. The global population is aging at a rapid pace due to rising life expectance and over 47 million people live with dementia in 2016. The prevalence of dementia results in negative impacts on people's life quality and economy according to the 2016 World Alzheimer Report [1]. To our knowledge, there is no effective anti-dementia drug available for the management of dementia. Therefore, it is in great need to identify modifiable risk factors for preventing cognitive impairment.

Tooth loss is prevalent in patients with dementia and it is a worldwide public health issue in older adults [2], impacting negatively on their quality of daily life, such as chewing, swallowing, and social life [3–5]. Evidence has shown that tooth loss is not only associated with oral health, but also with systemic health [6]. Recently, increasing studies have focused on the link between tooth loss and the risk of dementia [7–12]. There are several potential mechanisms by which tooth loss can negatively impact cognitive function. Periodontitis is one of the main causes of tooth

* Correspondence: docliujun@hotmail.com
†Wen-li Fang and Mu-jun Jiang contributed equally to this work.
[1]Department of Neurology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yanjiang West Road, Guangzhou 510120, Guangdong, China
[5]Laboratory of RNA and Major Diseases of Brain and Heart, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China
Full list of author information is available at the end of the article

Journal section: Oral Surgery
Publication Types: Research

doi:10.4317/medoral.23082
http://dx.doi.org/doi:10.4317/medoral.23082

Descriptive retrospective study analyzing relevant factors related to dental implant failure

Lizet Castellanos-Cosano [1], Antonio Rodriguez-Perez [2], Sergio Spinato [3], Marcel Wainwright [3], Guillermo Machuca-Portillo [4], Maria Angeles Serrera-Figallo [4], Daniel Torres-Lagares [4]

[1] Associate Professor. Oral Surgery, School of Dentistry, University of Seville. University of Fernando Pessoa Canarias
[2] Full-time Professor, School of Dentistry, University of Fernando Pessoa Canarias
[3] Private Practice in Dentistry
[4] Full-time Professor, School of Dentistry, University of Seville

Correspondence:
Department of Stomatology, School of Dentistry
University of Seville:
C/ Avicena s/n 41009, Seville, Spain
danieltl@us.es

Received: 03/03/2019
Accepted: 16/09/2019

Castellanos-Cosano L, Rodriguez-Perez A, Spinato S, Wainwright M, Machuca-Portillo G, Serrera-Figallo MA, Torres-Lagares D. Descriptive retrospective study analyzing relevant factors related to dental implant failure. Med Oral Patol Oral Cir Bucal. 2019 Nov 1;24 (6):e726-38.
http://www.medicinaoral.com/pubmed/medoralv24_i6_p726.pdf

Article Number: 23082 http://www.medicinaoral.com/

eMail: medicina@medicinaoral.com
Indexed in:
Science Citation Index Expanded
Journal Citation Reports
Index Medicus, MEDLINE, PubMed
Scopus, Embase and Emcare
Indice Médico Español

Abstract

Background: The objective of this retrospective descriptive study was to analyze the characteristics of incident reports provided by dentists while using a specific brand of dental implants.

Material and Methods: The study was carried out in collaboration with Oxtein Iberia S.L.®, with the company providing access to the incident database in order to evaluate the characteristics of incidents from January 2014 to December 2017 (a total of 917 over four years). The data sheet recorded different variables during each of the stages of implant treatment, from initial implant placement to subsequent prosthetic rehabilitation. These variables included age, sex, systemic pathologies, smoking habits, bone quality, implant type, prosthesis type, and type of load applied, among others. SPSS Statistics was used to perform statistical analysis of the qualitative variables (univariate logistic regressions, $\chi 2$ test, Haberman's adjusted standardized residuals).

Results: The total study sample consisted of 44,415 implants shipped from Oxtein® warehouses on the dates indicated, of which 917 implants (2.1%) were flagged due to reports of lack of primary stability, failed osseointegration, or implant failure within one year of placement. When analyzing incident reports, it was observed that 61.6% of incidents occurred in male patients, compared to 38.4% in female patients. The average age of patients in the reported cases was 56.12 ± 12.15 years. A statistically significant correlation was discovered between incidents of implant failure and tobacco use, diabetes, heart disease, poor oral hygiene, previous infection, poor bone quality, and bruxism ($p < 0.05$). A (statistically significant) higher rate of incidents was also observed in tapered, internal connection, Grade IV titanium, narrow, and short implants.

Conclusions: Analysis of these implants reveals a higher rate of complication in short, tapered, internal connection and narrow-diameter implants. These data can help and encourage clinicians to use the utmost surgical precautions when placing these implants.

Key words: *Pharmacovigilance, dental implant, dental implant failure.*

Journal section: Oral Surgery
Publication Types: Review

doi:10.4317/medoral.16.e42
http://dx.doi.org/doi:10.4317/medoral.16.e42

Implant failure: Etiology and complications

Salah Sakka [1], Paul Coulthard [2]

[1] DDS MSc PhD. Senior Clinical Lecturer in Oral and Maxillofacial Surgery, Faculty of Dentistry, The University of Albath, Homs, Syria
[2] BDS MDS PhD. Professor in Oral and Maxillofacial Surgery, School of Dentistry, The University of Manchester, Manchester, United Kingdom

Correspondence:
Abdul Hameed Droubi St
Plaza Building, Floor 2
PO Box 2137 Homs, Syria
salah.sakka@hotmail.com

Sakka S, Coulthard P. Implant failure: Etiology and complications. Med Oral Patol Oral Cir Bucal. 2011 Jan 1;16 (1):e42-4.
http://www.medicinaoral.com/medoralfree01/v16i1/medoralv16i1p42.pdf

Article Number: 16870 http://www.medicinaoral.com/

eMail: medicina@medicinaoral.com
Indexed in:
Science Citation Index Expanded
Journal Citation Reports
Index Medicus, MEDLINE, PubMed
Scopus, Embase and Emcare
Indice Médico Español

Received: 25/02/2010
Accepted: 02/04/2010

Abstract

The possible occurrence of implant failure is a major concern for implantologists and knowledge in such unavoidable fact is clinically essential. Periimplantitis is an inflammatory response in which there is a loss of the bony support of the implant. Diagnosis is based on the clinical signs of infection such as hyperplastic soft tissues, suppuration, colour changes of the marginal peri-implant tissues and gradual bone loss. This site-specific infection may have many features in common with chronic adult periodontitis. Surgical trauma, micromotion and overload are also considered to be associated with implant failures. The lack of osseointegration is generally distinguished by implant mobility and radiological radiolucency. Here, the implant is considered to be failed . Progressive marginal bone loss without marked mobility is referring to a failing implant. The purpose of this concise review was to discuss the implant complications and failure by highlighting the major etiologic factors as well as the parameters used for evaluating such failure.

Key words: *Implant failure, peri-implantitis, marginal bone loss, implant mobility.*

Introduction

Implantology is continually developing as new research results provide a better understanding of the biologic principles that direct the development of a dynamic interface between the living tissue and an artificial structure. However, in spite of high success rate, occurrence of implants failure has been reported (1).

Implant failure may be referred to as the status of the implant performance that when using some quantitative measurements, falls below an acceptable level. This definition encompasses clinical situations, ranging from all symptomatic mobile implants to implants show more than 0.2mm of peri-implant bone loss after the first year of loading (2) or bleeding depth exceeding 5mm of probing depth (3). The distinction between failed implant and failing implant is clinically important. The lack of osseointegration is generally characterized by implant mobility and peri- fixtural radiolucency. In this situation, the implant is considered to be "failed" (4). On the other hand, the failure process might be slow and continuing (5). Therefore, an implant characterized by progressive marginal bone loss without marked mobility is considered to be "failing" (4).

MEDICAL JOURNAL ARMED FORCES INDIA 72 (2016) S24–S30

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Review Article

Mapping the milestones in tooth regeneration: Current trends and future research

Atanu Bhanja [a,*], *Col D.S.J. D'Souza (Retd)* [b]

[a] Assistant Professor, Dept of Dental Surgery, Sikkim Manipal Institute of Medical Sciences, Gangtok, India
[b] Professor & HOD, Dept of Dental Surgery, Sikkim Manipal Institute of Medical Sciences, Gangtok, India

ARTICLE INFO

Article history:
Received 26 February 2016
Accepted 10 May 2016
Available online 14 June 2016

Keywords:
Odontogenesis
Regeneration
Stem cells
Tissue engineering
Tooth

ABSTRACT

Research into finding the perfect replacement for lost dentition is an ever-evolving and rapidly advancing subject involving many scientific disciplines. The present consensus appears to be that regeneration of tooth in morphological and functional form is the ideal answer to lost tooth replacement. This article traces the milestones in this elusive search for the ultimate tooth replacement. The various research developments are highlighted that are aimed at the final goal of being able to "re-grow a natural tooth". Whole tooth regeneration is technically challenging and further research into this field of complex molecular biology, embryology, biomaterials and stem cells is required to answer the unsolved questions. However, the milestones that have been crossed in the attempts at whole tooth regeneration have been remarkable and the future is quite promising. This article highlights the noteworthy research work that is being done in the field of whole tooth regeneration with a view to not only inform the clinicians of the significant developments but also inspire them to actively participate in this rapidly evolving field.

Introduction

Regeneration of lost tissue or organs for rehabilitation of patients has been the ultimate dream of every clinician and healthcare researcher. Due to the unique, multifarious role of the teeth and associated structures there has been a sustained effort to replace the missing dentition over many centuries. From the prehistoric attempts of wiring together extracted teeth, various denture designs and materials to the development of dental implants the search has had many unique breakthroughs and path-breaking developments. Despite all its advantages, dental implants (currently considered to be the best alternative) have certain inherent drawbacks. They lack the 3-dimensional structure of natural teeth and consequently their functionality, such as a periodontal ligament which gives a sense of proprioception and cushioning effect; pulpal tissue that act as a reparative source of cells and the lack of thermal stimuli through nerve endings' that all of which have got a protective role teleologically.

The consensus, therefore, has been that regeneration of tooth in morphological and functional form is the only perfect

* *Corresponding author*. Tel.: +91 9434142646.
E-mail address: doc.abhanja@gmail.com (A. Bhanja).
http://dx.doi.org/10.1016/j.mjafi.2016.05.004
0377-1237/

ARTICLE IN PRESS

Journal of Dentistry xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Developing a questionnaire to measure psychological disturbance associated with tooth loss

Zaki Kudsi[a,*], Michael R Fenlon[b], Kirsty Hill[c], Aylin Baysan[d]

[a] Emerson Green NHS Treatment Center, The Brooms, Emerson Green, BS16 7FH, United Kingdom
[b] Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, United Kingdom
[c] The School of Dentistry Edgbaston Birmingham, B5 7 EG, United Kingdom
[d] Institute of Dentistry, Queen Mary University of London, United Kingdom

ARTICLE INFO

Keywords:
Tooth loss
Dentures
Depression
Anxiety
Stress

ABSTRACT

Objectives: To develop and validate a self-reporting measure to assess the psychological disturbance in adult patients with tooth loss and dentures

Methods: Ethical approval obtained from the Health Research Authority NHS England (Ref:17/NI/0098). 128 participants (100 patients - 28 clinicians) were recruited to participate in the development and validation of the questionnaire. Inclusion criteria included adults (age ≥18) with tooth loss/dentures. Exclusion criteria included patients with a history of psychotic mental illness or patients who had treatment with dental implants. The development processes included: Phase 1. Development of questionnaire: describing the aims/target population of the questionnaire, generating a pool of items, defining the constructs to be measured, adapting psychological morbidity screening tools, Items reduction and producing a preliminary questionnaire. Phase 2. Validation of questionnaire: content validation, face validation, establishing construct validity, pilot testing and establishing reliability.

Results: Face and content validation indicated that the questionnaire was an appropriate tool to measure the impact of tooth loss and related psychological morbidities. Reliability analysis (Test re-test reliability/internal consistency) indicated the questionnaire has satisfactory reliability (correlation > 0.7). Testing the theoretical hypothesis structure of the impact of tooth loss has also enhanced the construct validity of the questionnaire (domains correlated mildly (r > 5 & < 3) to strongly (r > 5). Pilot testing confirmed the scale adequacy and wording clarity (> 90 % of respondents). Results indicated that the developed questionnaire has adequate psychometric properties.

Conclusion: A disease-specific measure that assesses the psychological impact of tooth loss and the effectiveness of interventions (i.e. dentures) has been developed and validated.

Clinical significant: A patient outcome measure was developed which could be used to assess the psychological impact of tooth loss and compare the effectiveness of various interventions like dentures and implants.

1. Introduction

Adult Oral Health in the UK has been gradually improving, and the prevalence of tooth loss has been in decline in the last 30 years. Nevertheless, it is estimated that 6% of the population remain edentulous, a further 14 % have experienced significant tooth loss (> 11 tooth loss) and "one in every five" adults have removable dentures (either partial or complete). [1] Previous research has shown that tooth loss can have a significant impact on the general and oral health-related quality of life [2,3]. Edentulous or partially dentate patients may require either removable dentures or osseointegrated dental implants to restore their dentition. Dentures could restore function and is a non-invasive treatment option. Whilst some patients cope with and adapt well to tooth loss and dentures; others experience emotional distress as they might have less psychological resilience and ability to adapt to changes [4]. Some authors also reported that tooth loss could cause significant emotional and psychological distress in some patients despite being successful denture wearers [5]. Therefore, it is important to assess the psychological disturbance and wellbeing in those patients.

Screening tools have widely been used for depression, anxiety and distress in patients with various medical conditions, such as amputations, artificial prosthesis replacements, chronic illness, cancer and palliative care [6–8].

Different methods were suggested to develop and test

* Corresponding author.
E-mail addresses: z.kudsi@nhs.net (Z. Kudsi), michael.fenlon@kcl.ac.uk (M.R. Fenlon), k.b.hill@bham.ac.uk (K. Hill), a.baysan@qmul.ac.uk (A. Baysan).

https://doi.org/10.1016/j.jdent.2020.103353
Received 20 January 2020; Received in revised form 23 March 2020; Accepted 23 April 2020

Please cite this article as: Zaki Kudsi, et al., Journal of Dentistry, https://doi.org/10.1016/j.jdent.2020.103353

THE JOURNAL OF PROSTHETIC DENTISTRY

REVIEW

Removable partial dentures: The clinical need for innovation

Stephen D. Campbell, DDS, MMSc,[a] Lyndon Cooper, DDS, PhD,[b] Helen Craddock, PhD, MDent Sci, BDS, FDS(Rest Dent), MRD(Pros), MFDS RCS(Edin), MGDS RCS(Eng), DGDP(UK), PGCTLHE(Leeds), FHEA,[c] T. Paul Hyde, BChD, PhD, DGDP RCS(Eng), MGDS RCS(Eng), FHEA,[d] Brian Nattress, BChD, PhD, MRD RCS(Edin), FDS RCS(Edin),[e] Sue H. Pavitt, BSc, PhD,[f] and David W. Seymour, BChD, MFDS RCS(Ed), MSc ClinDent(Rest Dent), FDS(Rest Dent), RCS(Ed)[g]

ABSTRACT

Statement of problem. The number of partially dentate adults is increasing, and many patients will require replacement of missing teeth. Although current treatment options also include fixed partial dentures and implants, removable partial dentures (RPDs) can have advantages and are widely used in clinical practice. However, a significant need exists to advance materials and fabrication technologies because of the unwanted health consequences associated with current RPDs.

Purpose. The purpose of this review was to assess the current state of and future need for prosthetics such as RPDs for patients with partial edentulism, highlight areas of weakness, and outline possible solutions to issues that affect patient satisfaction and the use of RPDs.

Material and methods. The data on treatment for partial edentulism were reviewed and summarized with a focus on currently available and future RPD designs, materials, means of production, and impact on oral health. Data on patient satisfaction and compliance with RPD treatment were also reviewed to assess patient-centered care.

Results. Design, materials, ease of repair, patient education, and follow-up for RPD treatment all had a significant impact on treatment success. Almost 40% of patients no longer use their RPD within 5 years because of factors such as sociodemographics, pain, and esthetics. Research on RPD-based treatment for partial edentulism for both disease-oriented and patient-centered outcomes is lacking.

Conclusions. Future trials should evaluate new RPD materials and design technologies and include both long-term follow-up and health-related and patient-reported outcomes. Advances in materials and digital design/production along with patient education promise to further the application of RPDs and improve the quality of life for patients requiring RPDs. (J Prosthet Dent 2017;118:273-280)

The proportion of partially dentate adults is increasing, partly as a result of increased life expectancy, a rise in the number of elderly individuals within the population, and a shift from total tooth loss/total edentulism toward partial edentulism.[1-3] The prevalence of partial edentulism is already estimated at greater than 20% in some regions,[4] and the number of individuals with partial edentulism could increase to more than 200 million in the United States alone in the next 15 years.[5] In the United States, the average adult over the age of 20 has 24.9 remaining teeth, and 43.7% of all U.S. adults have had a tooth extracted. Individuals over 65 have an average of 18.9 remaining teeth, with 43.1% missing 6 or more teeth.[6,7] In the United Kingdom, the 2009 Adult Dental Health Survey found that "nearly one in five adults wore removable dentures of some description

Supported by Solvay Dental 360. This article was prepared according to the International Society for Medical Publication Professionals' "Good Publication Practice for Communication Company-Sponsored Medical Research: the GPP3 Guidelines."
[a]Professor and Head, Department of Restorative Dentistry, Director, Implant and Innovations Center, Director, Center for Digital Excellence, University of Illinois at Chicago College of Dentistry, Chicago, Ill.
[b]Associate Dean for Research, Department Head, Oral Biology, University of Illinois at Chicago College of Dentistry, Chicago, Ill.
[c]Professor Emeritus, University of Leeds, School of Dentistry, Leeds, United Kingdom.
[d]Clinical Associate Professor, University of Leeds, School of Dentistry, Leeds, United Kingdom.
[e]Honorary consultant in restorative dentistry, University of Leeds, School of Dentistry, Leeds, United Kingdom.
[f]Professor of Translational and Applied Health Research, Director of Dental Translational and Clinical Research Unit (DenTCRU), and Division Head of Applied Health & Clinical Translation, University of Leeds, School of Dentistry, Leeds, United Kingdom.
[g]Consultant, Restorative Dentistry, York Teaching Hospital NHS Foundation Trust, York, United Kingdom.

International Journal of
Environmental Research and Public Health

MDPI

Article

Development and Validation of a Questionnaire Evaluating the Impact of Prosthetic Dental Treatments on Patients' Oral Health Quality of Life: A Prospective Pilot Study

Eitan Mijiritsky [1,2], Yael Lerman [3], Ori Mijiritsky [4], Asaf Shely [5,*,†], Joseph Meyerson [6] and Maayan Shacham [7,†]

1 Department of Otolaryngology, Head and Neck and Maxillofacial Surgery, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv 6139001, Israel; mijiritsky@bezeqint.net
2 The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv 6997801, Israel
3 Private Practice, Tel Aviv 6971915, Israel; yaelerman@gmail.com
4 Department of Psychology, Tel Aviv-Yafo Academic College, Tel Aviv 6818543, Israel; orimijiritsky2@gmail.com
5 Department of Prosthodontics, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv 6997801, Israel
6 Department of Psychology, Bar Ilan University, Ramat Gan 5290002, Israel; hypnoclinic10@gmail.com
7 School of Social Work, Ariel University, Ariel 40700, Israel; Drmaayanshacham@gmail.com
* Correspondence: asafshely@gmail.com; Tel.: +972-052-8787472
† These authors contributed equally to the work.

Received: 10 June 2020; Accepted: 10 July 2020; Published: 13 July 2020

check for updates

Abstract: Objectives: the aims of this study were the development of a novel questionnaire to assess the impact of prosthetic treatments on oral health-related quality of life (OHRQoL) and the performance of a prospective pilot study. Background: the currently preferred OHRQoL measurement tool is the oral health impact profile-49 (OHIP-49), a self-report questionnaire which mainly focuses on general effects related to oral health. Materials and methods: A total of 24 adult participants (9 females and 15 males) were recruited and asked to complete the novel questionnaire twice: once before the prosthetic treatment began and 4–6 weeks post-treatment. The assessment of the change in OHRQoL was based on the differences in participants' answers before and after treatment. Data were analyzed using ANOVA with a repeated-measures method and *t*-tests. The reliability of the questionnaire was tested using Cronbach's alpha and intraclass coefficient (ICC). Results: The questionnaire was found to be reliable ($\alpha \geq 0.6$), with "social disability" having the highest score ($\alpha = 0.868$). All domains showed an improvement ($\alpha < 0.005$) in OHRQoL scores after treatment. Conclusions: the novel questionnaire tested in this study was found to be reliable and convenient to use, and demonstrated that prosthetic treatments have a significant positive effect on OHRQoL post-treatment scores.

Keywords: oral health related quality of life (OHRQoL); psychological impacts; prosthodontics; esthetics

1. Introduction

Dental and oral health both seem to have a significant impact on self-perception, psychological status, and human social status [1,2]. These factors affect self-esteem and the individual's overall quality of life (QoL) [1–3]. Patients' expectations of good oral health include a number of specific characteristics related but not limited to function, comfort, and appearance [4,5]. Dental aesthetics

International Journal of Oral and Craniofacial Science
Peertechz

Laura de Sousa-Romero* and Ana María Moreno-Fernández

Department of Cytology and Histology, school of Medicine, University of Seville, Spain

Dates: **Received:** 07 April, 2016; **Accepted:** 18 April, 2016; **Published:** 29 April, 2016

***Corresponding author:** Laura de Sousa Romero, Department of Cytology and Histology, School of Medicine, University of Seville, Avda Sanchez Pizjuán sn, 41009, Spain, Tel. +34954551798; E-mail: lauradesousaromero@gmail.com

www.peertechz.com

ISSN: 2455-4634

Keywords: Cell differentiation; Cell proliferation; Intercellular signalling peptides and proteins; Odontogenesis: Tooth; Transcription factors

Review Article

Growth and Transcription Factors in Tooth Development

Abstract

Odontogenesis is a complex embryonic process originated by the interaction between two main embryonic components, dental epithelium and ectomesenchyme. This ectomesenchymal interaction is mediated by growth and transcription factors controlling the different aspects of tooth development such as tooth initiation, enamel knot formation and/or cell proliferation and differentiation. The aim of this review was to establish which factors are, how they interact and their functions in Odontogenesis. We have described several signaling pathways which are essential for correct tooth development and organized all available information. Our conclusion is that instead of large amount of information about tooth development, further studies are necessary to clear several essential mechanisms which still remain unknown and/or unclear.

Introduction

The embryonic process of odontogenesis is originated by two main embryonic tissues which are ectoderm and the underlying ectomesenchyme. The interaction between both two components leads tooth development throughout different phases known as initial stage, bud stage, cap stage, bell stage, appositional stage and root development [1].

Signal molecules, growth and transcription factors among other factors, are responsible of this interaction between epithelium and ectomesenchyme, and the communication in a one tissue layer [1].

Nowadays there are several researches which show the expression and functions of these factors during tooth development, but it is necessary to collect and organize this information improving the quality of the future studies. Therefore the aim of this review has been the collection and organization of all information about these factors during Odontogenesis.

Discussion

Initial stage

The first morphological signal of tooth development is the formation of a serie of epithelial thickenings into ectomesenchyme at sites corresponding to the position of presumptive teeth [2].

In mice the number of thickenings which appear is fewer than human. Mice has only one incisor, which is continuously growing throughout their live, and three molars separated by a diastema region in each quadrant [3].

During this stage the cranial ectoderm produces the signals which initiate tooth development, until E12.5 the underlying ectomesenchyme has not yet been specified for tooth development [4,5].

Early markers of tooth position and tooth type: Prior to thickening of dental epithelium various factors are expressed in dental epithelium and mesenchyme determining the position and pattern of prospective tooth.

The earliest marker of tooth position is Pixt2 appears in the stomatodeal and is progressively restricted to dental placode determining the request of Pixt2 for early specification of odontogenic epithelium [6]. Pax9 is another early marker of tooth position and its function might be necessary for establishing the competence of future tooth mesenchyme to respond to epithelial signals [7]. The same study proposes an alternative explanation about Pax9 function during initial stage suggesting that it plays a more direct role in the regulation of signaling molecules' production by the mesenchyme [7]. Wnt7b and Shh act as early markers of tooth position and are expressed in oral ectoderm and dental epithelium, respectively, interacting to keep cell boundaries between oral ectoderm and dental epithelium from E9.5 until E11.5 [8].

In Table 1 [9-13] we can see which factors are implicated in molar and/or incisor formation such as Lhx6 and Lhx7 which control the acquisition of odontogenic potential by molar mesenchyme [14,15], in response to epithelial FGF-8 [15], or Dlx1 and Dlx2 which specify a subpopulation of neural crest derived mesenchymal cells as odontogenic for the upper molar region [10].

In Figure 1 [9,12,16-18], it has been shown interaction between some factors which determine tooth type and position.

Thickening of dental epithelium and mesenchymal condensation: Epithelial BMP-4 and FGF-8 are essential in control of target genes transcription at this stage. They interact, BMP-4 as inhibitor and FGF-8 as inducer, and lead to different responses (Figure 2) [7,16,19] controlling epithelial proliferation.

Table 1: Factors implicated in the determination of tooth type. Here we show factors known which act in determination of tooth type [9–13].

	Barx1	Msx1	Dlx1	Dlx2	dHAND2	Isl1	Lhx6	Lhx7	Activinβ
Upper Incisor	-	+	-	-	-	+	-	-	+
Lower Incisor	-	+	-	-	+	+	-	-	+
Upper Molar	+	-	+	+	-	-	+	+	-
Lower Molar	+	-	-	-	-	-	+	+	+

Citation: de Sousa-Romero L, Moreno-Fernández AM (2016) Growth and Transcription Factors in Tooth Development. Int J Oral Craniofac Sci 2(1): 015-029. DOI: 10.17352/2455-4634.000014

Stem Cell and Biomaterials Research in Dental Tissue Engineering and Regeneration

Orapin V. Horst, DDS, PhD[a], Miquella G. Chavez, PhD[b,c], Andrew H. Jheon, DDS, PhD[c], Tejal Desai, PhD[b,d], Ophir D. Klein, MD, PhD[c,e,*]

KEYWORDS

• Tissue engineering • Regenerative medicine • Dental tissues • Scaffold

KEY POINTS

- Dental caries and periodontal disease are the most common diseases resulting in tissue loss. To replace or regenerate new tissues, various types of stem cells have been identified, including embryonic, somatic/adult, and induced pluripotent stem cells. Somatic and induced pluripotent stem cells can be obtained from teeth and periodontium.
- Endothelial cells and their paracrine factors mediate the formation of vasculature into engineered tissues or organs.
- Growth factors and bioactive molecules dictate various aspects of tooth morphogenesis and maturation and thus can be used to guide the formation of engineered tooth tissues in the manner recapitulating development.
- Various biomaterials can be chosen when designing a scaffold, including synthetic, natural, degradable and non-degradable materials.
- Advances in biomaterial sciences including microfabrication, self-assembled biomimetic peptides, and three-dimensional printing hold great promise for whole organ or partial tissue regeneration to replace teeth and periodontium.

Funding: The authors are supported by the NIH/NIDCR.

[a] Division of Endodontics, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, Box 0758, 521 Parnassus Avenue, Clinical Science Building 627, San Francisco, CA 94143-0758, USA; [b] Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, Box 2330, 1700 4th Street, San Francisco, CA 94158-2330, USA; [c] Department of Orofacial Sciences, University of California, San Francisco, Box 0442, 513 Parnassus Avenue, San Francisco, CA 94143-0442, USA; [d] Department of Physiology, University of California, San Francisco, Byers Hall Room 203C, MC 2520, 1700 4th Street, San Francisco, CA 94158-2330, USA; [e] Department of Pediatrics, University of California, San Francisco, Box 0442, 513 Parnassus Avenue, San Francisco, CA 94143-0442, USA
* Corresponding author. Department of Orofacial Sciences, University of California, San Francisco, Box 0442, 513 Parnassus Avenue, San Francisco, CA 94143-0442.
E-mail address: Ophir.Klein@ucsf.edu

Dent Clin N Am 56 (2012) 495–520
doi:10.1016/j.cden.2012.05.009 dental.theclinics.com
0011-8532/12/$ – see front matter

PULP BIOLOGY AND REGENERATION GROUP SATELLITE MEETING

Clinical Perspectives of Pulp Regeneration

Gottfried Schmalz, DDS, PhD,[*†] *Matthias Widbiller, DDS, PhD,*[*] *and Kerstin M. Galler, DDS, PhD*[*]

ABSTRACT

Introduction: A sound and vital pulp is an essential prerequisite for long-term tooth survival and preservation. However, current endodontic treatment concepts are based on the removal of inflamed or necrotic pulp tissue and the replacement by a synthetic biomaterial. Recently, total or partial pulp regeneration has been proposed as an alternative treatment concept. The aim of this review was to evaluate the current options of pulp treatment and regenerative approaches, both for immature and mature teeth, in a clinical context. **Methods**: Clinical success rates of classic treatment options such as pulpotomy or root canal filling after pulpectomy or the removal of necrotic tissue are compared with recent reports on regenerative approaches like revitalization or partial and total pulp regeneration. **Results**: Revitalization in immature teeth with pulp necrosis is an additional treatment option besides placing an apical plug, leading to clinically acceptable outcomes, although with low predictability regarding the completion of root formation. Coronal regeneration of the amputated pulp in immature teeth constitutes a promising scientific approach, but data from clinical studies are missing. Mature teeth display a reduced potential for regeneration. Regenerative procedures using cell transplantation or cell homing are mainly in the experimental phase with only 2 clinical studies on cell transplantation. In parallel to the further development of regenerative therapies, the classification of pulp diseases should be revised, and the diagnostic tools need improvement. **Conclusions**: The rethinking of current concepts for biology-based treatments and improved diagnostic concepts might postpone the point of root canal filling depending on the clinical situation. *(J Endod 2020;46:S161–S174.)*

KEY WORDS

Dental pulp necrosis; pulpectomy; pulpitis; pulpotomy; regenerative endodontics; root canal obturation

SIGNIFICANCE

Innovative treatment options for immature and mature teeth have been emerging over the last years, and the feasibility of pulpal regeneration has been proven for some clinical situations. However, it is debatable whether these methods can already be considered a suitable alternative for conventional endodontic treatment methods. This review aims to compile the available evidence and outlines the clinical perspectives for regenerative endodontic treatment approaches.

Over the past decade, the number of studies describing different treatment methods for dental pulp regeneration has significantly increased. These methods include the treatment of immature and mature teeth with so-called revitalization procedures; partial and total pulp regeneration procedures have also been proposed. However, considerable controversy still exists as to whether these methods may already be considered a suitable alternative or even a replacement for conventional endodontic treatment procedures.

In this review, innovative pulp regeneration procedures are compared with classic treatment modalities, each in the context of specific clinical situations. Classic vital pulp therapies like pulp capping or pulpotomy applying bioactive materials on the vital pulp may lead to the generation of new hard tissue. Therefore, these therapies also show a regenerative or reparative aspect; however, in this review, we focus on pulp regeneration and used the term for any induced new formation of pulp or pulplike tissue.

Furthermore, the question of what a desirable, or at least a clinically acceptable, outcome of these regenerative procedures is is addressed. Thus, this review focuses on different clinical situations, such as immature and mature permanent teeth, as well as different stages from early dental pulp inflammation to the loss of vitality (pulp necrosis), with or without apical periodontitis (AP), as a basis for the assessment of the suitability of present regenerative endodontic procedures.

IMMATURE TEETH

Endodontic treatment of immature teeth is a challenge, mainly because of the incompletely developed roots, both in length and thickness, and the wide open apical foramen. Clinical situations that may

From the *Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany; and †Department of Periodontology, University of Bern, Bern, Switzerland

Address requests for reprints to Dr Gottfried Schmalz, Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Franz-Josef-Strauss Allee 11, D-93053 Regensburg, Germany.
E-mail address: Gottfried.Schmalz@klinik.uni-regensburg.de
0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2020.06.037

HHS Public Access

Author manuscript

Biomaterials. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

Biomaterials. 2016 November ; 106: 167–179. doi:10.1016/j.biomaterials.2016.08.024.

Dental Cell Sheet Biomimetic Tooth Bud Model

Nelson Monteiro[1], **Elizabeth E. Smith**[1], **Shantel Angstadt**[1], **Weibo Zhang**[1], **Ali Khademhosseini**[2], and **Pamela C. Yelick**[1]

Nelson Monteiro: Nelson.Monteiro@tufts.edu; Elizabeth E. Smith: Elizabeth.Smith@tufts.edu; Shantel Angstadt: Shantel.Angstadt@tufts.edu; Weibo Zhang: Weibo.Zhang@tufts.edu; Ali Khademhosseini: alik@bwh.harvard.edu; Pamela C. Yelick: Pamela.Yelick@tufts.edu

[1]Department of Orthodontics, Division of Craniofacial and Molecular Genetics, Tufts University, Boston, Massachusetts, United States of America. 136 Harrison Avenue, M824 Boston MA 02111

[2]Division of Health Sciences and Technology, Harvard-MIT, Biomaterials Innovations Research Center, Division of Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School. 65 Landsdowne Street Cambridge, MA 02139

Abstract

Tissue engineering and regenerative medicine technologies offer promising therapies for both medicine and dentistry. Our long-term goal is to create functional biomimetic tooth buds for eventual tooth replacement in humans. Here, our objective was to create a biomimetic 3D tooth bud model consisting of dental epithelial (DE) – dental mesenchymal (DM) cell sheets (CSs) combined with biomimetic enamel organ and pulp organ layers created using GelMA hydrogels. Pig DE or DM cells seeded on temperature-responsive plates at various cell densities (0.02, 0.114 and 0.228 cells $10^6/cm^2$) and cultured for 7, 14 and 21 days were used to generate DE and DM cell sheets, respectively. Dental CSs were combined with GelMA encapsulated DE and DM cell layers to form bioengineered 3D tooth buds. Biomimetic 3D tooth bud constructs were cultured in vitro, or implanted *in vivo* for 3 weeks. Analyses were performed using micro-CT, H&E staining, polarized light (Pol) microscopy, immunofluorescent (IF) and immunohistochemical (IHC) analyses. H&E, IHC and IF analyses showed that *in vitro* cultured multilayered DE-DM CSs expressed appropriate tooth marker expression patterns including SHH, BMP2, RUNX2, tenascin and syndecan, which normally direct DE-DM interactions, DM cell condensation, and dental cell differentiation. *In vivo* implanted 3D tooth bud constructs exhibited mineralized tissue formation of specified size and shape, and SHH, BMP2 and RUNX2and dental cell differentiation marker expression. We propose our biomimetic 3D tooth buds as models to study optimized DE-DM cell interactions leading to functional biomimetic replacement tooth formation.

Keywords

Tooth development; dental stem cells; tissue engineering; regenerative medicine; biomaterials

Correspondence to: Pamela C. Yelick, `Pamela.Yelick@tufts.edu`.

The development of a bioengineered organ germ method

Kazuhisa Nakao[1,2], Ritsuko Morita[1,2], Yasumitsu Saji[1,2], Kentaro Ishida[1,2], Yusuke Tomita[1,2], Miho Ogawa[1,2], Masahiro Saitoh[3], Yasuhiro Tomooka[1,2] & Takashi Tsuji[1,2]

To bioengineer ectodermal organs such as teeth and whisker follicles, we developed a three-dimensional organ-germ culture method. The bioengineered tooth germ generated a structurally correct tooth, after both *in vitro* organ culture as well as transplantation under a tooth cavity *in vivo*, showing penetration of blood vessels and nerve fibers. Our method provides a substantial advance in the development of bioengineered organ replacement strategies and regenerative therapies.

The approaches that have been adopted in regenerative medicine are influenced by our understanding of embryonic development, stem-cell biology and tissue-engineering technology[1–3]. To restore the partial loss of organ function, stem cell transplantation therapies have been developed[1–3]. The ultimate goal of regenerative therapy, however, is to develop fully functioning bioengineered organs that can replace lost or damaged organs after disease, injury or aging[1–3]. Almost all organs arise from the organ germ, which is induced by the reciprocal interactions between the epithelium and mesenchyme in the developing embryo[4–7]. Therefore, it has been suggested that to properly reproduce the developmental process of organogenesis, it will be necessary to fully reconstitute these events in an artificially bioengineered organ[7].

The purpose of our study was to improve bioengineering methods for three-dimensional organ germs using completely dissociated epithelial and mesenchymal cells. For this purpose, we adopted the tooth and whisker follicle germs as model ectodermal organs. Although previous studies have demonstrated three-dimensional reconstruction of an artificial organ germ from dissociated single cells *in vitro*, improvement in bioengineering technology is needed before reconstitution of a primordial organ precisely replicates tooth organogenesis as observed in embryonic development[7–11]. The first step in multicellular aggregation of epithelial and mesenchymal cells is multicellular assembly by self-reorganization in each cell type through cell movement and selective cell adhesion until the cells reach an equilibrium configuration[12]. Next the reciprocal interactions between epithelial and mesenchymal cell layers initiate organogenesis and regulate differentiation and morphogenesis[5,6]. The cell potential for self-reorganization and tissue reconstitution, however, is different among cell types of various organs[10]. Here we describe a bioengineered organ germ method with cell compartmentalization *in vitro*, which is applicable to not only *in vitro* organ culture but also *in vivo* transplantation. Our model improves our understanding of the principles by which organ reconstitution can be achieved with tissues that have been bioengineered *in vitro* and increases the potential for the use of bioengineered organ replacement in future regenerative therapies.

We first investigated the possibility of developing a bioengineered tooth germ using completely dissociated single cells from epithelial and mesenchymal tissues of incisor tooth germ at cap stage from the lower jaw in ED14.5 mice (**Fig. 1a**, **Supplementary Methods** online and **Supplementary Fig. 1** online). The explants reconstituted by epithelial or mesenchymal cells alone generated keratinized oral epithelium-like structures or bone, respectively, but not a complete tooth (**Supplementary Fig. 1**). The explants that reconstituted the cell compartmentalization between epithelial and mesenchymal cells at a low-cell density ($0.5–1 \times 10^8$ cells/ml) or that did not form cell compartmentalization at high-cell density (5×10^8 cells/ml), also could not generate a correct tooth structure (**Supplementary Fig. 1**). To reconstitute a bioengineered tooth germ with the correct cell compartmentalization between epithelium- and mesenchyme-derived single cells, we injected the cells in turn at high cell density (5×10^8 cells/ml) into adjacent regions within a collagen gel drop (**Fig. 1a** and **Supplementary Methods**). Within 1 d of organ culture, we observed formation of a tooth germ with the appropriate compartmentalization between epithelial and mesenchymal cells and cell-to-cell compaction (**Fig. 1b**). We then performed transplantations of this bioengineered tooth germ into subrenal capsules in mice and over a 10-d period observed by histology that this primordium could generate plural incisors, in which tissue elements such as odontoblasts, dentin, dentinal tubules, ameloblasts, enamel, Tomes' process, dental pulp, root analog, blood vessels, alveolar bone and periodontal ligaments, were arranged appropriately when compared with a natural tooth (**Fig. 1c**, **Supplementary Fig. 1** and **Supplementary Fig. 2** online). We also found that this occurred with a frequency of 100% in 50 separate transplants. *In situ* hybridization analysis of the reconstituted teeth showed mRNA for dentin sialoprotein, amelogenin and periostin, specific markers for odontoblasts, ameloblasts and periodontal ligaments, respectively (**Supplementary Fig. 1**). We next examined the origin of the cell types derived from epithelium or mesenchyme using bioengineered tooth germ from GFP-transgenic mice. GFP-transgenic mouse–derived mesenchymal cells generated

[1]Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, Noda, Chiba, 278-8510, Japan. [2]Tissue Engineering Research Center, Tokyo University of Science, Noda, Chiba, 278-8510, Japan. [3]Department of Molecular and Cellular Biochemistry, Osaka University Graduate School of Dentistry, Osaka, Japan. Correspondence should be addressed to T.T. (t-tsuji@rs.noda.tus.ac.jp).

RECEIVED 23 OCTOBER 2006; ACCEPTED 9 JANUARY 2007; PUBLISHED ONLINE 18 FEBRUARY 2007; DOI:10.1038/NMETH1012

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Regrowing a tooth: *in vitro* and *in vivo* approaches

Liwen Li, Qinghuang Tang, Amy Wang and YiPing Chen

Biologically oriented regenerative dentistry in an attempt to regrow a functional tooth by harnessing the natural healing capabilities of dental tissues has become a recent trend challenging the current dental practice on repairing the damaged or missing tooth. In this review, we outline the conceptual development on the *in situ* revitalization of the tooth replacement capability lost during evolution, the updated progress in stem-cell-based *in vivo* repair of the damaged tooth, and the recent endeavors for *in vitro* generation of an implantable bioengineered tooth germ. Thereafter, we summarize the major challenges that need to be overcome in order to provide the rationale and directions for the success of fully functional tooth regeneration in the near future.

Address
Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118, USA

Corresponding authors: Li, Liwen (lli17@tulane.edu), Chen, YiPing (ychen@tulane.edu)

Current Opinion in Cell Biology 2019, **61**:126–131

This review comes from a themed issue on **Differentiation and disease**

Edited by **Sara Wickstrom** and **Yingzi Yang**

https://doi.org/10.1016/j.ceb.2019.08.002

Introduction

According to The National Health and Nutrition Examination Survey on oral health and dental care in the United States between 1999 and 2004, adults aged from 20 to 64 have an average of 3.28 decayed or missing permanent teeth. The loss or damage of natural teeth affects numbers of aspects of human's daily life, and is widespread, especially in older people, becoming a global health problem in the aging societies [1]. While conservative approaches in dental practice using inorganic materials [2] that would fail with time and could not provide the full function of teeth, regrowing or repairing one's own teeth is an ultimate alternative [2]. Recently, with the advanced understanding of tooth evolution and the underlying mechanisms regulating the morphogenesis of natural tooth *in vivo*, stem cell-based bioengineering is making it possible for *in vivo* and *in vitro* regeneration of a functional tooth. This review provides an overview of various paradigms of tooth tissue regeneration and outlines the challenges facing the field.

Tooth regeneration *in situ* by revitalizing the tooth replacement capability lost during evolution

Teeth and dermal denticles were previously recognized as derivatives of a homologous developmental unit, odontode [3]. Recent studies found that vertebrate teeth may emerge phylogenetically from the expansion of the odontogenic competence from the external dermal denticles [4]. When probably the first teeth emerged in Condonts as a series of odontodes throughout the oro-pharyngeal cavity in lower vertebrate [3], they had the regenerative capacity in adults. Almost all the vertebrates, including most teethed fishes, many reptiles and amphibians, regenerate their teeth continuously throughout entire lives (so-called polyphyodonts, Figure 1a) [5,6]. Although mammalian ancestors were polyphyodont, most extant mammals, such as humans [7] and miniature pigs [8], can regrow their teeth only once (diphyodont, Figure 1b). Some mammals, like mice, have only a single set of dentition, classified as monophyodont [5]. The history of vertebrate teeth brings us a consensus that the successional tooth replacement capability has been reduced over evolution [9], which wonders scientists that whether revitalizing the lost regenerative potential could achieve biological replacement of dental tissues, or even whole tooth regeneration *in vivo*. The capacity for tooth replacement resides in the dental lamina (DL) on the lingual side of the first tooth, known as the successional dental lamina (SDL) [10]. It was demonstrated that an odontogenic source of stem cells residing in the lingual DL gives rise to replacement tooth in lizard [11]. Further evidence indicates that the DL and SDL, which possess the capacity for tooth renewal in several species of mammals and reptiles, can be recognized by the stem cell marker Sox2 [12]. Moreover, it was shown that the SDL can be activated by increasing Wnt/β-catenin signaling, resulting in the initiation of tooth formation in alligator [13], and even the early emergence of multiple-generations of teeth in snakes [14]. Interestingly, in mice, which never replace their teeth, a transient rudimentary successional dental lamina (RSDL) still forms during tooth development [12]. Remarkably, stabilizing Wnt/β-catenin signaling in the RSDL revitalizes the rudimentary replacement teeth. Moreover, the RSDL has the potential to form a tooth when isolated from the first molar (Figure 1c) [15••]. These atavistic-like effects in the SDLs suggest that Wnt signaling can revitalize, or 'unlock' the tooth regenerative power lost during evolution. Therefore, understanding

Harnessing the Natural Regenerative Potential of the Dental Pulp

Anthony J. Smith, PhD*, James G. Smith, BMedSc,
Richard M. Shelton, BDS, PhD, Paul R. Cooper, PhD

KEYWORDS

• Dentin • Pulp • Regeneration • Stem cells • Tissue engineering • Injury
• Wound healing

KEY POINTS

- Biological solutions to the repair and regeneration of the dental tissues offer significant potential for improved clinical treatment outcomes.
- Translation of dental tissue-engineering approaches to the clinic will make considerable contributions to these outcomes in the future, but exploiting the natural regenerative potential of dentin-pulp to enhance wound-healing responses offers solutions for maintaining pulp vitality now.
- Strategies to harness the natural regenerative potential of the pulp must be based on a sound biological understanding of the cellular and molecular events taking place, and require careful consideration of the interplay of infection, inflammation, and regeneration.

Regenerative medicine offers many advantages for the treatment of disease with its aim of "replacing or regenerating human cells, tissues or organs to restore or establish normal function,"[1] and within dentistry there is exciting future potential for engineering whole teeth.[2–8] Nevertheless, several challenges still exist before such tooth-replacement therapies can be clinically implemented in dental practice. Paramount among these challenges is programmed development of appropriate crown and cuspal morphology for occlusal function and engineering of the tooth/periodontium interface to allow normal oral function. Use of scaffolds of defined morphology may assist with overcoming some of the morphologic challenges. Recently the authors generated a mineralized tissue construct, retaining the morphology of the human tooth

Financial Disclosure and Conflict of Interest: The authors declare no conflict of interest or financial interest with any commercial company in relation to the content of this article. The University of Birmingham was the only body/organization that provided funding support for the preparation of this article.
School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham B4 6NN, UK
* Corresponding author.
E-mail address: a.j.smith@bham.ac.uk

Dent Clin N Am 56 (2012) 589–601
doi:10.1016/j.cden.2012.05.011 dental.theclinics.com
0011-8532/12/$ – see front matter

Dental Pulp Tissue Engineering with Stem Cells from Exfoliated Deciduous Teeth

Mabel M. Cordeiro, DDS, MS, PhD, Zhibong Dong, MD, PhD,* Tomoatsu Kaneko, DDS, PhD,*† Zhaocheng Zhang, MD, PhD,* Marta Miyazawa, DDS, MS, PhD,* Songtao Shi, DDS, PhD,‡ Anthony J. Smith, BSc, DDS, PhD,§ and Jacques E. Nör, DDS, MS, PhD*‖*

Abstract

Stem cells from human exfoliated deciduous teeth (SHED) have been isolated and characterized as multipotent cells. However, it is not known whether SHED can generate a dental pulp-like tissue in vivo. The purpose of this study was to evaluate morphologic characteristics of the tissue formed when SHED seeded in biodegradable scaffolds prepared within human tooth slices are transplanted into immunodeficient mice. We observed that the resulting tissue presented architecture and cellularity that closely resemble those of a physiologic dental pulp. Ultrastructural analysis with transmission electron microscopy and immunohistochemistry for dentin sialoprotein suggested that SHED differentiated into odontoblast-like cells in vivo. Notably, SHED also differentiated into endothelial-like cells, as demonstrated by B-galactosidase staining of cells lining the walls of blood-containing vessels in tissues engineered with SHED stably transduced with LacZ. This work suggests that exfoliated deciduous teeth constitute a viable source of stem cells for dental pulp tissue engineering. *(J Endod 2008;34:962–969)*

Key Words

Angiogenesis, endodontics, multipotency, odontoblast, scaffold

From the *Department of Cariology, Restorative Sciences, and Endodontics, University of Michigan School of Dentistry, Ann Arbor, Michigan; †Department of Restorative Sciences, Tokyo Medical and Dental University, Tokyo, Japan; ‡Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California; Los Angeles, California; §Unit of Oral Biology, University of Birmingham School of Dentistry; Birmingham, United Kingdom; and ‖Department of Biomedical Engineering, University of Michigan College of Engineering; Ann Arbor, Michigan.

Address requests for reprints to Jacques E. Nör, DDS, MS, PhD, Department of Cariology, Restorative Sciences, and Endodontics; School of Dentistry, Department of Biomedical Engineering; College of Engineering, University of Michigan, 1011 N University, Room 2309, Ann Arbor, MI 48109-1078. E-mail address: jenor@umich.edu.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2008.04.009

Regenerative medicine offers exciting opportunities to replace or restore tissues of the body after disease and trauma. Tissue engineering approaches aim to fabricate new replacement body tissues, and such approaches commonly involve seeding of cells at various stages of differentiation within scaffolds, which can then be implanted (1). The complexity of architecture and function of many tissues, however, provides significant challenges to engineering replacement tissues resembling their physiologic counterparts. Use of stem cells, either of embryonic or postnatal derivation, for tissue engineering is attractive because it offers greater scope for cell fate to try and mimic physiologic tissue architecture. However, ethical constraints associated with use of embryonic stem cells (ESCs) and limitations of readily accessible sources of autologous postnatal stem cells with multipotentiality pose significant challenges for use of stem cells in tissue engineering. Furthermore, the requirement for good vascularization of any tissue construct is of paramount importance to its vitality.

The discovery of stem cells in the pulp of permanent teeth (2) and also in deciduous teeth (3) raised the intriguing possibility of using dental pulp stem cells for tissue engineering (4–6). The dental pulp stem cells have been shown to be capable of self-renewal and multilineage differentiation (7). These stem cells can be isolated from patients with relatively minimal morbidity, especially when they are retrieved noninvasively from the pulps of exfoliated deciduous teeth (3). The first successful attempt to engineer complex whole tooth structures used single-cell suspensions dissociated from porcine third molar tooth buds and suggested the existence of dental pulp stem cells in this tissue (8). Others have successfully used a similar approach for the bioengineering of organs for regenerative therapies (9). The concept of using stem cells for dental tissue engineering was explored by Sharpe and Young (10). They and others demonstrated that it is possible to engineer murine teeth by using adult stem cells of nondental or dental origin (10–12). Recently, mesenchymal stem cells isolated from the root apical papilla of human teeth were shown to be capable of mediating tooth regeneration with recovery of tooth strength and appearance (13).

Stem cells from human exfoliated deciduous teeth (SHED) have become an attractive alternative for dental tissue engineering (3). The use of SHED might bring advantages for tissue engineering over the use of stem cells from adult human teeth as follows: (1) SHED were reported to have higher proliferation rate and increase cell population doublings as compared with stem cells from permanent teeth (3). This might facilitate the expansion of these cells in vitro before replantation. (2) SHED cells are retrieved from a tissue that is "disposable" and readily accessible in young patients, ie, exfoliated deciduous teeth. (3) We have previously proposed that dental pulp tissue engineering with stem cells could be ideally suited for young patients who have suffered pulp necrosis in immature permanent incisors as consequence of trauma (14). Such treatment could potentially allow for the completion of vertical and lateral root development and perhaps improve the long-term outcome of these teeth. The fact that these patients are in mixed dentition, and therefore their deciduous molars are at various degrees of exfoliation, makes SHED a timely and opportune stem cell source for the engineering of dental pulps in immature permanent teeth.

Although the concept of engineering whole tooth structures offers exciting potential, significant clinical challenges still remain, and engineering or regeneration of component tissues of the tooth might be a more realistic shorter-term goal. In partic-

Thematic Review Series 2013

Respiration

Respiration 2013;85:3–10
DOI: 10.1159/000345615

Published online: December 13, 2012

Introduction to Stem Cells and Regenerative Medicine

George Kolios[a] Yuben Moodley[b–d]

[a]Laboratory of Pharmacology, Faculty of Medicine, Democritus University of Thrace, Alexandroupolis, Greece; [b]School of Medicine and Pharmacology, University of Western Australia, and [c]Department of Respiratory and Sleep Medicine, Royal Perth Hospital, and [d]Lung Institute of Western Australia, Perth, W.A., Australia

Key Words
Tissue-resident stem cells · Induced pluripotent stem cells · Mesenchymal stem cells

Abstract
Stem cells are a population of undifferentiated cells characterized by the ability to extensively proliferate (self-renewal), usually arise from a single cell (clonal), and differentiate into different types of cells and tissue (potent). There are several sources of stem cells with varying potencies. Pluripotent cells are embryonic stem cells derived from the inner cell mass of the embryo and induced pluripotent cells are formed following reprogramming of somatic cells. Pluripotent cells can differentiate into tissue from all 3 germ layers (endoderm, mesoderm, and ectoderm). Multipotent stem cells may differentiate into tissue derived from a single germ layer such as mesenchymal stem cells which form adipose tissue, bone, and cartilage. Tissue-resident stem cells are oligopotent since they can form terminally differentiated cells of a specific tissue. Stem cells can be used in cellular therapy to replace damaged cells or to regenerate organs. In addition, stem cells have expanded our understanding of development as well as the pathogenesis of disease. Disease-specific cell lines can also be propagated and used in drug development. Despite the significant advances in stem cell biology, issues such as ethical controversies with embryonic stem cells, tumor formation, and rejection limit their utility. However, many of these limitations are being bypassed and this could lead to major advances in the management of disease. This review is an introduction to the world of stem cells and discusses their definition, origin, and classification, as well as applications of these cells in regenerative medicine.

Introduction

Stem cells are undifferentiated cells that are present in the embryonic, fetal, and adult stages of life and give rise to differentiated cells that are building blocks of tissue and organs. In the post-natal and adult stages of life, tissue-specific stem cells are found in differentiated organs and are instrumental in repair following injury to the organ. The major characteristics of stem cells are: (a) self-renewal (the ability to extensively proliferate), (b) clonality (usually arising from a single cell), and (c) potency (the ability to differentiate into different cell types). These properties may differ between various stem cells. For example, embryonic stem cells (ESCs)

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/13/0851–0003$38.00/0

Accessible online at:
www.karger.com/res

Prof. George Kolios, MD, PhD
Laboratory of Pharmacology
Faculty of Medicine, Democritus University of Thrace
Dragana, GR–68100 Alexandroupolis (Greece)
E-Mail gkolios@med.duth.gr

TISSUE ENGINEERING: Part B
Volume 17, Number 2, 2011

DOI: 10.1089/ten.teb.2010.0549

Prospects of Induced Pluripotent Stem Cell Technology in Regenerative Medicine

Feng Zhang, B.S., Fudiman Citra, B.S., and Dong-An Wang, Ph.D.

Induced pluripotent stem (iPS) cells are derived from adult somatic cells via reprogramming with ectopic expression of four transcription factors (Oct3/4, Sox2, c-Myc and Klf4; or, Oct3/4, Sox2, Nanog, and Lin28), by which the resultant cells regain pluripotency, namely, the capability exclusively possessed by some embryonic cells to differentiate into any cell lineage under proper conditions. Given the ease in cell sourcing and a waiver of ethical opponency, iPS cells excel embryonic pluripotent cells in the practice of drug discovery and regenerative medicine. With an *ex vivo* practice in regenerative medicine, many problems involved in conventional medicine dosing, such as immune rejection, could be potentially circumvented. In this article, we briefly summarize the fundamentals and status quo of iPS-related applications, and emphasize the prospects of iPS technology in regenerative medicine.

Introduction

Embryonic stem cells (ESCs), as a type of pluripotent stem cells, have been extensively studied in recent years owing to their high plasticity. They have been a promising therapeutic agent since their derivation in 1998.[1] However, the complicated procedures involved in their derivation, immunological issues, as well as the ethical problems involved, have greatly hindered their clinical applications. The emergence of induced pluripotent stem (iPS) technology has presented a breakthrough as an encouraging alternative for ESCs. The work in iPS cells was pioneered by a Japanese group, which successfully obtained pluripotency from mouse and human adult/embryonic fibroblasts by introducing four transcriptional factors- Oct3/4, Sox2, c-Myc, and Klf4, via ectopic expression using retroviral vectors.[2,3] Alternatively, the U.S. group generated iPS cells from human somatic cells with Oct4, Sox2, Nanog, and Lin28 using lentiviral vectors.[4] Since then, a great many publications have emerged following these pioneering studies.[4–6] Using somatic cells as a source, ethical dispute and immunological problems can be circumvented, whereas, in the meantime, the obtained pluripotency will render great hope in regenerative medicine by giving rise to any cell type in need.

In this review, the fundamentals of iPS cells involving their creation will be briefly reviewed, followed by a detailed description of the efforts aiming at inducing iPS cells into specific target cell types as potential regenerative medicine. Some examples of current research using iPS cells in some pathological models will also be provided. Besides, efforts to solve some technical problems encountered, as well as prospects in the application of iPS cells in regenerative medicine will also be concisely covered.

Brief Overview of iPS Technology

The generation of iPS cells was first reported by Takahashi *et al.* and Yu *et al.*, who identified Oct3/4, Sox2, c-Myc, and Klf4, or Oct4, Sox2, Nanog, and Lin28 as four essential transcriptional factors sufficient to induce fibroblasts to gain pluripotency.[2,4] After their innovation, more and more researchers have achieved the same objective with some alterations in the selection of source cells, various inducing agents and the vectors used to deliver such agents. The details of these items as well as the evidences for the resulting pluripotency are listed in Table 1. Reviews about iPS biological fundamentals have been contributed by Selvaraj *et al.* and Das *et al.*,[7,8] whereas we focus on the application of iPS technology in regenerative medicine in this article.

iPS cells have broad potential applications as ESCs do, with some work having been done or being carried out to exercise these applications. Since iPS cells can be derived from patients with specific diseases, such as Shwachman-Bodian-Diamond syndrome, adenosine deaminase deficiency-related severe combined immunodeficiency, Parkinson disease, and Gaucher disease type III, they can serve as perfect disease models for biological and pharmacological studies and drug screening.[9–11] The application of iPS cells as *in vitro* models of neurogenetic disorders has also been reviewed.[12] Cardiomyocytes induced from iPS cells were used for screening purposes by measuring the responsiveness of the cells to a variety of drugs for arrhythmic diseases.[13,14] iPS cells can also be a target in

Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, Singapore.

International Journal of
Molecular Sciences

MDPI

Review

Tooth Formation: Are the Hardest Tissues of Human Body Hard to Regenerate?

Juliana Baranova [1], Dominik Büchner [2], Werner Götz [3], Margit Schulze [2] and Edda Tobiasch [2,*]

[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, Avenida Professor Lineu Prestes 748, Vila Universitária, São Paulo 05508-000, Brazil; jbaranova@usp.br
[2] Department of Natural Sciences, Bonn-Rhein-Sieg University of Applied Sciences, von-Liebig-Straße 20, 53359 Rheinbach, NRW, Germany; dominik.buechner@h-brs.de (D.B.); margit.schulze@h-brs.de (M.S.)
[3] Oral Biology Laboratory, Department of Orthodontics, Dental Hospital of the University of Bonn, Welschnonnenstraße 17, 53111 Bonn, NRW, Germany; wgoetz@uni-bonn.de
* Correspondence: edda.tobiasch@h-brs.de; Tel.: +49-2241-865-576

Received: 29 April 2020; Accepted: 3 June 2020; Published: 4 June 2020

check for updates

Abstract: With increasing life expectancy, demands for dental tissue and whole-tooth regeneration are becoming more significant. Despite great progress in medicine, including regenerative therapies, the complex structure of dental tissues introduces several challenges to the field of regenerative dentistry. Interdisciplinary efforts from cellular biologists, material scientists, and clinical odontologists are being made to establish strategies and find the solutions for dental tissue regeneration and/or whole-tooth regeneration. In recent years, many significant discoveries were done regarding signaling pathways and factors shaping calcified tissue genesis, including those of tooth. Novel biocompatible scaffolds and polymer-based drug release systems are under development and may soon result in clinically applicable biomaterials with the potential to modulate signaling cascades involved in dental tissue genesis and regeneration. Approaches for whole-tooth regeneration utilizing adult stem cells, induced pluripotent stem cells, or tooth germ cells transplantation are emerging as promising alternatives to overcome existing in vitro tissue generation hurdles. In this interdisciplinary review, most recent advances in cellular signaling guiding dental tissue genesis, novel functionalized scaffolds and drug release material, various odontogenic cell sources, and methods for tooth regeneration are discussed thus providing a multi-faceted, up-to-date, and illustrative overview on the tooth regeneration matter, alongside hints for future directions in the challenging field of regenerative dentistry.

Keywords: dentogenesis; amelogenesis; dentinogenesis; cementogenesis; drug release materials; scaffolds; odontogenic cells; stem cells; whole-tooth regeneration

1. Introduction

Dental injuries and diseases such as caries and periodontitis are affecting significant fractions of populations worldwide and are the main reason for dental tissue regeneration efforts [1,2]. Caries lesions cause local enamel resorption and dentin damage due to oral microbiota activities in the morbid tooth. Although relatively easily manageable at early stages, if left untreated caries causes excessive dentin damage and poses a need for reparative treatment [3]. Periodontitis is a complex inflammatory disease, where pathogenic oral microbiota and host immune response dysregulation lead to the gingiva, periodontal ligament, cementum, and alveolar bone damage [4]. Excessive periodontitis damage cannot be regenerated naturally, thus requires specialized soft and hard calcified tissues regeneration approaches. Next to infectious/inflammatory oral diseases, several heritable disorders of dental tissue formation exist (e.g., amelogenesis imperfecta, dentinogenesis imperfecta, and tooth agenesis), which affect tooth formation, eruption, calcification, or maturation [5–8]. In addition to disrupted teeth

Multipotent Stem Cell and Current Application

Aligholi Sobhani[1], Neda Khanlarkhani[1], Maryam Baazm[2], Farzaneh Mohammadzadeh[1], Atefeh Najafi[1], Shayesteh Mehdinejadiani[1], and Fereydoon Sargolzaei Aval[3]

[1] Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Anatomy, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[3] Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

Received: 13 Dec. 2015; Accepted: 17 Dec. 2016

Abstract- Stem cells are self-renewing and undifferentiated cell types that can be differentiate into functional cells. Stem cells can be classified into two main types based on their source of origin: Embryonic and Adult stem cells. Stem cells also classified based on the range of differentiation potentials into Totipotent, Pluripotent, Multipotent, and Unipotent. Multipotent stem cells have the ability to differentiate into all cell types within one particular lineage. There are plentiful advantages and usages for multipotent stem cells. Multipotent Stem cells act as a significant key in procedure of development, tissue repair, and protection. Multipotent Stem cells have been applying in treatment of different disorders such as spinal cord injury, bone fracture, autoimmune diseases, rheumatoid arthritis, hematopoietic defects, and fertility preservation.

Acta Med Iran 2017;55(1):6-23.

Keywords: Multipotent; Stem cell; Fertility; Preservation; Regenerative medicine

Introduction

How to define stem cells?

Stem cells are self-renewing and undifferentiated cell types that can be differentiate into functional cells (1-5). Self-renewal is the process by which stem cells generate unfirentiated daughter cells. It is required for preserve stem cell populations in different tissues (3,6).

Classification of stem cells

Stem cells can be classified into two main types based on their source of origin: (1) Embryonic stem cell, which derived from the inner cell mass of preimplantation embryos and has the ability to form all three embryonic germ layers (i.e., ectoderm, endoderm and mesoderm); (2) Adult stem cells, which scattered in various tissues and organs, and has the capability to produce at least one type of differentiated functional progeny (4,7-10). Although the later type is thought to have limited differentiation capability previously, recent evidence have shown the capacity of differentiation into the 3 embryonic layers (11). Such as induced pluripotent stem cells that can be able to generate from a variety of somatic cells and give rise into endodermal-, mesodermal-, and ectodermal-lineage cells (12,13).

Stem cells also classified based on the range of differentiation potentials (3): Totipotent, Pluripotent, Multipotent, and Unipotent. Totipotent cells such as Zygote and early Blastomeres (1-3 d from oocyte fertilization) have the ability to produce all types of cells while pluripotent cells such as inner cell mass of blastocysts (days 4-14 after oocyte fertilization) could generate all cell types excluding extra embryonic trophoblast lineage (3,9,14,15). Telomerase (Tert) catalytic subunit which is a landmark of pluripotent and germ cells, is express extensively in mouse and human oogonial stem cells (16). Also multilineage-differentiating stress-enduring (Muse) cells are one of the other Pluripotent stem cells examples that have capacity to generate cell types from all three germ layers (17). Multipotent stem cells have the ability to differentiate into all cell types within one particular lineage (14,15) and unipotent stem cells, are defined as cells that have the competency of differentiating into only one lineage (Figure 1) (3).

Corresponding Author: A. Sobhani
Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 64053308, Fax: +98 21 66419072, E-mail address: sobhania@tums.ac.ir

World Journal of Stem Cells

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4252/wjsc.v7.i5.839

World J Stem Cells 2015 June 26; 7(5): 839-851
ISSN 1948-0210 (online)

REVIEW

Human dental pulp stem cells: Applications in future regenerative medicine

Pravin D Potdar, Yogita D Jethmalani

Pravin D Potdar, Yogita D Jethmalani, Department of Molecular Medicine and Biology, Jaslok Hospital and Research Centre, Mumbai 400026, Maharashtra, India

Author contributions: Both authors contributed to this manuscript.

Supported by Jaslok Hospital and Research Centre, Mumbai, India, Project ni 491, A/C 27814.

Conflict-of-interest: No.

Correspondence to: Dr. Pravin D Potdar, MSc, PhD, DMLT, DHE, DMS, Head, Department of Molecular Medicine and Biology, Jaslok Hospital and Research Centre, 15 Dr. G Deshmukh Marg, Mumbai 400026, Maharashtra, India. ppotdar@jaslokhospital.net
Telephone: +91-22-66573445
Fax: +91-22-23520508

Received: October 24, 2014
Peer-review started: October 27, 2014
First decision: January 20, 2015
Revised: April 2, 2015
Accepted: April 16, 2015
Article in press: April 20, 2015
Published online: June 26, 2015

Abstract

Stem cells are pluripotent cells, having a property of differentiating into various types of cells of human body. Several studies have developed mesenchymal stem cells (MSCs) from various human tissues, peripheral blood and body fluids. These cells are then characterized by cellular and molecular markers to understand their specific phenotypes. Dental pulp stem cells (DPSCs) are having a MSCs phenotype and they are differentiated into neuron, cardiomyocytes, chondrocytes, osteoblasts, liver cells and β cells of islet of pancreas. Thus, DPSCs have shown great potentiality to use in regenerative medicine for treatment of various human diseases including dental related problems. These cells can also be developed into induced pluripotent stem cells by incorporation of pluripotency markers and use for regenerative therapies of various diseases. The DPSCs are derived from various dental tissues such as human exfoliated deciduous teeth, apical papilla, periodontal ligament and dental follicle tissue. This review will overview the information about isolation, cellular and molecular characterization and differentiation of DPSCs into various types of human cells and thus these cells have important applications in regenerative therapies for various diseases. This review will be most useful for postgraduate dental students as well as scientists working in the field of oral pathology and oral medicine.

Key words: Human dental pulp stem cells; Mesenchymal stem cells; Dentin; Pluripotency; Stem cell therapy; Molecular markers

Core tip: Human dental pulp stem cells (DPSCs) have shown a potentiality for the treatment of various human diseases including dental related problems. The review will overview the information about DPSCs, their isolation, cellular and molecular characterization, differentiation into various types of cells and their applications in regenerative therapies for various diseases. This review will be most useful for postgraduate dental students as well as the scientists working in the field of oral pathology, oral medicine and regenerative medicine.

Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*

S. Gronthos, M. Mankani, J. Brahim, P. Gehron Robey, and S. Shi*

Craniofacial and Skeletal Diseases Branch, National Institute on Dental Research, National Institutes of Health, Bethesda, MD 20892

Edited by Darwin J. Prockop, Tulane University, New Orleans, LA, and approved September 26, 2000 (received for review July 5, 2000)

Dentinal repair in the postnatal organism occurs through the activity of specialized cells, odontoblasts, that are thought to be maintained by an as yet undefined precursor population associated with pulp tissue. In this study, we isolated a clonogenic, rapidly proliferative population of cells from adult human dental pulp. These DPSCs were then compared with human bone marrow stromal cells (BMSCs), known precursors of osteoblasts. Although they share a similar immunophenotype *in vitro*, functional studies showed that DPSCs produced only sporadic, but densely calcified nodules, and did not form adipocytes, whereas BMSCs routinely calcified throughout the adherent cell layer with clusters of lipid-laden adipocytes. When DPSCs were transplanted into immunocompromised mice, they generated a dentin-like structure lined with human odontoblast-like cells that surrounded a pulp-like interstitial tissue. In contrast, BMSCs formed lamellar bone containing osteocytes and surface-lining osteoblasts, surrounding a fibrous vascular tissue with active hematopoiesis and adipocytes. This study isolates postnatal human DPSCs that have the ability to form a dentin/pulp-like complex.

odontoblast | dentin | *in vivo* transplantation

During tooth formation, interactions between epithelial and dental papilla cells promote tooth morphogenesis by stimulating a subpopulation of mesenchymal cells to differentiate into odontoblasts, which in turn form primary dentin. Morphologically, odontoblasts are columnar polarized cells with eccentric nuclei and long cellular processes aligned at the outer edges of dentin (1). After tooth eruption, reparative dentin is formed by odontoblasts in response to general mechanical erosion or disruption, and through dentinal degradation caused by bacteria (2). These odontoblasts are thought to arise from the proliferation and differentiation of a precursor population, residing somewhere within the pulp tissue (3). Despite extensive knowledge of tooth development, and of the various specialized tooth-associated cell types, little is known about the characteristics and properties of their respective precursor cell populations in the postnatal organism.

To date, the identification and isolation of an odontogenic progenitor population from adult dental pulp tissue has never been done. It is known that in certain conditions, cultures of pulp cells derived from early developing dental root tissue and pulp tissue can develop an odontoblast-like appearance with the capacity to form mineralized nodules *in vitro* (4), a trait normally attributed to cultures of bone or bone marrow cells (5, 6). More is known about the characteristics of multipotent bone marrow stromal cells (BMSCs) and their potential to develop into osteoblasts, chondrocytes, adipocytes, myelosupportive fibrous-stroma, and perhaps even muscle and neural tissues (7–12). They are characterized by their high proliferative capacity *ex vivo*, whereas maintaining their ability to differentiate into multiple stromal cell lineages. The tissue-specific differentiation of BMSCs seems to be dependent on their state of differentiation and commitment, and the microenvironment in which they are located. By analogy, we speculated that adult dental pulp tissue might also contain a population of multipotential stem cells.

In the present study, clonogenic and highly proliferative cells were derived from enzymatically disaggregated adult human dental pulp, which we have termed DPSCs, and compared with BMSCs, cells with known stem cell character (13). We have previously shown that human bone is generated after xenogeneic transplantation of BMSCs with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier vehicle (9). We therefore explored the possibility that isolated *ex vivo*-expanded human DPSCs would also be capable of regenerating a dentin/pulp-like structure *in vivo* under similar conditions.

Materials and Methods

Subjects and Cell Culture. Normal human impacted third molars were collected from adults (19–29 years of age) at the Dental Clinic of the National Institute of Dental and Craniofacial Research under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. Tooth surfaces were cleaned and cut around the cementum-enamel junction by using sterilized dental fissure burs to reveal the pulp chamber. The pulp tissue was gently separated from the crown and root and then digested in a solution of 3 mg/ml collagenase type I (Worthington Biochem, Freehold, NJ) and 4 mg/ml dispase (Boehringer Mannheim) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-μm strainer (Falcon). Bone marrow cells, processed from marrow aspirates of normal human adult volunteers (20–35 years of age), were purchased from Poietic Technologies (Gaithersburg, MD) and then washed in growth medium. Single-cell suspensions (0.01 to 1 $\times$ 10^5/well) of dental pulp and bone marrow were seeded into 6-well plates (Costar) with alpha modification of Eagle's medium (GIBCO/BRL) supplemented with 20% FCS (Equitech-Bio, Kerrville, TX)/100 μM L-ascorbic acid 2-phosphate (Wako Pure Chemicals, Osaka)/2 mM L-glutamine/100 units/ml penicillin/100 μg/ml streptomycin (Biofluids, Rockville, MD), and then incubated at 37°C in 5% CO_2. To assess colony-forming efficiency, day 14 cultures were fixed with 4% formalin, and then stained with 0.1% toluidine blue. Aggregates of $\geq$50 cells were scored as colonies. Conditions for the induction of calcified bone matrix deposition *in vitro* were as reported (6). The proliferation rate of subconfluent cultures (first passage) of DPSCs and BMSCs was assessed by bromodeoxyuridine (BrdUrd) incorporation for 24 h by using a Zymed BrdUrd staining kit (Vector Laboratories).

Immunohistochemistry. Primary DPSCs and BMSCs were subcultured into 8-chamber slides (2 $\times$ 10^4 cells/well) (Nunc). The

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: DPSC, dental pulp stem cell; BMSC, bone marrow stromal cell; HA/TCP, hydroxyapatite/tricalcium phosphate; BrdUrd, bromodeoxyuridine; DSPP, dentin sialophosphoprotein; CFU-F, colony-forming unit-fibroblast.

*To whom reprint requests should be addressed. E-mail: sshi@dir.nidr.nih.gov.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.240309797. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.240309797

Dental Stem Cells and Their Sources

Christine M. Sedgley, MDS, MDSc, FRACDS, MRACDS(ENDO), PhD[a,*],
Tatiana M. Botero, DDS, MS[b]

KEYWORDS

- Dental pulp stem cells (DPSCs)
- Stem cells from human exfoliated deciduous teeth (SHED cells)
- Stem cells from root apical papilla (SCAP cells)
- Periodontal ligament stem cells (PDLSCs) • Dental follicle precursor cells (DFPCs)

KEY POINTS

- The search for more accessible mesenchymal stem cells than those found in bone marrow has propelled interest in dental tissues, which are rich sources of stem cells. Human dental stem/progenitor cells (collectively termed dental stem cells [DSCs]) that have been isolated and characterized include dental pulp stem cells, stem cells from exfoliated deciduous teeth, stem cells from apical papilla, periodontal ligament stem cells, and dental follicle progenitor cells.
- The common characteristics of these cell populations are the capacity for self-renewal and the ability to differentiate into multiple lineages (multipotency). In vitro and animal studies have shown that DSCs can differentiate into osseous, odontogenic, adipose, endothelial, and neural-like tissues.
- In recent studies, third molar dental pulp somatic cells have been reprogrammed to become induced pluripotent stem cells, and dental pulp pluripotentlike stem cells have been isolated from the pulps of third molar teeth.

INTRODUCTION

The aim of regenerative medicine and tissue engineering is to replace or regenerate human cells, tissue or organs, to restore or establish normal function.[1] The 3 key elements for tissue engineering are stem cells, scaffolds, and growth factors. Cell-based therapies are integral components of regenerative medicine that exploit the

The authors have nothing to disclose.

[a] Department of Endodontology, School of Dentistry, Oregon Health and Science University, 611 Southwest Campus Drive, Portland, OR 97239, USA; [b] Cariology, Restorative Sciences and Endodontics, School of Dentistry, University of Michigan, 1011 North University, Room 1376D, Ann Arbor, MI 48108-1078, USA
* Corresponding author.
E-mail address: sedgley@ohsu.edu

Dent Clin N Am 56 (2012) 549–561
http://dx.doi.org/10.1016/j.cden.2012.05.004 **dental.theclinics.com**

DOI: http://dx.doi.org/10. 18363/rbo.v73n4.p.331

:: Review Literature Article/ Basic Sciences

Dental stem cells and their application in Dentistry: a literature review

Paula Nascimento Almeida,[1,2] Karin Soares Cunha[1,2]

[1]Programa de Pós-Graduação em Patologia, Faculdade de Medicina, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brasil

[2]Centro Nacional de Neurofibromatose, Rio de Janeiro, Rio de Janeiro, Brasil

• Conflicts of interest: none declared.

Abstract

Objective: the aim of this study was to conduct a literature review of the types of stem cells of dental origin and their applications in Dentistry. Material and Methods: for this, we selected scientific articles published between 2000 and 2016 through the databases PUBMED and LILACS. Results: there are five main sources of stem cells of dental origin: stem cells from dental pulp of permanent teeth and deciduous teeth, apical papilla, periodontal ligament and dental follicle. These cells have been studied for the treatment of periodontitis, bone repair, regeneration of the pulp after necrosis as well as the development of new teeth. Conclusion: stem cells from dental origin are an interesting alternative for research and application in regenerative therapies in Dentistry.

Keywords: Stem cells; Tissue engineering; Dentistry.

Introduction

Stem cells (SCs) are undifferentiated cells with self-renewal ability and capacity to differentiate into specialized cell types.[1,2] Regarding the origin, they can be classified as embryonic stem cells (ESCs) and adult stem cells (ASCs).[3] Embrionic stem cells (ESCs) are derived from the inner cell mass of the blastocyst and form all cell types, derived from the three germ layers, and are therefore pluripotent.[3,4] The zygote and cells derived from the first two cellular divisions constitute the most primitive cells (totipotent cells) that are capable of forming the embryo and the embryonic annexes (e.g. placenta, amniotic membranes etc).

ASCs are present in a number of posnatal tissues and are responsible for normal tissue renewal as well as for regeneration and healing after injuries. Due to the ability to self-renew and to differentiate into cells that are found throughout the body, there is a great interest in using stem cells for the regeneration of injured tissues as well as to develop tissue-engineered implants and bio-hybrid organs, in order to restore tissue function. The use of ASCs in regenerative medicine and tissue engineering research has important advantages in comparison with ESCs, since there are no ethical complications and the process of differentiation of these cells is better controlled.[5]

Mesenchymal stem cells (MSCs) are ASCs, and were first described in 1966 by Friedenstein *et al.*[6] Since then, clinical and biological interest in MSCs have increased and the Mesenchymal Stem Cells Committee of the International Society for Cellular Therapy proposed a minimum criteria for the identification of these cells: adherence to plastic culture surfaces, potential of osteogenic, adipogenic and chondrogenic differentiation *in vitro* as well as expression of surface antigens CD73, CD90 and CD105 and lack of expression of hematopoietic and endothelial markers CD14 or CD11b, CD34, CD45, CD79alpha or CD19 and human leukocyte antigen-DR (HLA-DR).[7]

MSCs can be isolated from different locations, such as bone marrow, umbilical cord, placenta, adipose and dental tissues.[8,9] Because dental stem cells (DSCs) are easy to obtain and present a great potential of differentiation, there has been a growing interest in their use in regenerative medicine for treatment of various human diseases.[10]

In human postnatal dental tissues, five main sources of DSCs have been identified: dental pulp stem cells (DPSCs),[11] stem cells from human exfoliated deciduous teeth (SHEDs),[12] periodontal ligament stem cells (PDLSCs),[13] dental follicle stem cells (DFSCs)[14] and stem cells from apical papilla (SCAPs).[15] Regardless the tooth tissue of origin, DSCs can be isolated either disaggregating the tissue enzymatically and/or mechanically, or also by explant. After enzymatic and/or mechanical dissociation, cells are placed in culture medium for growing on plastic flasks or dishes. In explant method, the dental tissue is placed on a plastic surface and the cells migrate out from the tissue fragment adhering to culture flasks or dishes (Figure 1).

Biol. Cell (2007) 99, 465–474 (Printed in Great Britain) doi:10.1042/BC20070013

Research article

Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells

Jinhua Yu*†‡[1], Yijing Wang*†[1], Zhihong Deng§, Liang Tang†[1], Yuanfei Li‖, Junnan Shi*[2] and Yan Jin†¶[2]

*Department of Endodontics, School of Stomatology, Fourth Military Medical University, Xi'an, Shaanxi 710032, China, †Department of Oral Histology and Pathology, School of Stomatology, Fourth Military Medical University, Xi'an, Shaanxi 710032, China, ‡Department of Endodontics, School of Stomatology, Nanjing Medical University, Nanjing, Jiangsu 210029, China, §Department of Otolaryngology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China, ‖State Key Laboratory of Cancer Biology, Fourth Military Medical University, Xi'an, Shaanxi 710032, China, and ¶Research and Development Center for Tissue Engineering, Fourth Military Medical University, Xi'an, Shaanxi 710032, China

Background information. Although adult bone-marrow-derived cell populations have been used to make teeth when recombined with embryonic oral epithelium, the differences between dental and non-dental stem-cell-mediated odontogenesis remain an open question.

Results. STRO-1$^+$ (stromal precursor cell marker) DPSCs (dental pulp stem cells) and BMSSCs (bone marrow stromal stem cells) were isolated from rat dental pulp and bone marrow respectively by magnetic-activated cell-sorting techniques. Their odontogenic capacity was compared under the same inductive microenvironment produced by ABCs (apical bud cells) from 2-day-old rat incisors. Co-cultured DPSCs/ABCs *in vitro* showed more active odontogenic differentiation ability than mixed BMSSCs/ABCs, as indicated by the accelerated matrix mineralization, up-regulated alkaline phosphatase activity, cell-cycle modification, and the expression of tooth-specific proteins and genes. After cultured for 14 days in the renal capsules of rat hosts, recombined DPSC/ABC pellets formed typical tooth-shaped tissues with balanced amelogenesis and dentinogenesis, whereas BMSSC/ABC recombinants developed into atypical dentin–pulp complexes without enamel formation. DPSC and BMSSC pellets *in vivo* produced osteodentin-like structures and fibrous connective tissues respectively.

Conclusions. DPSCs presented more striking odontogenic capability than BMSSCs under the induction of postnatal ABCs. This report provides critical insights into the selection of candidate cells for tooth regeneration between dental and non-dental stem cell populations.

Introduction

Adult stem cells, with the capacity of self-renewal and multi-lineage differentiation, play a crucial role in postnatal tissue development and provide an attractive progenitor cell source for tissue engineering and regenerative medicine (Mimeault and Batra, 2006). Stem-cell-based tissue engineering has been performed in the animal model for many types of tissue regeneration, such as articular cartilage, bone, tendon, muscle and adipose tissues. Studies, including direct cell-pellet implantation (Iohara et al., 2004; Ohazama et al., 2004) and tissue engineering in combination with biocompatible scaffolds (Young et al., 2005; Rezwan et al., 2006), have enabled us to contemplate new and promising strategy for hard-tissue repair, particularly for tooth reconstruction.

DPSCs (dental pulp stem cells) and BMSSCs (bone marrow stromal stem cells), which can differentiate

[1] These authors contributed equally to this work.
[2] To whom correspondence should be addressed (email junnan@fmmu.edu.cn or yanjin@fmmu.edu.cn).

Key words: bone marrow stromal stem cell, dental pulp stem cell, epithelial–mesenchymal interaction, odontogenesis, tissue engineering.

Abbreviations used: ABC, apical bud cell; AGS, absorbable gelatin sponge; ALP, alkaline phosphatase; AMBN, ameloblastin; BMSC, bone marrow stromal cell; BMSSC, bone marrow stromal stem cell; BSP, bone sialoprotein; CK, cytokeratin; Col I, type I collagen; DMEM, Dulbecco's modified Eagle's medium; DMP-1, dentin matrix protein 1; DPSC, dental pulp stem cell; DSPP, dentin sialophosphoprotein; EMA, epithelial membrane antigen; FBS, fetal bovine serum; (b)FGF, (basic) fibroblast growth factor; GFAP, glial fibrillary acidic protein; HNK-1, human natural killer antigen-1; OC, osteocalcin; OPN, osteopontin; RT-PCR, reverse transcriptase PCR; α-SMA, α-smooth muscle actin; STRO, stromal precursor cell marker.

RESEARCH REPORTS
Biological

M. Buchtová[1,3]*, J. Štembírek[2], K. Glocová[3], E. Matalová[1,4], and A.S. Tucker[5]

[1]Institute of Animal Physiology and Genetics, v.v.i., Academy of Sciences of the Czech Republic, Veveri 97, 602 00 Brno, Czech Republic; [2]Department of Oral and Maxillofacial Surgery, University Hospital Ostrava, Ostrava, Czech Republic; [3]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; [4]Department of Physiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; and [5]Department of Craniofacial Development and Stem Cell Biology and Department of Orthodontics, King's College London, Dental Institute, London, SE1 9RT, UK; *corresponding author, buchtova@iach.cz

J Dent Res 91(5):491-498, 2012

Early Regression of the Dental Lamina Underlies the Development of Diphyodont Dentitions

ABSTRACT

Functional tooth germs in mammals, reptiles, and chondrichthyans are initiated from a dental lamina. The longevity of the lamina plays a role in governing the number of tooth generations. Monophyodont species have no replacement dental lamina, while polyphyodont species have a permanent continuous lamina. In diphyodont species, the dental lamina fragments and regresses after initiation of the second tooth generation. Regression of the lamina seems to be an important mechanism in preventing the further development of replacement teeth. Defects in the complete removal of the lamina lead to cyst formation and has been linked to ameloblastomas. Here, we show the previously unknown mechanisms behind the disappearance of the dental lamina, involving a combination of cell migration, cell-fate transformation, and apoptosis. Lamina regression starts with the loss of the basement membrane, allowing the epithelial cells to break away from the lamina and migrate into the surrounding mesenchyme. Cells deactivate epithelial markers (E-cadherin, cytokeratin), up-regulate Slug and MMP2, and activate mesenchymal markers (vimentin), while residual lamina cells are removed by apoptosis. The uncovering of the processes behind lamina degradation allows us to clarify the evolution of diphyodonty, and provides a mechanism for future manipulation of the number of tooth generations.

KEY WORDS: developmental biology, dental morphology, tooth development, odontogenesis, apoptosis, epithelial-mesenchymal interactions.

DOI: 10.1177/0022034512442896

Received November 25, 2011; Last revision February 2, 2012; Accepted February 27, 2012

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

INTRODUCTION

The dental lamina (DL) has been described in many different species of vertebrates and starts as an invagination of the epithelium growing deeply into the mesenchyme (Buchtová *et al*., 2008; Jarvinen *et al*., 2009; Fraser and Smith, 2011). Teeth bud off from the DL, with replacement teeth originating from the free end of the lamina. There are significant variations in the morphology of the DL during development in monophyodont, diphyodont, and polyphyodont species that relate to their ability to form replacement teeth. However, detailed accounts of the processes are unknown.

The mouse is the main model for the study of odontogenesis, but it forms only one tooth generation. Our knowledge of the processes involved in replacement tooth development is therefore limited. The mouse DL is very short; therefore, the teeth develop at the oral surface, and there is no evidence of a replacement lamina (Fig. 1A). In diphyodont species, such as the pig and ferret, the replacement lamina is evident as the primary dentition reaches the late bell stage, lying lingual to the deciduous tooth (Jarvinen *et al*., 2009; Stembirek *et al*., 2010). A second generation of teeth then buds off from this replacement lamina (Fig. 1B). After initiation of this second generation during mid-gestation, the pig lamina, like that of humans, undergoes degradation, preventing the initiation of further tooth generations (Moskow and Bloom, 1983; Stembirek *et al*., 2010). In humans, incomplete lamina degradation has been linked to the formation of epithelial pearls, which can lead to the development of oral cysts or tumors (Moskow and Bloom, 1983; Eversole, 1999). In contrast to the pig and human, in species with multiple generations of teeth, such as snakes, the lamina persists, linking the developing teeth in a chain, and providing further generations from its leading edge (Fig. 1C) (Buchtová *et al*., 2008).

Here, we have investigated the mechanisms behind the regression of the lamina in a diphyodont species, the pig. The pig has a dentition very similar to that of humans and therefore represents an excellent model.

There are three possible processes that have been proposed to play a role in loss of epithelial cells during development: apoptosis, migration, and epithelial-mesenchymal transformation (Prindull and Zipori, 2004; Ahlstrom and Erickson, 2009). We therefore proposed that a combination of these processes might be involved in loss of the replacement dental lamina in the pig.

The Molecular Circuit Regulating Tooth Development in Crocodilians

Journal of Dental Research
2016, Vol. 95(13) 1501–1510

DOI: 10.1177/0022034516667724
jdr.sagepub.com

S. Tsai[1,2,3,4], A. Abdelhamid[5], M.K. Khan[5], A. Elkarargy[5], R.B. Widelitz[1], C.M. Chuong[1,4,6], and P. Wu[1]

Abstract

Alligators have robust regenerative potential for tooth renewal. In contrast, extant mammals can either renew their teeth once (diphyodont dentition, as found in humans) or not at all (monophyodont dentition, present in mice). Previously, the authors used multiple mitotic labeling to map putative stem cells in alligator dental laminae, which contain quiescent odontogenic progenitors. The authors demonstrated that alligator tooth cycle initiation is related to β-catenin/Wnt pathway activity in the dental lamina bulge. However, the molecular circuitry underlying the developmental progression of polyphyodont teeth remains elusive. Here, the authors used transcriptomic analyses to examine the additional molecular pathways related to the process of alligator tooth development. The authors collected juvenile alligator dental laminae at different developmental stages and performed RNA-seq. This data shows that Wnt, bone morphogenetic protein (BMP), and fibroblast growth factor (FGF) pathways are activated at the transition from pre-initiation stage (bud) to initiation stage (cap). Intriguingly, the activation of Wnt ligands, receptors and co-activators accompanies the inactivation of Wnt antagonists. In addition, the authors identified the molecular circuitry at different stages of tooth development. The authors conclude that multiple pathways are associated with specific stages of tooth development in the alligator. This data shows that Wnt pathway activation may play the most important role in the initiation of tooth development. This result may offer insight into ways to modulate the genetic controls involved in mammalian tooth renewal.

Keywords: polyphyodont, tooth cycle, stem cell, niche, RNA-seq, molecular pathway

Introduction

Non-mammalian vertebrates can renew their teeth repeatedly throughout their lifetime. However, extant mammals either renew their teeth once (diphyodont dentition) or not at all (monophyodont dentition; Richman and Handrigan 2011). For example, in humans, the deciduous "milk" teeth are replaced with permanent teeth, but a third renewal of dentition is not possible. Mice by comparison, never replace their teeth.

Adult alligators have 80 teeth. Each tooth position contains a complex tooth family unit that includes a functional tooth (ft), a replacement tooth (RT) and a dental lamina (dl) (Westergaard and Ferguson 1990; Wu et al. 2013). Each adult alligator ft lasts for about 1 y (Edmund 1962) and is then replaced by an RT. Previously, we described that a normal tooth family unit progresses through a cycle of pre-initiation stage to initiation stage to growth stage (Wu et al. 2013; Fig. 1A). The developing dl at these stages corresponds to the mammalian tooth at bud-, cap- and bell-stages, respectively. At bell-stage, the lingual outer epithelium splits from the RT to form a new dl for subsequent renewal cycles (Fig. 1A). The tooth cycle may involve dynamic molecular circuitry that regulates tissue remodeling for tooth replacement.

In dyphyodont mammals (e.g., human), the dl degenerates completely after the permanent teeth develop into the late bell-stage, and the capacity for tooth renewal is lost. However, abnormal spatial or temporal retention of epithelial cell rests of the dl may interact with the ectomesenchyme and cause odontogenic cyst or tumor formation (Neville et al. 2002). Thus, it is important to understand the proper molecular circuitry modulating tooth development in order to properly activate dl remnants for the purposes of human tooth regeneration.

Previously, we studied the molecular and cellular activities regulating how the alligator tooth family unit is built and maintained.

[1]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[2]Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, USA
[3]Graduate School of Clinical Dentistry, National Taiwan University, Taipei, Taiwan
[4]Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan
[5]Qassim College of Dentistry, Qassim University, Saudi Arabia
[6]Integrative Stem Cell Center, China Medical University Hospital, China Medical University, Taichung, Taiwan

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

Corresponding Author:
P. Wu, Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA.
Email: pingwu@usc.edu

Regenerative and Technological Section / Mini-Review

Gerontology

Gerontology
DOI: 10.1159/000486592

Received: October 18, 2017
Accepted: January 4, 2018
Published online: March 13, 2018

Biology Explaining Tooth Repair and Regeneration: A Mini-Review

Anamaria Balic

Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Keywords
Tooth development · Aging · Tooth stem cells · Tooth regeneration · Tooth repair

Abstract
The tooth is an intricate composition of precisely patterned, mineralized matrices and soft tissues. Mineralized tissues include enamel (produced by the epithelial cells called ameloblasts), dentin and cementum (produced by mesenchymal cells called odontoblasts and cementoblasts, respectively), and soft tissues, which include the dental pulp and the periodontal ligament along with the invading nerves and blood vessels. It was perceived for a very long time that teeth primarily serve an esthetical function. In recent years, however, the role of healthy teeth, as well as the impact of oral health on general well-being, became more evident. Tooth loss, caused by tooth decay, congenital malformations (tooth agenesis), trauma, periodontal diseases, or age-related changes, is usually replaced by artificial materials which lack many of the important biological characteristics of the natural tooth. Human teeth have very low to almost absent regeneration potential, due to early loss of cell populations with regenerative capacity, namely stem cells. Significant effort has been made in recent decades to identify and characterize tooth stem cells, and to unravel the developmental programs which these cells follow in order to generate a tooth.

Introduction

Teeth are ectodermal organs generated by most vertebrates, and they are produced in various sizes, shapes, and numbers. With the exception of mammals, the majority of vertebrates replace their teeth continuously (polyphyodonts). Mammals, on the other hand, have restricted tooth regeneration capacity and they replace their teeth only once (diphyodonts) or not at all (monophyodonts). What restricts the arrest of tooth replacement to only one round in most mammals is not known, and the purpose or benefit of having such restriction in terms of evolution and development is not fully understood. It should be noted that most monophyodonts have continuously growing teeth, in which mineralized matrices are unceasingly produced to compensate for tooth wear.

Humans are diphyodonts which develop two dentitions during life. First is the primary dentition (also referred to as deciduous teeth), which is initiated around the 6th week of gestation. Deciduous teeth are replaced by permanent teeth, whose development is initiated between the 10th and 13th week of gestation [1]. Detailed histological analyses of human dentition have implied that humans could potentially generate a third dentition, but this potential has been inhibited by an unknown molecular mechanism. Individual teeth within the primary and secondary dentitions do not form simultaneously,

KARGER

E-Mail karger@karger.com
www.karger.com/ger

Anamaria Balic
Institute of Biotechnology, University of Helsinki
Viikinkaari 5D
FI–00790 Helsinki (Finland)
E-Mail anamaria.balic@helsinki.fi

Principles and Applications of Cell Delivery Systems for Periodontal Regeneration

PM Bartold[1], Y Xiao[2], SP Lyngstaadas[3], ML Paine[4], ML Snead[4]

[1]Colgate Australian Clinical Dental Research Centre, University of Adelaide, Adelaide, Australia,

[2]Tissue BioRegeneration and Integration, Science Research Centre, Queensland University of Technology, Brisbane, Australia,

[3]Faculty of Dentistry, University of Oslo, Oslo, Norway,

[4]School of Dentistry, University of Southern California, Los Angeles, California

Short title: Cell Delivery for Regeneration

Address for Correspondence

PM Bartold
University of Adelaide
Colgate Australian Clinical Dental Research Centre,
Dental School
Frome Road
Adelaide, South Australia 5005
AUSTRALIA

International Journal of Oral Science www.nature.com/ijos

REVIEW ARTICLE OPEN

Enamel biomimetics—fiction or future of dentistry

Mirali Pandya[1] and Thomas G. H. Diekwisch[1]

Tooth enamel is a complex mineralized tissue consisting of long and parallel apatite crystals configured into decussating enamel rods. In recent years, multiple approaches have been introduced to generate or regenerate this highly attractive biomaterial characterized by great mechanical strength paired with relative resilience and tissue compatibility. In the present review, we discuss five pathways toward enamel tissue engineering, (i) enamel synthesis using physico-chemical means, (ii) protein matrix-guided enamel crystal growth, (iii) enamel surface remineralization, (iv) cell-based enamel engineering, and (v) biological enamel regeneration based on de novo induction of tooth morphogenesis. So far, physical synthesis approaches using extreme environmental conditions such as pH, heat and pressure have resulted in the formation of enamel-like crystal assemblies. Biochemical methods relying on enamel proteins as templating matrices have aided the growth of elongated calcium phosphate crystals. To illustrate the validity of this biochemical approach we have successfully grown enamel-like apatite crystals organized into decussating enamel rods using an organic enamel protein matrix. Other studies reviewed here have employed amelogenin-derived peptides or self-assembling dendrimers to re-mineralize mineral-depleted white lesions on tooth surfaces. So far, cell-based enamel tissue engineering has been hampered by the limitations of presently existing ameloblast cell lines. Going forward, these limitations may be overcome by new cell culture technologies. Finally, whole-tooth regeneration through reactivation of the signaling pathways triggered during natural enamel development represents a biological avenue toward faithful enamel regeneration. In the present review we have summarized the state of the art in enamel tissue engineering and provided novel insights into future opportunities to regenerate this arguably most fascinating of all dental tissues.

International Journal of Oral Science (2019)11:8 ; https://doi.org/10.1038/s41368-018-0038-6

TOOTH ENAMEL—AN IMPOSSIBLE MATERIAL TO REGENERATE?

Tooth enamel is a highly unique tissue-specific biomaterial characterized by exceptional structural and mechanical properties as well as esthetic beauty.[1–4] The unique physico-chemical properties of enamel are due to its high content in hydroxyapatite, the parallel arrangement of individual elongated apatite crystals into enamel prisms, and the interwoven alignment of perpendicular prisms in a picket-fence resembling three-dimensional order (Fig. 1). Together, these characteristics result in a biomaterial of great hardness and physical resilience. Due to its toughness and relative fracture resistance, enamel-like biomaterials hold great promise as structural components for future biomedical and engineering applications, including tooth enamel repair, orthopedic defect restoration, and as functional components of insulators, brakes, and exhaust pollutant filters.[5–9]

As desirable as the regeneration or fabrication of tooth enamel may seem, de novo enamel tissue engineering and its potential future clinical implementation remain a daunting task.[10–13] In biological organisms, enamel is manufactured only once prior to tooth eruption, and the capacity to form new enamel in each individual tooth organ is lost forever, once the tooth is fully erupted.[14,15] The high ion concentrations and dramatic pH changes involved in initial amelogenesis pose a formidable hurdle in cell-based approaches toward tooth enamel regeneration.[16–18] And even though the synthesis of hydroxyapatite blocks may appear straight-forward from a manufacturing perspective, the faithful fabrication of true enamel with its parallel-aligned filigree apatite crystals and decussating prism bundles has rarely been accomplished so far.[19–23]

The cells at the core of nature's ability to manufacture tooth enamel are called ameloblasts. Ameloblasts are highly specialized epithelial cells originally derived from the enamel organ. After differentiating from inner enamel organ cells and thereafter pre-ameloblasts, ameloblasts turn into highly polarized and elongated prismatic cells with a pronounced endoplasmic reticulum and Golgi apparatus to synthesize and secrete amelogenin and other enamel proteins and transport calcium and phosphate ions into the enamel matrix. Once a sufficient amount of enamel matrix has been synthesized, ameloblasts function to resorb large quantities of water and degraded enamel matrix proteins during the resorptive stage of enamel formation. While it appears logical to culture ameloblasts for the in vitro manufacture of tooth enamel, ameloblast culture approaches have encountered numerous difficulties, perhaps due to the highly differentiated status of these secretory cells or due to the lack of a suitable tissue context and/or related physical cues. In comparison, ameloblast precursor cells and stratum intermedium ameloblast progenitor cells have been relatively easier to maintain in vitro, but so far have not demonstrated any evidence of enamel matrix secretion in culture. In contrast, maintenance of postsecretory ameloblasts in vitro has remained challenging because of their reduced proliferative capability. Finally, cells from the papillary layer and junctional epithelium would require extensive reprogramming

[1]Center for Craniofacial Research and Diagnosis, Texas A&M College of Dentistry, Dallas, TX, USA
Correspondence: Thomas G. H. Diekwisch (diekwisch@tamhsc.edu)

Received: 29 August 2018 Accepted: 19 September 2018
Published online: 05 January 2019

TRANSPLANTATION OF TOOTH GERM ELEMENTS AND THE EXPERIMENTAL HETEROTOPIC FORMATION OF DENTIN AND ENAMEL*

By C. B. HUGGINS, M.D., H. R. McCARROLL, M.D., and A. A. DAHLBERG, D.D.S.

(From the Department of Surgery, The University of Chicago, Chicago)

PLATES 9 TO 12

(Received for publication, May 26, 1934)

The histologic proximity of epithelium to such superficial appendages of the organism as the scales, shell, hair, nails and the developing tooth has been discovered within the last century. Moreover, experiments in recent years (2–4) have shown that the epithelium of certain organs in mammals, notably the gall bladder and the urinary tract distal to the kidney, when transposed to certain connective tissue areas regularly causes the formation of osteoblasts and bone in these loci. Since the relationship of the calcified elements of the tooth to epithelium in the developmental stage is strikingly similar to bone forming after epithelial transplantation, and since the inorganic crystallites of teeth and bone are chemically identical even to X-ray diffraction studies (5, 6), it was considered advisable to investigate the odontogenic properties of tooth germ elements in an attempt to induce the extra-oral formation of dentin and enamel. The relatively common heterotopic occurrence of teeth in pathological situations, as in the pituitary (7) and elsewhere, especially in the ovary, as a result of teratomatous tumor formation lent support to the conception that this could be accomplished experimentally.

The idea of the ontogenetic relationship of epithelium to the developing tooth arose mostly as a result of the anatomical studies of Kölliker. Hertwig (8) de-

* This work was done under a grant from the Douglas Smith Foundation for Medical Research. A preliminary report was read before the Society for Experimental Biology and Medicine (1) and at the annual meeting of the American Society for Experimental Pathology (1934).

Original Paper

Cells Tissues Organs

Cells Tissues Organs 2013;198:99–110
DOI: 10.1159/000353116

Accepted after revision: May 13, 2013
Published online: July 30, 2013

Inductive Ability of Human Developing and Differentiated Dental Mesenchyme

Liwei Zheng[a, b] Rungnapa Warotayanont[a] Jonathan Stahl[a] Ryo Kunimatsu[a]
Ophir Klein[a] Pamela K. DenBesten[a] Yan Zhang[a]

[a]Department of Orofacial Sciences, University of California, San Francisco, Calif., USA; [b]State Key Laboratory of Oral Diseases, Sichuan University, Chengdu, China

Key Words
Ameloblasts · Amelogenin · Dental mesenchymal cells · Human embryonic stem cells · Odontogenic competence · Reprogramming · Transcription factors

Abstract
The development of cell-based therapeutic strategies to bioengineer tooth tissue is a promising approach for the treatment of lost or damaged tooth tissue. The lack of a readily available cell source for human dental epithelial cells (ECs) severely constrains the progress of tooth bioengineering. Previous studies in model organisms have demonstrated that developing dental mesenchyme can instruct nondental epithelium to differentiate into enamel-forming epithelium. In this study, we characterized the ability of fetal and adult human dental mesenchyme to promote differentiation of human embryonic stem cell (hESC)-derived ECs (ES-ECs) into ameloblast-lineage cells. ES-ECs were co-cultured either with human fetal dental mesenchymal cells (FDMCs) or with adult dental mesenchymal cells (ADMCs) in either a three-dimensional culture system, or in the renal capsules of SCID mice. When co-cultured with FDMCs in vitro, ES-ECs polarized and expressed amelogenin. Tooth organ-like structures assembled with epithelium and encased mesenchyme and developing enamel-like structures could be detected in the complexes resulting from in vitro and ex vivo co-culture of ES-ECs and FDMCs. In contrast, co-cultured ES-ECs and ADMCs formed amorphous spherical structures and occasionally formed hair. Transcription factors were significantly upregulated in FDMCs compared to ADMCs including *MSX1*, *GLI1*, *LHX6*, *LHX8*, *LEF1* and *TBX1*. In summary, FDMCs but not ADMCs had the capacity to induce differentiation of ES-ECs into ameloblast lineage cells. Further characterization of the functional differences between these two types of dental mesenchyme could enable reprogramming of ADMCs to enhance their odontogenic inductive competence.

Introduction

Ameloblasts, highly specialized enamel-forming dental epithelial cells (ECs), go through multiple stages of differentiation during tooth development. As ameloblasts differentiate from the presecretory stage to the secretory stage, enamel matrix proteins consisting primarily of amelogenins are synthesized and secreted. Amelogenins are alternatively spliced matrix proteins required for nucleation and growth of enamel hydroxyapatite crys-

KARGER

E-Mail karger@karger.com
www.karger.com/cto

1422–6405/13/1982–0099$38.00/0

Dr. Yan Zhang
Department of Orofacial Sciences
University of California, 513 Parnassus Avenue
San Francisco, CA 94143-0422 (USA)
E-Mail yan.zhang2@ucsf.edu

Review
TheScientificWorldJOURNAL (2010) 10, 901–916
TSW Development & Embryology
ISSN 1537-744X; DOI 10.1100/tsw.2010.81

TheScientificWorldJOURNAL
www.thescientificworld.com

Stem Cell–Based Dental Tissue Engineering

Petar Zivkovic, Vladimir Petrovic, Stevo Najman, and Vladisav Stefanovic*
University School of Medicine, 18000 Nis, Serbia

E-mail: petarz83@yahoo.com; vlada@medfak.ni.ac.rs; stevo@medfak.ni.ac.rs; stefan@ni.ac.rs

Received December 21, 2009; Revised April 1, 2010; Accepted April 13, 2010; Published May 18, 2010

The development of biological and biomaterial sciences profiled tissue engineering as a new and powerful tool for biological replacement of organs. The combination of stem cells and suitable scaffolds is widely used in experiments today, in order to achieve partial or whole organ regeneration. This review focuses on the use of tissue engineering strategies in tooth regeneration, using stem cells and stem cells/scaffold constructs. Although whole tooth regeneration is still not possible, there are promising results. However, to achieve this goal, it is important to understand and further explore the mechanisms underlying tooth development. Only then will we be able to mimic the natural processes with the use of stem cells and tissue engineering techniques.

KEYWORDS: tooth development, stem cells, tooth engineering, dentin-pulp complex, periodontium

INTRODUCTION

The loss of teeth is one of the most common functional and esthetic defects in society today. The increased number of cases of periodontal diseases among the older population, as well as caries and traumas, are the leading causes of tooth loss[1]. Tooth transplantation and dental implants are the most used therapeutic approaches in restorative dentistry today in order to preserve the masticatory function of patients. However, complete restoration therapy to compensate for complete tooth loss has not been achieved[1].

The development of biological sciences, the profiling of tissue engineering, and the discovery of stem cells have offered new possible solutions in the therapy of tooth loss. Although the ideal solution to the problem would be the creation of the whole tooth by using the patient's own stem cells, we are still far from that goal because the biological processes underlying tooth development and differentiation are still not completely clear.

TIMETABLE OF TOOTH DEVELOPMENT: A POSSIBLE TEMPLATE FOR TOOTH REGENERATION

The tooth develops through complex reciprocal interactions between the ectodermal-derived oral epithelium and neural crest–derived ectomesenchyme[2]. Considering that there is only a slight difference in the gene expression between murine and human tooth development, mice are commonly used as a model for tooth development[1]. The mediators in these processes are signal molecules belonging mostly

*Corresponding author.

Published by TheScientificWorld; www.thescientificworld.com

Bioengineered Tooth Buds Exhibit Features of Natural Tooth Buds

Journal of Dental Research
2018, Vol. 97(10) 1144–1151

DOI: 10.1177/0022034518779075
journals.sagepub.com/home/jdr

E.E. Smith[1], S. Angstadt[2], N. Monteiro[2], W. Zhang[2], A. Khademhosseini[3], and P.C. Yelick[1,2]

Abstract

Tooth loss is a significant health issue currently affecting millions of people worldwide. Artificial dental implants, the current gold standard tooth replacement therapy, do not exhibit many properties of natural teeth and can be associated with complications leading to implant failure. Here we propose bioengineered tooth buds as a superior alternative tooth replacement therapy. We describe improved methods to create highly cellularized bioengineered tooth bud constructs that formed hallmark features that resemble natural tooth buds such as the dental epithelial stem cell niche, enamel knot signaling centers, transient amplifying cells, and mineralized dental tissue formation. These constructs were composed of postnatal dental cells encapsulated within a hydrogel material that were implanted subcutaneously into immunocompromised rats. To our knowledge, this is the first report describing the use of postnatal dental cells to create bioengineered tooth buds that exhibit evidence of these features of natural tooth development. We propose future bioengineered tooth buds as a promising, clinically relevant tooth replacement therapy.

Keywords: tissue engineering, odontogenesis, stem cell, ameloblast, odontoblast, regeneration

Introduction

Synthetic dental implants are susceptible to peri-implantitis, gingival recession, and bone resorption at the implant site, leading to implant failure (Chrcanovic et al. 2014, 2016; Esposito et al. 2012; Greenstein et al. 2008). To create vitalized teeth for human tooth replacement, bioengineered tooth regeneration has emerged as an innovative field of translational dentistry. Studies have shown that postnatal dental stem cells (DSCs) retain the ability to form small, anatomically correct whole tooth crowns in animal models (Duailibi et al. 2004; Young et al. 2005), supporting the feasibility of this approach.

Currently, a variety of biodegradable scaffolds are being tested for utility in whole-tooth regeneration therapies (Smith and Yelick 2016). In particular, gelatin methacryloyl (GelMA) hydrogel scaffolds were shown to support DSCs and human umbilical vein endothelial cell (HUVEC) differentiation into mineralized dental tissues of specified size and shape (Smith et al. 2017). To improve upon this model, here we investigated 3 ways to optimize GelMA tooth bud constructs to facilitate their use in humans. First, we tested whether sequentially photocrosslinking GelMA bilayers would better maintain distinct dental epithelial (DE) and dental mesenchymal (DM) cell layers. Next, to optimize initial cell seeding densities, we compared 3.0×10^7, 6.0×10^7, and 9.0×10^7 cells/mL and examined dental cell differentiation. Last, we tested whether extended culture in normal growth media, prior to culture in osteogenic differentiation media, resulted in improved tooth bud construct cellularity and mineralized dental tissue formation.

Materials and Methods

Dental and Endothelial Cell Culture

DE and DM cells were isolated from porcine tooth buds and cultured as previously described (Smith et al. 2017). Briefly, unerupted early bell stage tooth buds were extracted from 5-mo-old porcine jaws. The enamel and pulp organs were dissected apart and used to prepare single-cell suspensions of DE and DM cells, respectively, which were cultured and expanded in vitro in appropriate media. HUVECs (PCS100010; ATCC) were grown in vascular basal media (PCS100030; ATCC) with vascular endothelial growth factor (VEGF) growth kit (PCS10004; ATCC). All cells were expanded in 5% CO_2 at 37°C and cryopreserved in 10% dimethyl sulfoxide (DMSO) until use.

[1]Program in Cell, Molecular, and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University School Medicine, Boston, MA, USA
[2]Department of Orthodontics, Tufts University School of Dental Medicine, Boston, MA, USA
[3]Department of Bioengineering, University of California Los Angeles, Los Angeles, CA, USA

A supplemental appendix to this article is available online.

Corresponding Author:
P.C. Yelick, Department of Orthodontics, Tufts University School of Dental Medicine, 136 Harrison Avenue, Room M824, Boston, MA 02111, USA.
Email: pamela.yelick@tufts.edu

European Cells and Materials Vol. 16 2008 (pages 1-9) DOI: 10.22203/eCM.v016a01 ISSN 1473-2262

STEM CELLS FOR TOOTH ENGINEERING

G. Bluteau[1]*, H-U. Luder[1], C. De Bari[2], T. A. Mitsiadis[1]*

[1]Department of Orofacial Development and Structure, Institute of Oral Biology, ZZMK, Faculty of Medicine, University of Zurich, Plattenstrasse 11, CH 8032 Zurich, Switzerland.
[2]Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, UK.

Abstract

Tooth development results from sequential and reciprocal interactions between the oral epithelium and the underlying neural crest-derived mesenchyme. The generation of dental structures and/or entire teeth in the laboratory depends upon the manipulation of stem cells and requires a synergy of all cellular and molecular events that finally lead to the formation of tooth-specific hard tissues, dentin and enamel. Although mesenchymal stem cells from different origins have been extensively studied in their capacity to form dentin *in vitro*, information is not yet available concerning the use of epithelial stem cells. The odontogenic potential resides in the oral epithelium and thus epithelial stem cells are necessary for both the initiation of tooth formation and enamel matrix production. This review focuses on the different sources of stem cells that have been used for making teeth *in vitro* and their relative efficiency. Embryonic, post-natal or even adult stem cells were assessed and proved to possess an enormous regenerative potential, but their application in dental practice is still problematic and limited due to various parameters that are not yet under control such as the high risk of rejection, cell behaviour, long tooth eruption period, appropriate crown morphology and suitable colour. Nevertheless, the development of biological approaches for dental reconstruction using stem cells is promising and remains one of the greatest challenges in the dental field for the years to come.

Keywords: Stem cells, odontoblast, dentin, ameloblasts, enamel, tooth, incisor, human.

*Address for correspondence:
Gilles Bluteau or Thimios Mitsiadis
University of Zurich, Faculty of Medicine,
Institute of Oral Biology, ZZMK,
Plattenstrasse 11, CH 8032 Zurich, Switzerland.
Tel: +41 446343197
Tel: +41 446343390
Fax: +41 446343310
E-mail: gilles.bluteau@zzmk.uzh.ch or
thimios.mitsiadis@zzmk.uzh.ch

Introduction

Teeth are highly mineralized organs resulting from sequential and reciprocal interactions between the oral epithelium and the underlying cranial neural crest-derived mesenchyme (Duailibi *et al.*, 2006; Mitsiadis, 2001) (Fig. 1). Tissue recombination experiments point out that the oral epithelium contains the inductive capability for odontogenesis. This potential allows conditioning of the underlying mesenchyme, which in turn regulates the differentiation of epithelial cells. The importance of cranial neural crest-derived cells in odontogenesis has been shown in experiments where transplantation of mouse neural crest cells into chick embryos allowed growth of tooth germs (Mitsiadis *et al.*, 2003). Numerous growth factors have been shown to be involved in different stages of the embryonic tooth development (i.e. initiation, morphogenesis, cytodifferentiation). Members of the Transforming Growth Factor beta (TGFβ) superfamily such as Bone Morphogenic Protein 2 (BMP-2) and BMP-4 are key signalling molecules in regulating epithelial-mesenchymal interactions during odontogenesis (Kratochwil *et al.*, 1996; Nadiri *et al.*, 2004; Vainio *et al.*, 1993). Molecules of the Fibroblast Growth Factor (FGF) family such as FGF-3, FGF-4, FGF-8 and FGF-10 are involved in cell proliferation and regulate expression of specific target genes in teeth (Bei and Maas, 1998; Kettunen *et al.*, 1998, 2000). Wnt proteins such as Wnt-3, Wnt-7b, Wnt-10a and Wnt-10b have essential roles as regulators of cell proliferation, migration and differentiation during tooth initiation and morphogenesis (Dassule and McMahon, 1998). Other diffusible factors such as sonic hedgehog (shh) also contribute to both initiation and subsequent dental morphogenesis (Khan *et al.*, 2007).

Two major cell types are involved in dental hard tissue formation: the mesenchyme-originated odontoblasts that are responsible for the production of dentin and the epithelium-derived ameloblasts that form the enamel (Fig. 1). Odontoblasts are columnar post-mitotic cells that form a layer in contact with the dentin. Odontoblastic processes are formed at their distal part, penetrate the dentin and participate in the secretion of dentin matrix and minerals. The matrix is composed of collagen (90%) and non-collagenous proteins such as Dentin Sialophosphoprotein (DSPP) and Dentin Matrix Protein 1 (DMP-1). The deposition of apatite minerals on this matrix gives rise to the mature calcified dentin.

Enamel is secreted by ameloblasts along the dentino-enamel junction. Enamel is mainly composed of hydrophobic proteins such as amelogenin, ameloblastin,

In Vitro Cell.Dev.Biol.—Animal (2010) 46:457–468
DOI 10.1007/s11626-009-9265-7

Newly established cell lines from mouse oral epithelium regenerate teeth when combined with dental mesenchyme

Chiho Takahashi · Hiroyuki Yoshida · Akihiko Komine · Kazuhisa Nakao · Takashi Tsuji · Yasuhiro Tomooka

Received: 9 June 2009 / Accepted: 10 November 2009 / Published online: 24 December 2009 / Editor: J. Denry Sato

Abstract The present study attempted to examine whether clonal cell lines of the oral epithelium can differentiate into ameloblasts and regenerate tooth when combined with dental germ mesenchyme. Clonal cell lines with a distinct morphology were established from the oral epithelium of *p53*-deficient fetal mice at embryonic day 18 (E18). The strain of mouse is shown to be a useful source for establishing clonal and immortalized cell lines from various tissues and at various stages of development. Tooth morphogenesis is almost completed and the oral epithelium is segregated from the dental epithelium at E18. In RT-PCR analysis of cell lines, mucosal epithelial markers (cytokeratin 14) were detected, but ameloblast markers such as amelogenin and ameloblastin were not detected when cells were cultured on plastic dish. They formed stratified epithelia and expressed a specific differentiation marker (CK13) in the upper layer when cultured on feeder layer or on collagen gel for 1–3 wk, demonstrating that they are of oral mucosa origin. Next, bioengineered tooth germs were prepared with cell lines and fetal molar mesenchymal tissues and implanted under kidney capsule for 2–3 wk. Five among six cell lines regenerated calcified structures as seen in natural tooth. Our results indicate that some oral epithelial cells at E18 possess the capability to differentiate into ameloblasts. Furthermore, cell lines established in the present study are useful models to study processes in tooth organogenesis and tooth regeneration.

C. Takahashi · H. Yoshida · A. Komine · K. Nakao · T. Tsuji · Y. Tomooka (✉)
Department of Biological Science and Technology and Tissue Engineering Research Center, Tokyo University of Science, Yamazaki 2641,
Noda, Chiba 278-8510, Japan
e-mail: tomoylab@rs.noda.tus.ac.jp

Keywords Oral epithelium · Immortalized cell lines · Tooth regeneration · *p53*-deficient mouse

Introduction

At the first stage of tooth development, odontogenic potential first appears in the presumptive dental epithelium which elicits the formation of a dental papilla in ectomesenchymal cells (reviewed by Mina and Kollar 1987; Lumsden 1988). The epithelial instructive information elicits odontogenic response from aggregates of non-dental stem cells (Ohazama et al. 2004). Once the determination occurs, the mesenchyme acts instructively to induce enamel organ formation on the epithelium, and the inductive activity is effective even on epithelia with non-oral sources (Kollar and Baird 1970a, b; Hu et al. 2006). These previous studies clearly demonstrated a series of reciprocal and sequential epithelial–mesenchymal interactions. The molecular mechanisms of the interactions have been extensively studied, and possible genes or factors are implied as candidates of signaling or acting (reviewed by Mass and Bei 1997; Miletich and Sharpe 2003; Thesleff 2003).

The dental epithelium is derived from the oral epithelium and differentiates into four cell types: (1) inner enamel epithelium (differentiate into ameloblasts), (2) stratum intermedium, (3) stellate reticulum, and (4) outer enamel epithelium (Kawano et al. 2002). Primary culture of dental epithelial cells has provided useful information, although it has inevitable heterogeneity and limited proliferation activity (Kukita et al. 1992; DenBesten et al. 1998; Matsumura et al. 1998). Immortalized cell lines of the dental epithelium were established spontaneously or with retrovirus transfection (Chen et al. 1992; DenBesten et al.

Chapter 8

Functional Tooth Regeneration

Masamitsu Oshima, Miho Ogawa, and Takashi Tsuji

Abstract

Three-dimensional organogenesis in vivo is principally regulated by the spatiotemporal developmental process that relies on the cellular behavior such as cell growth, migration, differentiation, and cell-to-cell interaction. Organ development and morphogenesis have been elucidated to be regulated by the proper transient expression of various signaling molecules including cytokines, extracellular matrix, and adhesion molecules based on the epithelial and mesenchymal interactions. Current bioengineering technology for regenerating three-dimensional organ has progressed to the replication of organogenesis, thereby enabling the development of fully functional bioengineered organs using bioengineered organ germs that are generated from immature stem cells via tissue engineering technology in vitro.

To achieve precise replication of organogenesis, we have developed a novel three-dimensional cell manipulation method designated the organ germ method, and enabled the generation of a structurally correct and fully functional bioengineered tooth in vivo. This method is also expected to be utilized for analyzing gene and protein functions during organogenesis. Here, we describe protocols for the tooth germ reconstitution by using the organ germ method and for the functional analysis of tooth development in vitro and in vivo.

Key words Tooth regeneration, Organ replacement regenerative therapy, Bioengineered tooth, Organ germ method, Cell manipulation, Transplantation

1 Introduction

The tooth is an ectodermal organ arising from a tooth germ, whose development is regulated by reciprocal epithelial-mesenchymal interactions [1, 2]. The tooth has a three-dimensional multicellular structure that includes enamel, dentin, cementum, pulp, and periodontal ligament (PDL) to establish functional cooperation with the maxillofacial region [3, 4]. In the tooth development, various dental-cell lineages, such as ameloblasts, odontoblasts, pulp cells, PDL cells, cementoblasts, and osteoblasts, occurred from the dental epithelium or mesenchyme [5]. These cells secrete a collagenous extracellular matrix to accumulate the enamel, dentin, cementum, PDL, and alveolar bone. In mature tooth after tooth development, immature cells seem to be maintained as dental stem cells that are

Takashi Tsuji (ed.), *Organ Regeneration: 3D Stem Cell Culture & Manipulation*, Methods in Molecular Biology, vol. 1597,
DOI 10.1007/978-1-4939-6949-4_8,

Fully functional bioengineered tooth replacement as an organ replacement therapy

Etsuko Ikeda[a,b,1], Ritsuko Morita[a,c,1], Kazuhisa Nakao[a,c], Kentaro Ishida[a,c], Takashi Nakamura[a,c], Teruko Takano-Yamamoto[d], Miho Ogawa[b], Mitsumasa Mizuno[a,c,d], Shohei Kasugai[e], and Takashi Tsuji[a,b,c,2]

[a]Department of Biological Science and Technology, Faculty of Industrial Science and Technology, and [c]Research Institute for Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan; [b]Organ Technologies Inc., Tokyo 101-0048, Japan; [d]Division of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Tohoku University, Sendai, Miyagi 980-8575, Japan; and [e]Oral and Maxillofacial Surgery, Department of Oral Restitution, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8510, Japan

Edited by Robert Langer, Massachusetts Institute of Technology, Cambridge, MA, and approved June 30, 2009 (received for review March 17, 2009)

Current approaches to the development of regenerative therapies have been influenced by our understanding of embryonic development, stem cell biology, and tissue engineering technology. The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs which work in cooperation with surrounding tissues to replace organs that were lost or damaged as a result of disease, injury, or aging. Here, we report a successful fully functioning tooth replacement in an adult mouse achieved through the transplantation of bioengineered tooth germ into the alveolar bone in the lost tooth region. We propose this technology as a model for future organ replacement therapies. The bioengineered tooth, which was erupted and occluded, had the correct tooth structure, hardness of mineralized tissues for mastication, and response to noxious stimulations such as mechanical stress and pain in cooperation with other oral and maxillofacial tissues. This study represents a substantial advance and emphasizes the potential for bioengineered organ replacement in future regenerative therapies.

regenerative therapy | transplantation

The current approaches being used to develop future regenerative therapies are influenced by our understanding of embryonic development, stem cell biology, and tissue engineering technology (1–4). One of the more attractive concepts under consideration in regenerative therapy is stem cell transplantation of enriched or purified tissue-derived stem cells (5), or in vitro manipulated embryonic stem (ES) and induced pluripotent stem (iPS) cells (6, 7). This therapy has the potential to restore the partial loss of organ function by replacing hematopoietic stem cells in hematopoietic malignancies (8), neural stem cells in Parkinson's disease (9), mesenchymal stem cells in myocardial infarction (10), and hepatic stem cells in cases of hepatic insufficiency (11).

The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs that can replace lost or damaged organs following disease, injury, or aging (4, 12–14). The feasibility of this concept has essentially been demonstrated by successful organ transplantations for various injuries and diseases (15). It is expected that bioengineering technology will be developed for the reconstruction of fully functional organs in vitro through the precise arrangement of several different cell species. However, these technologies have not yet achieved 3-dimensional reconstructions of fully functioning organs. To achieve the functional replacement of lost or damaged tissues and organs, the development of 3-dimensional bioengineered tissues comprising a single cell type is now being attempted using biodegradative materials (3), appropriate cell aggregation (16), or uniform cell sheets (17). These are now clinically applied for corneal dysfunction (18), myocardial infarction (19), and hepatic insufficiency (20) using oral mucosal epithelial cells, myocardial cells, and liver cells, respectively, with favorable clinical results.

A concept has also now been proposed to develop a bioengineered organ by reproducing the developmental processes during organogenesis (13, 21, 22). Almost all organs arise from their respective germs through reciprocal interactions between the epithelium and mesenchyme in the developing embryo (23–25). Therefore, it is predicted that a functional bioengineered organ could be produced by reconstituting organ germs between epithelial and mesenchymal cells in vitro, although the existence of organ-inductive stem cells in the adult body has not been fully elucidated yet with the exception of hair follicles (26) and the mammary gland (27). Tooth replacement regenerative therapy, which is also induced by typical reciprocal epithelial, and mesenchymal interactions (25, 28), is thought to be a feasible model system to evaluate the future clinical application of bioengineered organ replacement (13, 21). The strategy to develop a bioengineered third tooth after the loss of deciduous and permanent teeth is to properly reproduce the processes which occur during embryonic development through the reconstitution of a bioengineered tooth germ in vitro (21). We have recently developed a method for creating 3-dimensional bioengineered organ germ, which can be used as an ectodermal organ such as the tooth or whisker follicle (29). Our analyses have provided an effective method for reconstituting this organ germ and raised the possibility of tooth replacement with integrated blood vessels and nerve fibers in an adult oral environment (29). However, it remains to be determined whether a bioengineered tooth can achieve full functionality, including sufficient masticatory performance, biomechanical cooperation with tissues in the oral and maxillofacial regions, and proper responsiveness via sensory receptors to noxious stimulations in the maxillofacial region. There are currently no published reports describing successful replacement with a fully functional bioengineered organ.

In our current study, we describe a fully functioning tooth replacement achieved by transplantation of a bioengineered tooth germ into the alveolar bone of a lost tooth region in an adult mouse. We propose this as a model for future organ replacement therapy. The bioengineered tooth, which was erupted and reached occlusion in the oral environment, had the correct tooth structure, hardness of mineralized tissues for mastication, and responsiveness to experimental orthodontic treatment and noxious stimulation in cooperation with tissues in the oral and maxillofacial regions. Our results thus demonstrate

Author contributions: T.T. designed research; E.I., R.M., and K.N. performed research; E.I., K.N., T.T.-Y., and S.K. contributed new reagents/analytic tools; E.I., R.M., K.N., K.I., T.N., M.O., and M.M. analyzed data; and E.I., R.M., K.N., and T.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]E.I. and R.M. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: t-tsuji@rs.noda.tus.ac.jp.

This article contains supporting information online at www.pnas.org/cgi/content/full/0902944106/DCSupplemental.

BIOMEDICAL JOURNAL 43 (2020) 124–133

Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Review Article

Banking on teeth – Stem cells and the dental office

*Benjamin D. Zeitlin**

University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA

Dr. Benjamin D. Zeitlin

ARTICLE INFO

Article history:
Received 12 November 2019
Accepted 12 February 2020
Available online 4 May 2020

Keywords:
Dental pulp stem cell
Cryopreservation
Dentistry
Endodontic
Exfoliated

ABSTRACT

Science and commerce advance together and the stem cell field is no exception. With the promise of cures for conditions as diverse as cancer, autism, neural degeneration, organ replacement and addiction, long-term preservation of dental stem cells is a growth market. The discovery nearly twenty years ago, of viable, multipotent, stem cells in dental pulp from both baby and adult teeth initiated, and drives, this market.The dental stem cell preservation services, "tooth banks", focus on the collection of a child's baby teeth, as they are shed naturally, and storage of the stem cells from within the pulp for therapeutic use in later years should the child require them. This review focuses on the procedures related to these stem cell storage services and may serve as an introduction for many to the practice of "tooth banking".

Dentists have been changing lives for hundreds of years - removing pain, enabling people to eat normally, returning faces to their original splendor - but modern dentists are not only changing lives, they are saving them.

Over the last decade a service once solely the purview of hospital clinics, is becoming more and more popular in the dental office. The collection of stem cells for long-term storage for therapeutic use is a service now provided by dentists. More accurately, the dentist collects teeth and "tooth banking" services extract and preserve the stem cells within the pulp for the future benefit of the patient (Fig. 1).

Stem cell collection from bone marrow, blood, fetal material and umbilical cords present unique practical and conflicting ethical challenges [1,2]. However, the discovery of postnatal stem cell populations in the tooth pulp by Gronthos and Shi [3,4], around two decades ago, opened up new horizons to stem cell research and propelled the dental profession further into the exciting field of regenerative medicine. Post-natal stem cells are present in pulp from deciduous teeth (baby or milk teeth) lost - or exfoliated - by all children during the first 6–12 years of development and are also commonly available from orthodontic extraction of third molars (wisdom teeth) in adults.

* *Corresponding author*. University of the Pacific, Arthur A. Dugoni School of Dentistry, 155 Fifth St., San Francisco, CA 94103, USA.
E-mail address: bzeitlin@pacific.edu.
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2020.02.003

REVIEW

Mesenchymal Stem Cells and Tooth Engineering

Li Peng, Ling Ye, Xue-dong Zhou*
State Key Laboratory of Oral Disease, Sichuan University, Chengdu, China

Abstract

Li Peng, Ling Ye, Xue-dong Zhou. Mesenchymal Stem Cells and Tooth Engineering. *International Journal of Oral Science*, 1(1): 6–12, 2009

Tooth loss compromises human oral health. Although several prosthetic methods, such as artificial denture and dental implants, are clinical therapies to tooth loss problems, they are thought to have safety and usage time issues. Recently, tooth tissue engineering has attracted more and more attention. Stem cell based tissue engineering is thought to be a promising way to replace the missing tooth. Mesenchymal stem cells (MSCs) are multipotent stem cells which can differentiate into a variety of cell types. The potential MSCs for tooth regeneration mainly include stem cells from human exfoliated deciduous teeth (SHEDs), adult dental pulp stem cells (DPSCs), stem cells from the apical part of the papilla (SCAPs), stem cells from the dental follicle (DFSCs), periodontal ligament stem cells (PDLSCs) and bone marrow derived mesenchymal stem cells (BMSCs). This review outlines the recent progress in the mesenchymal stem cells used in tooth regeneration.

Keywords mesenchymal stem cell, tooth engineering, dental pulp stem cell

Document code: A **CLC number:** Q813.1 Received Dec.18,2008; Revision accepted Jan.21,2009

Introduction

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" (Langer and Vacanti, 1993). Tissue engineering has also been defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use" (MacArthur and Oreffo, 2005). Tissue engineering aims to stimulate the body either to regenerate tissue on its own or to grow tissue outside the body which can then be implanted as natural tissue.

Stem cells are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. According to developmental stages, stem cells can be divided into embryonic stem cells and adult stem cells. Differentiation and proliferation of embryonic stem cells constitute the basis of animal development. The further differentiation of adult stem cells is the prerequisite of tissues and organs' repair and regeneration. Embryonic stem cells are the progenitors of undifferentiated cells, which are "totipotent" (totipotency is the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues) and can differentiate into a variety of cells to form various organs, also known as the "all-competent cells". In the process of cell differentiation, they can gradually differentiate into a stable form of "pluripotent stem cells". With the features of highly proliferative capacity and plasticity, stem cells are regarded as a new source of seed cells in tissue engineering in a wide range of applications.

There is no doubt that the description of tissue engineer offers a new hope to both patients who

Herberts et al. Journal of Translational Medicine 2011, 9:29
http://www.translational-medicine.com/content/9/1/29

JOURNAL OF TRANSLATIONAL MEDICINE

REVIEW Open Access

Risk factors in the development of stem cell therapy

Carla A Herberts[1*], Marcel SG Kwa[2], Harm PH Hermsen[1]

Abstract

Stem cell therapy holds the promise to treat degenerative diseases, cancer and repair of damaged tissues for which there are currently no or limited therapeutic options. The potential of stem cell therapies has long been recognised and the creation of induced pluripotent stem cells (iPSC) has boosted the stem cell field leading to increasing development and scientific knowledge. Despite the clinical potential of stem cell based medicinal products there are also potential and unanticipated risks. These risks deserve a thorough discussion within the perspective of current scientific knowledge and experience. Evaluation of potential risks should be a prerequisite step before clinical use of stem cell based medicinal products.

The risk profile of stem cell based medicinal products depends on many risk factors, which include the type of stem cells, their differentiation status and proliferation capacity, the route of administration, the intended location, *in vitro* culture and/or other manipulation steps, irreversibility of treatment, need/possibility for concurrent tissue regeneration in case of irreversible tissue loss, and long-term survival of engrafted cells. Together these factors determine the risk profile associated with a stem cell based medicinal product. The identified risks (*i.e.* risks identified in clinical experience) or potential/theoretical risks (*i.e.* risks observed in animal studies) include tumour formation, unwanted immune responses and the transmission of adventitious agents.

Currently, there is no clinical experience with pluripotent stem cells (i.e. embryonal stem cells and iPSC). Based on their characteristics of unlimited self-renewal and high proliferation rate the risks associated with a product containing these cells (e.g. risk on tumour formation) are considered high, if not perceived to be unacceptable. In contrast, the vast majority of small-sized clinical trials conducted with mesenchymal stem/stromal cells (MSC) in regenerative medicine applications has not reported major health concerns, suggesting that MSC therapies could be relatively safe. However, in some clinical trials serious adverse events have been reported, which emphasizes the need for additional knowledge, particularly with regard to biological mechanisms and long term safety.

Introduction

Stem cells are undifferentiated cells that have the capacity to proliferate in undifferentiated cells both *in vitro* and *in vivo* (self-renewal) and to differentiate into mature specialized cells.

The field of stem cell therapy is rapidly developing, and many clinical trials have been initiated exploring the use of stem/progenitor cells in the treatment of degenerative diseases and cancer and for the repair of damaged or lost tissues. Despite the great promise, there are still many questions regarding the safe application of stem cell therapy. In this paper we will focus on risks associated with stem cell therapy, based on both theoretical concerns and examples of adverse observations.

Based on their characteristics different stem cells types have been described (table 1). The distinctive feature of different stem cell types is based on the capability of the cells to differentiate along multiple lineages and produce derivatives of cell types of the three germ layers or to produce multiple cell types. Below different stem cell types are briefly described.

Embryonal stem cells

In the early sixties researchers isolated a single cell type from a teratocarcinoma, a tumour derived from a germ cell. These embryonal carcinoma cells are the stem cells of teratocarcinomas which can be considered the

* Correspondence: carla.herberts@rivm.nl
[1]Centre for Biological Medicines and Medical Technology, National Institute for Public Health and the Environment, A. v. Leeuwenhoeklaan 9, P.O.Box 1, 3720 BA, Bilthoven, The Netherlands
Full list of author information is available at the end of the article

Wuputra et al. Journal of Experimental & Clinical Cancer Research (2020) 39:100
https://doi.org/10.1186/s13046-020-01584-0

Journal of Experimental & Clinical Cancer Research

REVIEW Open Access

Prevention of tumor risk associated with the reprogramming of human pluripotent stem cells

Kenly Wuputra[1,2], Chia-Chen Ku[1,2], Deng-Chyang Wu[2,3], Ying-Chu Lin[4], Shigeo Saito[5,6*] and Kazunari K. Yokoyama[1,2,5*]

Abstract

Human pluripotent embryonic stem cells have two special features: self-renewal and pluripotency. It is important to understand the properties of pluripotent stem cells and reprogrammed stem cells. One of the major problems is the risk of reprogrammed stem cells developing into tumors. To understand the process of differentiation through which stem cells develop into cancer cells, investigators have attempted to identify the key factors that generate tumors in humans. The most effective method for the prevention of tumorigenesis is the exclusion of cancer cells during cell reprogramming. The risk of cancer formation is dependent on mutations of oncogenes and tumor suppressor genes during the conversion of stem cells to cancer cells and on the environmental effects of pluripotent stem cells. Dissecting the processes of epigenetic regulation and chromatin regulation may be helpful for achieving correct cell reprogramming without inducing tumor formation and for developing new drugs for cancer treatment. This review focuses on the risk of tumor formation by human pluripotent stem cells, and on the possible treatment options if it occurs. Potential new techniques that target epigenetic processes and chromatin regulation provide opportunities for human cancer modeling and clinical applications of regenerative medicine.

Keywords: Cancer risk, Cell reprogramming, Pluripotent stem cells, Regenerative medicine, Therapeutic agents

Background

The first successful mammalian reprogramming of vegetal cells to totipotent cells using the technology of nuclear transfer generated the cloned sheep "Dolly" [1]. In recent decades, the problems caused by tumorigenesis generated by oocytes (embryos) created by nuclear transfer have been underestimated. The creation of induced pluripotent stem cells (iPSCs) requires the expression of stemness-related genes, such as the combination of *Oct4*, *Sox2*, *Klf4*, and c-*Myc* (OSKM) and that of *Oct4*, *Sox2*, *Nanog* and *Lin28* (OSNL) [2–5]. Studies of the risk of tumorigenesis and cancerous transformation have considered somatic cell reprogramming in the context of cancer patient-specific reprogramming [2–12].

Stem cells are putative candidates for cancerous transformation given their ability to self-renew and to dedifferentiate, which can lead to the acquisition of both the genetic and epigenetic modifications required for tumorigenesis [13, 14]. The stemness-related transcription factors are expressed in embryonic stem cells (ESCs) and adult stem cells, but they are not generally expressed in adult somatic cells. Abnormal expression of ESC-specific factors has recently been reported in human tumors [15–17]. A retrospective study of human patient cohorts has shown that the expression of these factors with survival outcomes in specific tumor types,

* Correspondence: Saict1@maple.ocn.ne.jp; kazu@kmu.edu.tw
[5]Waseda University Research Institute for Science and Engineering, Shinjuku, Tokyo 162-8480, Japan
[1]Graduate Institute of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Rd., San-Ming District, Kaohsiung 807, Taiwan
Full list of author information is available at the end of the article

Review Article

Stem Cell INVESTIGATION

Stem cell manipulation, gene therapy and the risk of cancer stem cell emergence

Flora Clément[1,2*], Elodie Grockowiak[1,2*], Florence Zylbersztejn[2], Gaëlle Fossard[1,2,3], Stéphanie Gobert[1,2], Véronique Maguer-Satta[1,2]

[1]Univ Lyon, Université Claude Bernard Lyon 1, Lyon, F-69008, France; [2]Univ Lyon, Université Claude Bernard Lyon 1, INSERM U1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon, F-69008, France; [3]Centre Hospitalier Lyon Sud, Hematology Department, Tours, France

Contributions: (I) Conception and design: F Clément, E Grockowiak, S Gobert, V Maguer-Satta; (II) Administrative support: V Maguer-Satta; (III) Provision of study material or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Véronique Maguer-Satta. CRCL, U1052-UMR5286, 28 rue Laennec, 69373 Lyon Cedex 08, France.
Email: veronique.maguer-satta@lyon.unicancer.fr.

Abstract: Stem cells (SCs) have been extensively studied in the context of regenerative medicine. Human hematopoietic stem cell (HSC)-based therapies have been applied to treat leukemic patients for decades. Handling of mesenchymal stem cells (MSCs) has also raised hopes and concerns in the field of tissue engineering. Lately, discovery of cell reprogramming by Yamanaka's team has profoundly modified research strategies and approaches in this domain. As we gain further insight into cell fate mechanisms and identification of key actors and parameters, this also raises issues as to the manipulation of SCs. These include the engraftment of manipulated cells and the potential predisposition of those cells to develop cancer. As a unique and pioneer model, the use of HSCs to provide new perspectives in the field of regenerative and curative medicine will be reviewed. We will also discuss the potential use of various SCs from embryonic to adult stem cells (ASCs), including induced pluripotent stem cells (iPSCs) as well as MSCs. Furthermore, to sensitize clinicians and researchers to unresolved issues in these new therapeutic approaches, we will highlight the risks associated with the manipulation of human SCs from embryonic or adult origins for each strategy presented.

Keywords: Cancer; clinical trials; regenerative medicine; stem cells (SCs); tissue engineering

Received: 28 February 2017; Accepted: 13 July 2017; Published: 25 July 2017.
doi: 10.21037/sci.2017.07.03
View this article at: http://dx.doi.org/10.21037/sci.2017.07.03

Introduction

The last few decades have witnessed major achievements in stem cell (SC) manipulation (*Figure 1*). This is especially true for hematopoietic stem cells (HSCs) due to the development of SC transplantation several decades ago, and more recently to that of gene therapy (GT) (*Figure 2*). Lately, SC researchers have made a tremendous breakthrough by artificially inducing cell reprogramming, thus increasing the probability of curing genetic diseases using GT. However, despite these new attractive concepts and exciting results, artificial modification of genes is also likely to generate unwanted consequences and requires caution. Therefore, safety procedures remain a fundamental issue in the field. To illustrate these progresses and remaining issues, we will present key examples of the use of HSCs and of GT. We will discuss the "duality" of using mesenchymal stem cells (MSCs) and provide perspectives on novel opportunities brought about by a new era of fetal, pluripotent and mature SCs. We will present the development of associated